# SYNTHESIS OF PYRIMIDINE, THIAZINE, DIAZEPINE AND QUINOXALINE DERIVATIVES *VIA* 3-COMPONENT REACTIONS (3-CR)

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I, Reagan Lehlogonolo Mohlala student number 795177, declare that the work presented in this thesis was done entirely by myself under the supervision of Prof Moira L. Bode and Dr E. Mabel Coyanis. It is being submitted for the degree of Doctoral philosophy of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.



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## Abstract

Preliminary molecular modeling (virtual screening) in search of bioactive heterocyclic compounds as potential disrupters of HIV-1 integrase and LEDGF/p75 interactions resulted in the identification of fused heterocyclic scaffolds such as hexahydroquinolines, pyrimidines quinoxalines and diazepines. The use of multicomponent reactions (MCRs) for synthesis of heterocyclic scaffolds in synthetic chemistry remains one of the preferred approaches due to atom economy. This thesis describes the use of three-component reactions for the synthesis of fused pyrimidines, thiazines, diazepines and quinoxalines as a strategic approach to accessing these fused heterocycles.

The hexahydroquinoline derivatives identified as potential ligands to disrupt the interaction of HIV-1 IN and LEDGF/p75 were chosen for initial synthesis based on availability of reagents and a facile synthetic route. This synthesis resulted in a library of fifteen novel hexahydroquinoline derivatives.

The first MCR approach applied involved was 3-CR of isocyanides with electron deficient alkynes (DMAD), giving rise to a zwitterion adduct that was reacted further with a range of prepared five-membered ring substrates containing an acidic proton. The resulting products were 5-6 fused ring heterocycles. When a thiazole derivative was used as a substrate the final racemic products obtained, containing a single stereogenic centre, were (*E*)-dimethyl 7-(*tert*-butylamino)-2-(2-methoxy-2-oxoethylidene)-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidine-5,6-dicarboxylates in good yields. The second five-membered ring substrate used was pyrrolidine which gave dimethyl 2-(*tert*-butylamino)-6-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]pyrimidine-3,4-dicarboxylates, also containing one stereogenic centre. Two substrates tested, 1*H*-1,2,4-triazole-5-thiol and 1*H*-1,2,4-triazol-5-amine each resulted in formation of two co-products; from a three-component reaction as well as a two-component reaction where the substrate reacted directly with DMAD. Substrate 1*H*-1,2,4-triazole-5-thiol gave rise to dimethyl 5-(*tert*-butylamino)-7*H*-[1,2,4]triazolo[5,1-*b*][1,3]thiazine-6,7-dicarboxylate and methyl 7-oxo-7*H*-[1,2,4]triazolo[5,1-*b*][1,3]thiazine-5-carboxylate while substrate 1*H*-1,2,4-triazol-5-amine gave rise to dimethyl 5-(*tert*-butylamino)-6,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6,7-dicarboxylate and methyl 5-oxo-5,8-dihydro-[1,2,4]triazolo[4,3-*a*]pyrimidine-7-carboxylate.

The substrate scope of the 3-CR was extended by using six-membered 2-amino-4*H*-1,3-thiazin-4-one derivatives to obtain 6-6 fused ring heterocycles. When this set of substrates was treated with zwitterion adducts this resulted in formation of 4-oxo-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate derivatives, containing one stereogenic centre, in good yields without use of a catalyst.

The Michael reaction of prepared five-membered ring substrates with electron deficient alkynes (DMAD\DEtAD\DTAD) gave rise to fused thiazole-pyrimidine derivatives containing no stereogenic centre. When thiazol-2-amine and its derivatives were treated with electron deficient alkynes they gave average yields of methyl 7-oxo-7*H*-thiazolo[3,2-*a*]pyrimidine-5-carboxylate derivatives. The alternative route of employing thiourea, α-haloketone and DMAD gave improved yields of the resulting thiazolo[3,2-*a*]pyrimidine-5-carboxylate derivatives *via* 3-CR. When DMAD\DEtAD were treated with 1*H*-1,2,4-triazol-5-amine or 1*H*-1,2,4-triazole-5-thiol this resulted in good yields from 2-CR. When 2-imino-1,3-oxazolidine was reacted with DMAD\DEtAD this resulted in a good yield of methyl 5-oxo-3,5-dihydro-2*H*-oxazolo[3,2-*a*]pyrimidine-7-carboxylates.

The unexpected incorporation of the solvent acetone in reaction with an isocyanide and benzimidazole *via* 3-CR resulted in fused diazepine scaffolds, 2-methyl-*N*-(2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[*b*][1,4]diazepin-10-ylidene)propan-2-amine derivatives, when using benzimidazole bearing neutral or activating groups on the benzene ring such as hydrogen and methyl. When using deactivating groups on the benzimidazole such as an ester group, reaction with acetone and isocyanide gave quinoxaline scaffolds instead. These reactions occurred without use of solvent or catalyst. The reaction of phenylenediamine or benzimidazole with DMAD\DEtAD\DTAD gave rise to diazepine scaffolds.

Various biological studies were conducted on the synthesised compounds, such as antimicrobial Minimum Inhibitory Concentration (MIC) assays for Gram-positive and Gram-negative organisms; antiviral HIV assays which were conducted in infected MT4 cells; cytotoxicity assays which were conducted in the MT4 cell line at 100 μM; and an assay was conducted on certain substrates to test for inhibition or disruption of the HIV-1 IN-LEDGF/p75 protein-protein interaction.

## Dedications

I dedicate this thesis to my family and close relatives:

To my parents

Andrew Mohlala, Monica Mohlala

To my siblings/close relatives

McDonald, Gregory, Tumelo, Onica, Lebo, Phethego, Andria.

Little kids and babies:

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## List of abbreviations

AlphaScreen: Amplified luminescent proximity pomogeneous assay

ADME: Absorption, distribution, metabolism and excretion

$Al(N_3)_3$: Aluminium azide

Al-MCM-41: Aluminium mobil composition of matter No.41

AFIR: Artificial force induced reaction

2-CR: Two-component reactions

3-CR: Three-component reactions

4-CR: Four-component reactions

CSA: Camphorsulfonic acid

CCD: Catalytic core domain

$CC_{50}$: 50% Cytotoxic concentration

$CDCl_3$: Deuterated chloroform

CDI: Carbonyldiimidazole

DCM: Dichloromethane

DDQ: 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone

DEtAD: Diethyl acetylenedicarboxylate

DHMP: 3,4-Dihydropyrimidin-2(1*H*)-one

DHP: Dihydropyridine

DMAD: Dimethyl acetylenedicarboxylate

DMF: *N*,*N*-Dimethylformamide

DMSO-$d_6$: Deuterated dimethyl sulfoxide

DTAD: Di*tert*-butyl acetylenedicarboxylate

EtOAc: Ethyl acetate

EtOH: Ethanol

ESI-LCMS: Electrospray ionization liquid chromatography mass spectrometry

ELISA: Enzyme-linked immunosorbent assay

FTIR: Fourier transform infrared spectroscopy

HRMS: High resolution mass spectrometry

HIV-1: Human immunodeficiency virus type-1

h: Hour(s)

$HN_3$: Hydrazoic acid

IBD: Integrase binding domain

IMCRs: Isocyanide-based multicomponent reactions

IN: Integrase enzyme

INT: Iodonitrotetrazolium

iPrOH: Isopropanol

LDA: Lithium diisopropylamide

LEDGF/p75: Lens epithelium-derived growth factor, or transcriptional coactivator

MT-4: Metallothionein 4 cell line

MeCN: Acetonitrile

MeOH: Methanol

MIC: Minimum inhibitory concentration

Min: Minute(s)

MMGBSA: Molecular mechanics/generalized born surface area

MCRs: Multicomponent reactions

MW: Microwave irradiation

NMR: Nuclear magnetic resonance

RNAi: Ribonucleic acid interference

TEMPO: 2,2,6,6-Tetramethyl-piperidin-1-oxyl

THF: Tetrahydrofuran

TFE: 2,2,2-Trifluoroethanol

TosMIC: *p*-Toluenesulfonylmethyl isocyanide

TLC: Thin layer chromatography

TSB: Tryptic soy broth

pTsOH: Toluenesulfonic acid

Wits: University of the Witwatersrand

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# CHAPTER 1

# Literature review

### 1.1 Introduction to multicomponent reactions

Multicomponent reactions (MCRs) are also known as one-pot reactions that comprise of at least three reactants chosen to form a single product containing building blocks of atoms from all the reactants.[1,2,3] The general practical concept of MCRs is depicted in Figure 1.1 adopted from Rocha and co-workers,[4] which shows at least four different reactants with different functional groups converging to form one product.

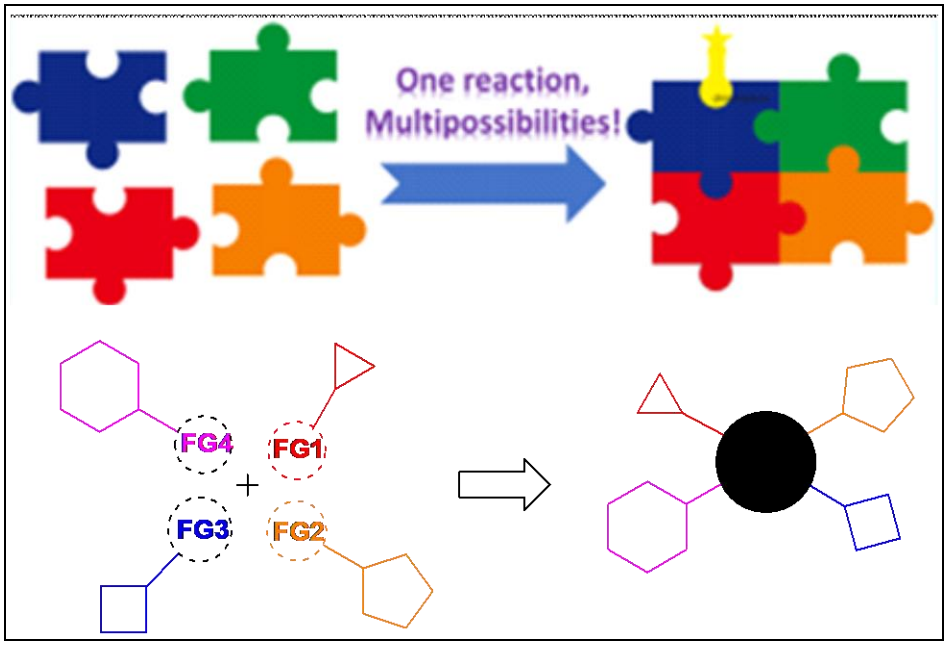



**Figure 1.1**: Schematic representation of multicomponent reactions (Rocha and co-workers[4])

There are a number of well-known MRCs such as a) the Ugi four-component reaction[5] (U-4CR), b) the Passerini three-component reaction[6, 7] (P-3CR), c) the Biginelli three-component reaction[8] (B-3CR) and d) the Hantzsch three-component reaction[9] (H-3CR) represented in Figure 1.2.

MCRs have been exploited for over 100 years; the initial discovery and report of an MCR remains difficult to identify, however, the Strecker[10] multicomponent reaction (S-3CR) was reported in 1850 followed by the Hantzsch dihydropyridine (DHP) synthesis in 1882. The Biginelli 3-CR was reported in 1891 while the Mannich[11] reaction (M-CR) was reported in

1912. The first isocyanide-based MCRs (IMCR) was reported in 1921 and 1959 by Passerini[6] (3-CR) and Ugi,[12] respectively.

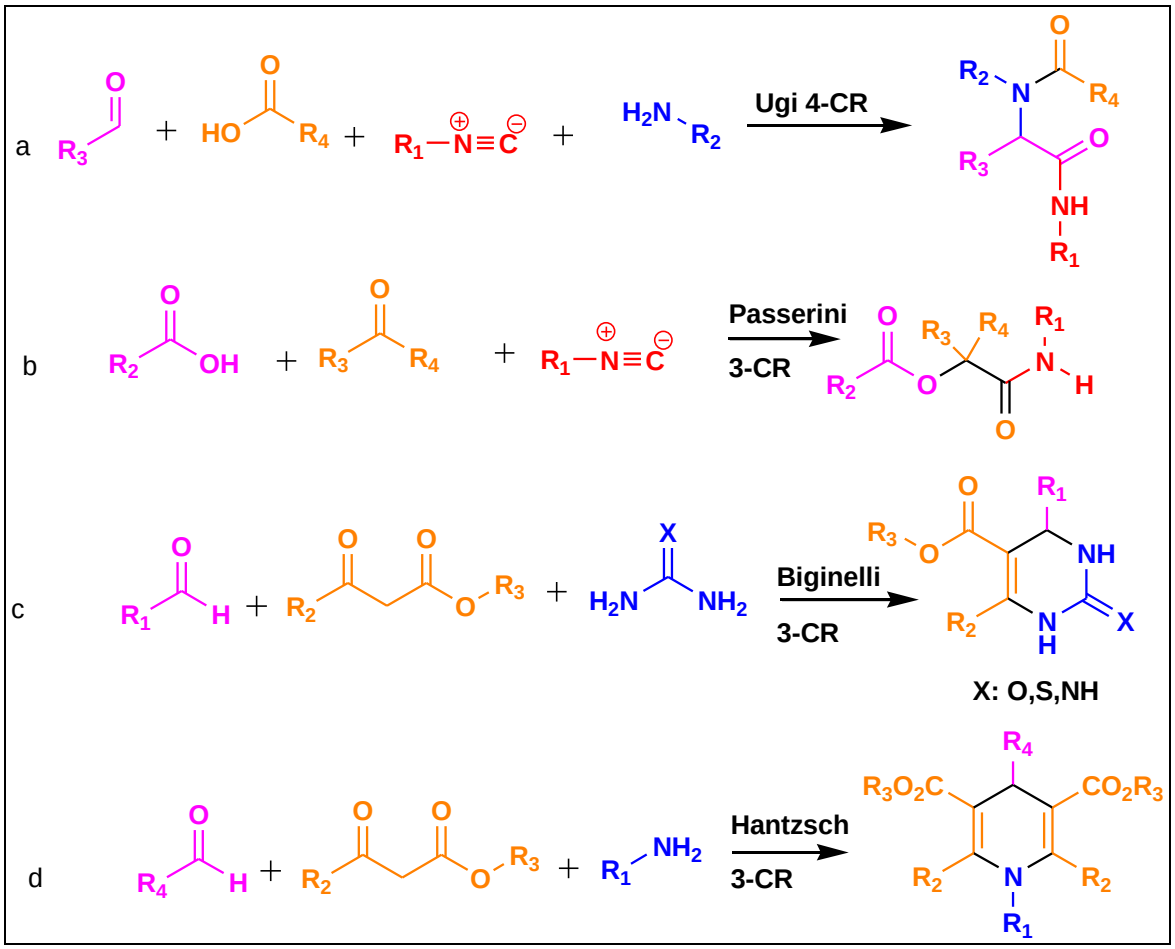



**Figure 1.2**: Examples of MRCs: a) Ugi, b) Passerini, c) Biginelli and d) Hantzsch.

MCRs are considered a convenient approach in synthetic chemistry and have many advantages over the traditional one or two component reactions by reducing the number of sequential multiple reactions required and often producing better yields.[13,14] The most famous MCRs are the Passerini and Ugi reactions which regained attention in the early 1990s.[15,16] These reactions rely on the dual electrophilic/nucleophilic reactivity of the isocyanide carbon to provide distinct complex products. The reactions of isocyanides in MCRs are well represented by the Passerini 3-CR, Ugi 3-CR and Ugi 4-CR.[17,18]

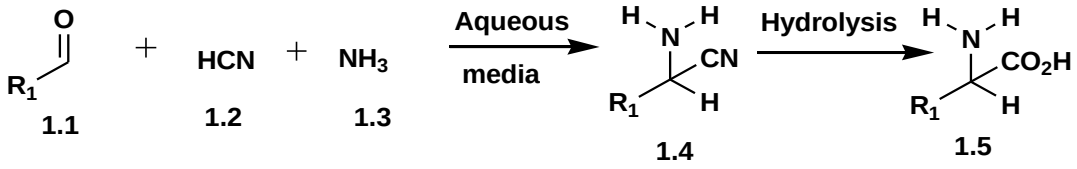
MCRs continue to attract attention in medicinal chemistry and drug discovery applications, due to the possibility of readily obtaining different heterocyclic frameworks of interest.[19] Interestingly, MCRs additionally possess considerable potential for contributing to green

production processes and protocols of pharmaceuticals, by generating complex products in a single step.[20,21] Compared to sequential multistep reactions, MCRs contribute significantly to minimising the large amount of waste generated daily in pharmaceutical and industrial production.[20,22,23, ,24]

The following sections summarise the history and use of MCRs, the elegant chemistry that they offer, the use of this approach in drug discovery, and the considerable use of MCRs in "green" chemical production and production of natural products.

### 1.2. Strecker reaction (S-3CR)

The Strecker reaction[10] was first reported in 1850 after condensation of an aldehyde **1.1** with a cyanide source **1.2** in the presence of ammonia **1.3** to gave intermediate **1.4** followed by hydrolysis of the nitrile group to form an amino acid **1.5** as shown in Scheme 1.1.



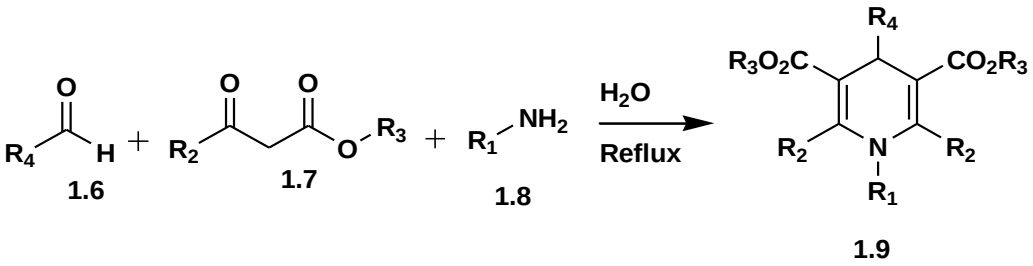

**Scheme 1.1**: The Strecker 3-CR (S-3CR)

The fact that the Strecker reaction occurs in aqueous medium and under catalyst-free conditions makes it one of the favourable methods for application in industries and laboratories because it is a convenient approach to produce amino acids, natural products and bioactive compounds. Another factor to consider is the commercial availability and affordability of the substrates required. [25,26,27] To date the Strecker reaction has produced a myriad of publications and is still under ongoing investigation. There are several biologically active compounds produced using the S-3CR, such as boron-containing retinoids,[28] hepatitis C virus $NS_3$ serine protease inhibitors[29] and (±)-phthalascidin 622.[30] Several methods used for the synthesis of amino acids and bioactive compounds involve the use of catalysts, such as Al-MCM-41,[31] nanocrystalline magnesium oxide,[32] BINOL-phosphoric acid,[33] *N*-heterocyclic carbene (NHC)-amidate palladium(II) complex,[34] gallium (III) triflate[35] and bisformamides.[36] However, the use of catalysts and non-green solvents results in additional work-up steps and the requirement for

additional waste treatments. Based on these disadvantages there is a continuous search for safer and more practical Strecker methods to synthesise bioactive compounds.

### 1.3. Hantzsch reaction (H-3CR)

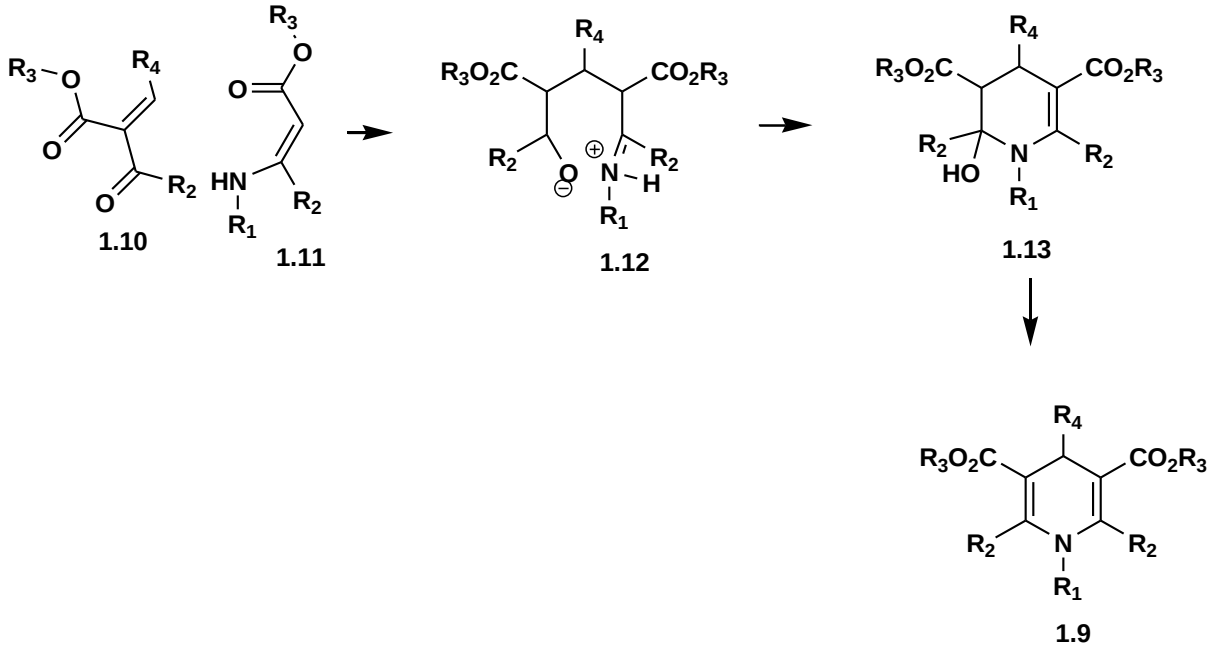
The first Hantzsch 3-CR was reported in 1882 after an aldehyde **1.6** and two equivalents of a β-ketoester **1.7** were condensed in the presence of a nitrogen donor **1.8** to give dihydropyridine (DHP) derivatives **1.9** [9, 37,38] as shown in Scheme 1.2.




**Scheme 1.2**: The Hantzsch 3-CR (H-3CR)

Dihydropyridines are considered a significant group of heterocyclic compounds in drug discovery and they can also be found in nature.[39,40,41] There are several catalysts which have also been comprehensively applied to promote the formation of the DPH ring such as ferric chloride ($Fe_3Cl$) or potassium permanganate, nano-γ-$Fe_2O_3$-$SO_3H$,[42] Al-MCM-41 and Fe/Al-MCM-41,[43] $CuBr_2$,[44] $SiO_2$/$P_2O_5$-$SeO_2$,[45] $H_2O_2$/$Co(OAc)_2$[46] and $Na_2S_2O_4$/TBHP.[47]

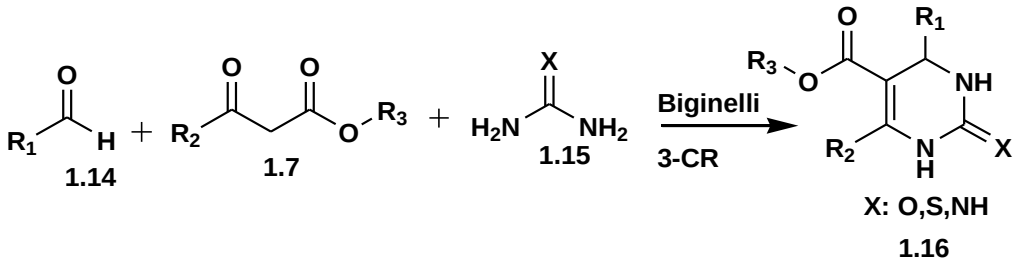
In consideration of green chemistry principles[48] and the use of safe practices in laboratories, functionalised Brønsted acidic ionic liquids are used to replace traditional catalysts used for synthesis of DPH derivatives: Sharma and co-workers[49] used a $SO_3H$-functionalised imidazolium ionic liquid, Liu and co-workers[50] used 3-(*N*,*N*-dimethyldodecylammonium) propanesulfonic acid hydrogen sulphate ([DDPA][$HSO_4$]), Hu and co-workers[51] used $H_2O_2$ catalysed by $V_2O_5$ in ionic liquid [$C_{12}$mim][$HSO_4$], and Lui and Lui[52] used 3-(*N*,*N*-dimethyldodecylammonium) propanesulfonic acid hydrogen sulphate ([DDPA][$HSO_4$]) with $NaNO_3$. The formation of the DPH ring as represented in Scheme 1.3 (see Katritzky and co-workers)[53] occurs by reaction of an aldehyde with a β-ketoester to give one intermediate **1.10**, and the nitrogen source reacts with another molecule of β-ketoester to form another intermediate **1.11**. These two intermediates subsequently react to generate intermediate **1.12**, leading to the formation of DPH **1.9**.




**Scheme 1.3**: Formation of DPH *via* H-3CR

### 1.4. Biginelli reaction (B-3CR)

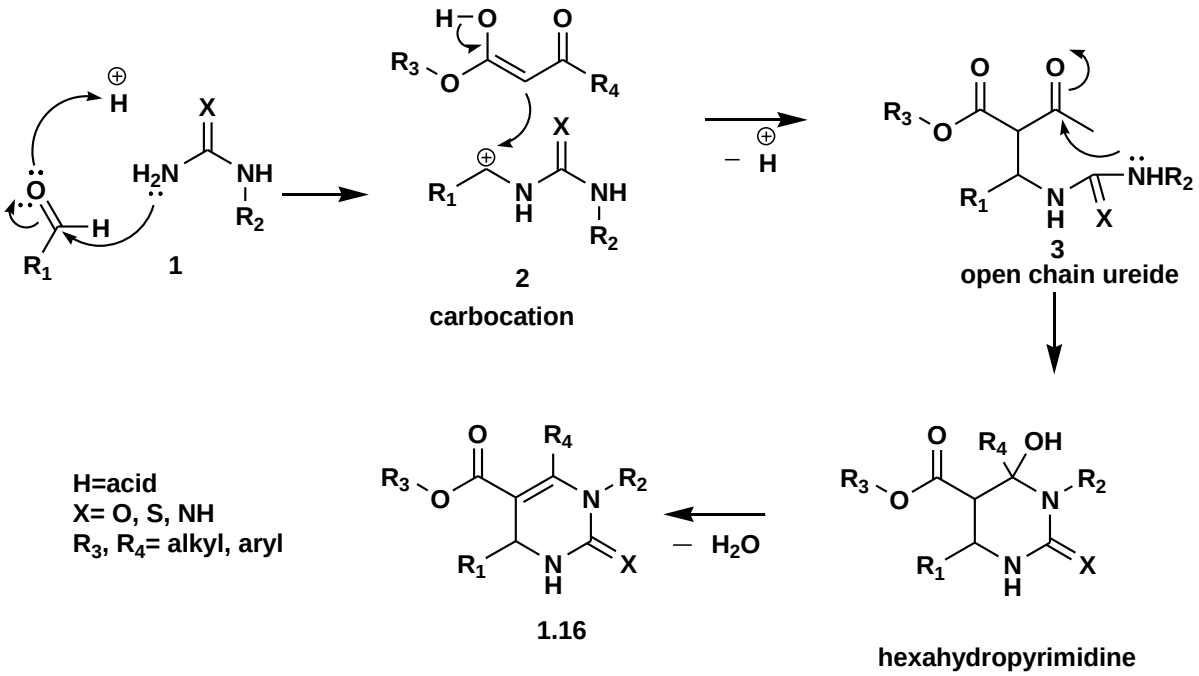
The 3-CR Biginelli reaction[8] was discovered in 1891 and involves condensation of an aldehyde **1.14** with a β-ketoester **1.7** in the presence of urea **1.15** or thiourea or guanidine hydrochloride under acidic catalysis to obtain 3,4-dihydropyrimidin-2(1*H*)-one (DHMP) **1.16** as shown in Scheme 1.4




**Scheme 1.4**: The Biginelli 3-CR (B-3CR)

The B-3CR initially had some challenges with poor yields and a very small substrate scope range. The discovery of the first B-3CR was followed by a thorough investigation into understanding the course of the reaction and structural variations. The reactions towards the formation of DHMP were explored by investigating solvent compatibility and effects, acid catalyst and increasing substrate scope.[54] The exploration was extended from solution reactions

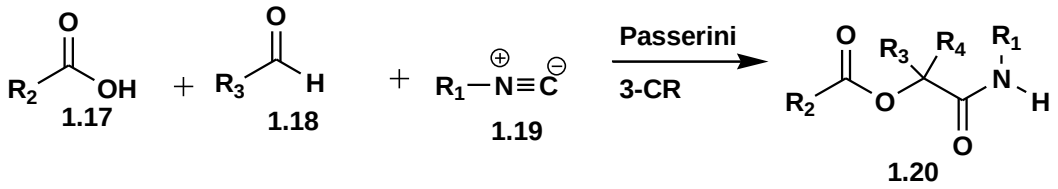
to solid phase reactions using microwave assistance. The DHMPs have interesting biological activities such as calcium channel modulation,[55] antimicrobial and antitubercular activity.[56] The proposed mechanism of the B-3CR (see Kappe)[57] is shown in Scheme 1.5. A Lewis or a Brønsted acid activates the aldehyde at event **1** in order to react with urea/thiourea to form a hemiaminal species which leads to the elimination of water forming an *N*-acyliminium cation at event **2**. The carbocation at event **2** readily reacts with the nucleophilic α-carbon atom centre of a β-ketoester to form an open chain ureide at intermediate **3**, followed by ring closing to form a hexahydropyrimidine intermediate and elimination of water to form DHMP. There are other alternative mechanisms reported and discussed in the literature.[58,59]




**Scheme 1.5**: Possible mechanism for the Biginelli 3-CR (B-3CR).

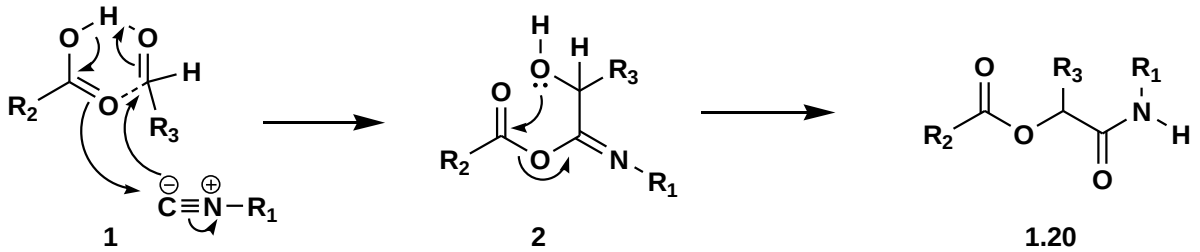
### 1.5. Passerini reaction (P-3CR)

Isocyanide-based multicomponent reactions (IMCRs) have gained much prestige, interest and growth over the last few decades. This area has become one of the exciting and robust tools for peptidomimetic synthesis. The first IMCR was reported by Passerini[6] as a 3-CR in 1921 after the reaction of a carboxylic acid **1.17** with an aldehyde or ketone **1.18** in the presence of an isocyanide **1.19** to give rise to an α-acyloxy carboxamide product **1.20** containing both amide and ester functionalities as shown in Scheme 1.6.



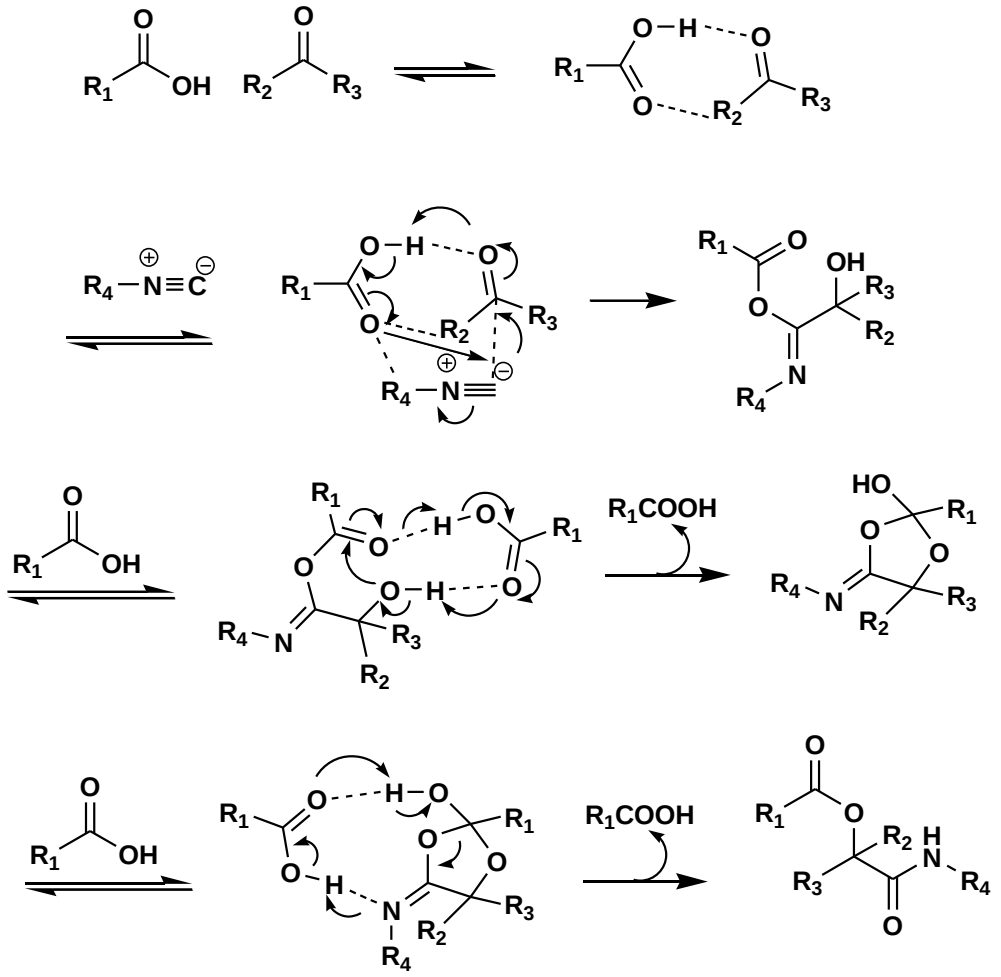

**Scheme 1.6**: The Passerini 3-CR (P-3CR)

The first proposal by Passerini was that the reaction mehanism might involve a zwitterion intermediate. The extensive research described in the literature to investigate the Passerini reaction mechanism has suggested various intermediates including carbocations, hemiacetals and hydrogen-bonded adducts.[60] However, the original Passerni reaction mechanism is generally considered to be a concerted reaction, especially in apolar solvents as shown in Scheme 1.7.[61] The reaction proceed by the activation of an aldehyde by the carboxylic acid and subsequent addition of an isocyanide to form nitrilium as shown at events **1** and **2**. The nitrilium is trapped by the carboxylate followed by a Mumm rearrangement leading to the α-acyloxy amide **1.20**



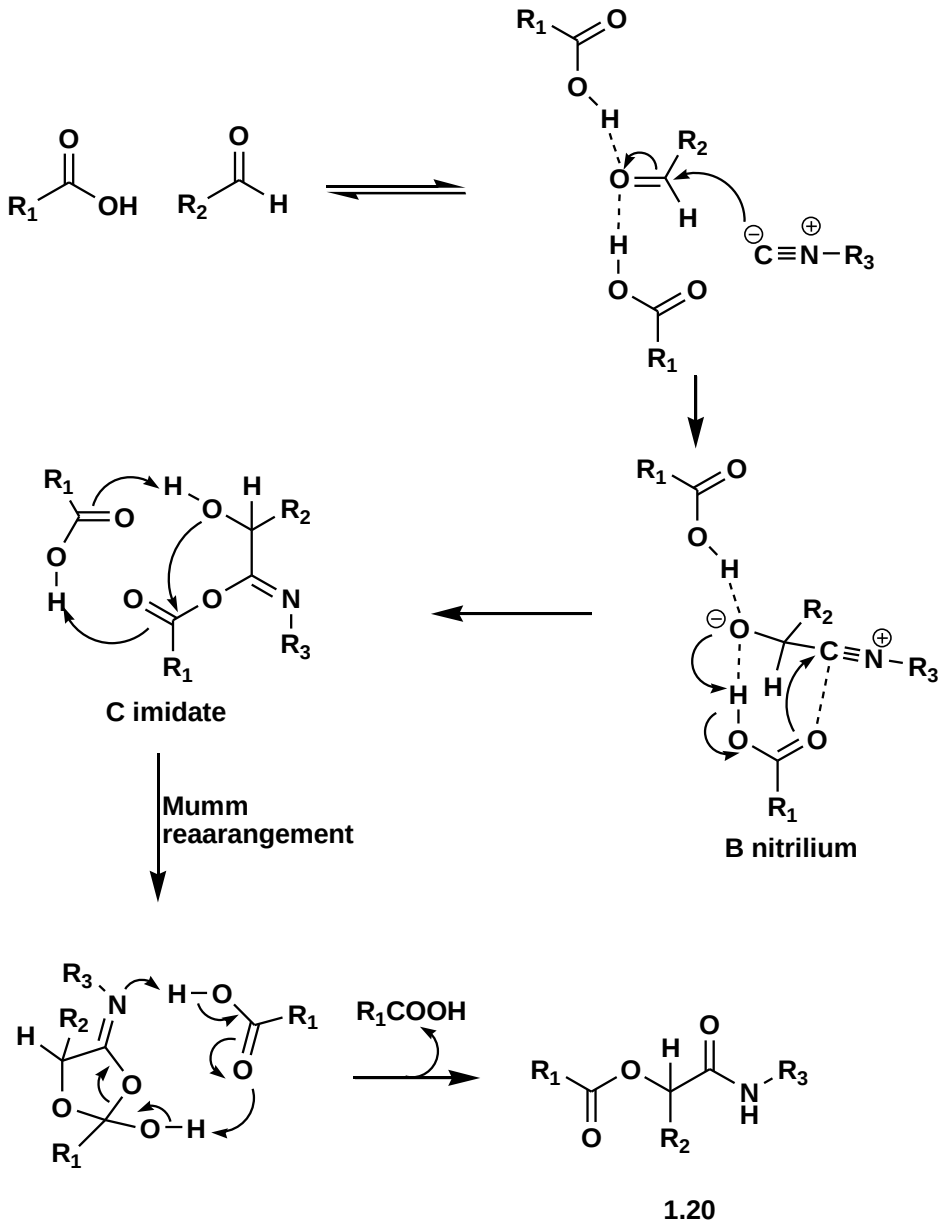

**Scheme 1.7**: Nucleophilic and electrophilic reactivity of the isocyanide carbon is illustrated for the Passerini reaction.

Maeda and co-workers[61] used the artificial force induced reaction (AFIR) method in gas phase for studdying the Passerini reaction mechanism. In their study it shows that the mechanism may involve an extra acidic component prior to the desired product, which shows the Passerini reaction as a pseudo four-component reaction shown in Scheme 1.8.



**Scheme 1.8**: Passerini reaction mechanism using AFIR method in gas phase (pseudo-four component reaction)

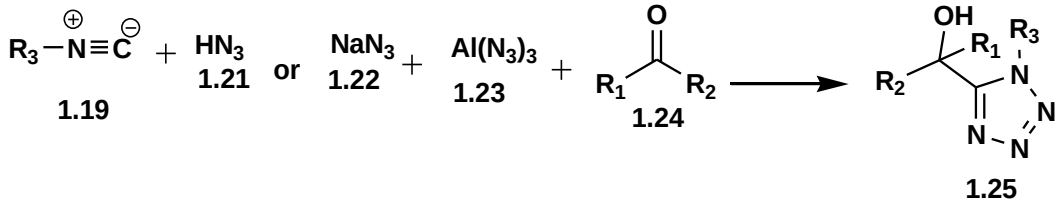
The mechanism proposed by Maeda and co-workers was supported by the studies done by Ramozzi and Morokuma[62] and also Alvim and co-workers[58] after perfoming high-level DFT calculations which also show a pseudo four-component reaction (Scheme 1.9). These studies also reveal that the nitrilium intermediate (B) is stable in solution and its generation is a rate-determing step, catalysed by an extra carboxylic acid molecule leading to Mumm rearrangement and affording **1.20** as the final product.




**Scheme 1.9**: Passerini reaction mechanism using high-level DFT in solution (pseudo-four component reaction)

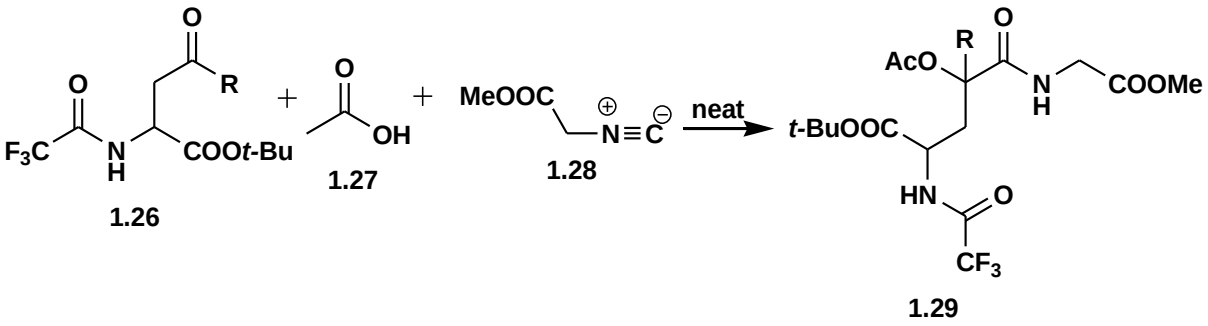
*1.5.1 Substrate scope in the Passerini reactions*

The scope of the Passerini reaction has been widely studied and some of the reaction components can be replaced by suitable isosteres. The first acid isostere used by Ugi[63] was hydrazoic acid ($HN_3$) **1.21** and aluminium azide $Al(N_3)_3$ **1.23** in the Passerini reaction, which was regarded as a template for the synthesis of α-hydroxy tetrazoles **1.25** (Scheme 1.10). Alternatively $NaN_3$ **1.22** can also be used in place of $HN_3$ **1.21**.



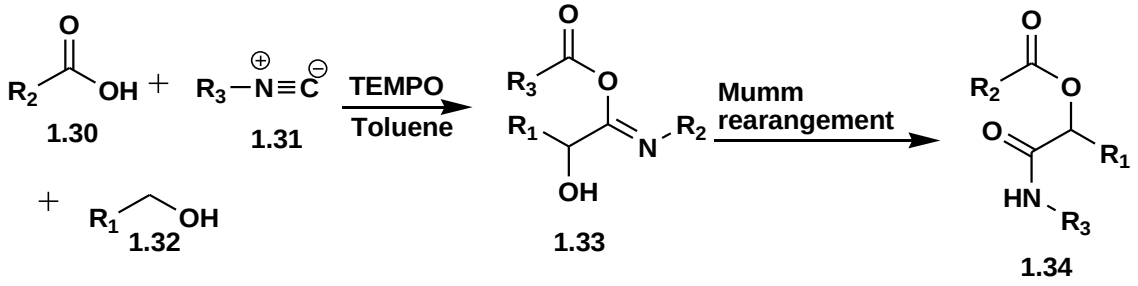

**Scheme 1.10**: Passerini reaction for synthesis of hydroxy tetrazoles.

Zahoor and co-workers[64] prepared peptidomimetic compounds **1.29** by reacting protected γ-oxo-amino acids **1.26**, carboxylic acid **1.27** and isocyanide **1.28** as a neat reaction (Scheme 1.11).



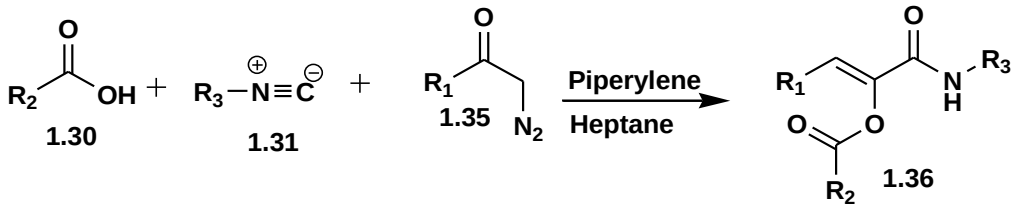

**Scheme 1.11**: Passerini reaction for preparation of peptidomimetic compounds.

Recyclable magnetic core-shell nanoparticle supported TEMPO (2,2,6,6-tetramethyl-piperidin-1-oxyl) catalyst was applied for the oxidative synthesis of Passerini products **1.34**, by reacting caboxylic acid **1.30**, isocyanide **1.31** and primary or secondary alcohol **1.32** using toluene as a solvent at room temperature (Scheme 1.12).[65] Presumably, *in situ* oxidation of the alcohol is followed by the 3-CR Passerini reaction.



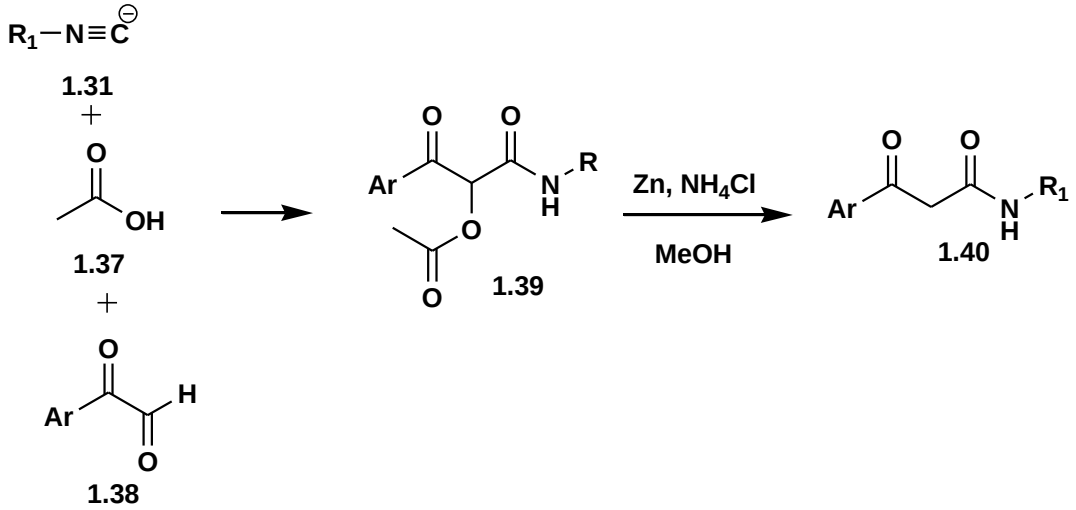

**Scheme 1.12**: Passerini reaction using TEMPO catalyst.

Basso and co-workers[66] reacted carboxylic acid **1.30**, isocyanide **1.31**, and diazoketones **1.35** under photocatalytic conditions to produce 3-CR product **1.36**. Under these conditions, the ketenes generated *in situ* from **1.35**, are able to participate in the three-component reaction (Scheme 1.13).




**Scheme 1.13**: Passerini reaction for synthesis of ketenes.

Neo and co-workers[67] reacted isocyanide **1.31**, carboxylic acid **1.37** and α-keto-aldehyde **1.38** to give product **1.39**. The desired β-keto-amides **1.40** were achieved *via* metal-mediated removal of the acetoxy group (Scheme 1.14).

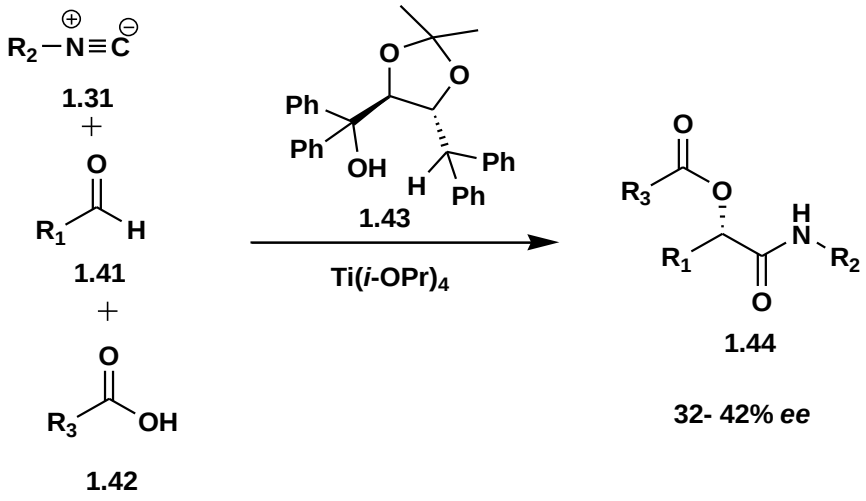



**Scheme 1.14**: Passerini reaction for synthesis of β-keto-amides

*1.5.2 Chirality in Passerini reactions*

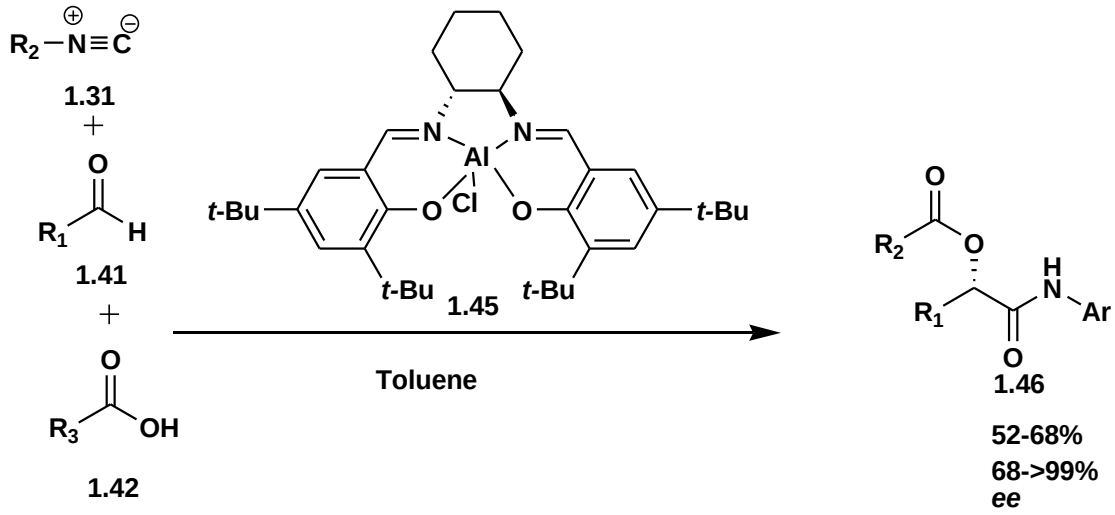
The strategies and methods for controlling the stereochemical outcome of carbon-carbon bond forming reactions from the achiral substrates used in Passerini reaction are finite. The generation of a highly enatioselective Passerini 3-CR is one of the most significant goals.

Kusebauch and co-workers[68] used stoichiometric amounts of a Ti-taddol complex **1.43** as a catalyst to obtain moderate enantioselectivity of α-acyloxyamides with 32–42% *ee* as shown in Scheme 1.15.



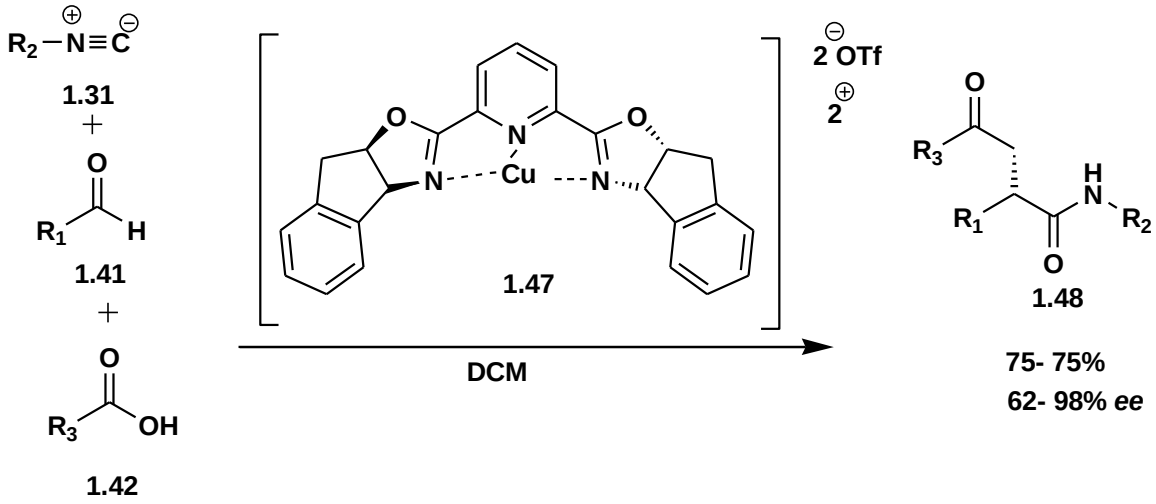

**Scheme 1.15**: Enantioselective Passerini reaction using Ti-taddol complex

Zhu and co-workers[69] obtained moderate to excellent yield of α-acyloxyamides enantioselectively using stable aluminium salen complex **1.45** as a chiral Lewis acid catalyst from isocyanides **1.31**, non chelating aldehydes **1.41** and carboxylic acids **1.42** (Scheme 1.16).



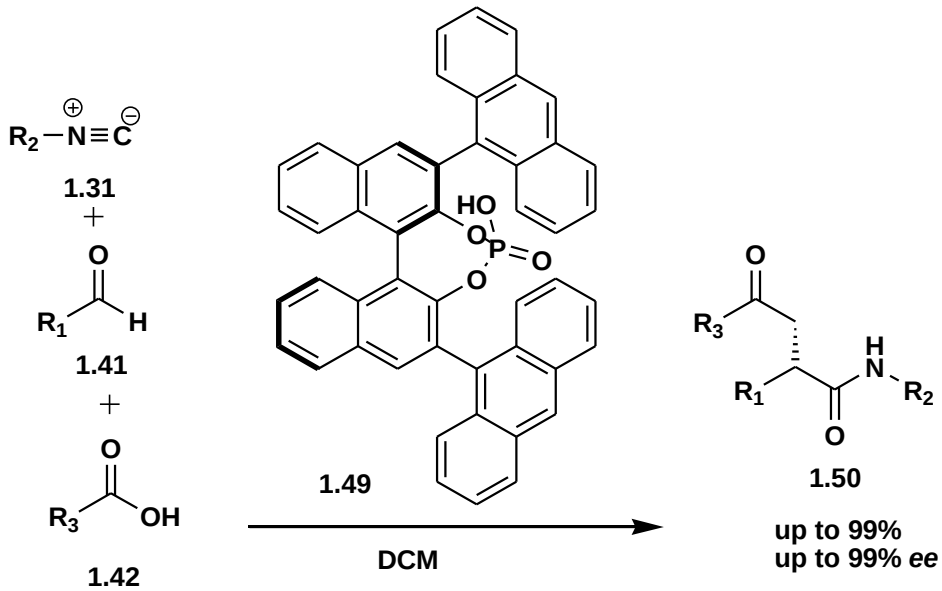

**Scheme 1.16**: Passerini enantioselective reaction using aluminium salen complex

Andreana and co-workers[70] managed to achieve good yields of product **1.48** with reasonable enantioselectivity using isocyanide **1.31**, chelating aldehydes **1.41** and carboxylic acid **1.42** after employing chiral tridentate Lewis acidic Cu-pybox complex **1.47** as a catalyst (Scheme 1.17).




**Scheme 1.17**: Enantioselective Passerini reaction using Cu-pybox complex

Zhang and co-workers[71] activated the reaction of isocyanide **1.31,** aldehyde **1.41**, and carboxylic acid **1.42** using chiral phosphoric acid **1.49** to obtain high yields of enantioselective products **1.50** (Scheme 1.18)

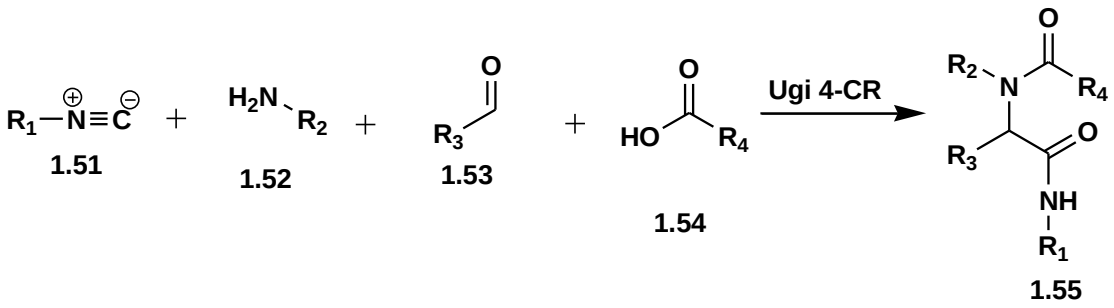



**Scheme 1.18**: Passerini enantioselective reaction using chiral phosphoric acid.

## 1.6. Ugi reaction: U-4CR and U-3CR

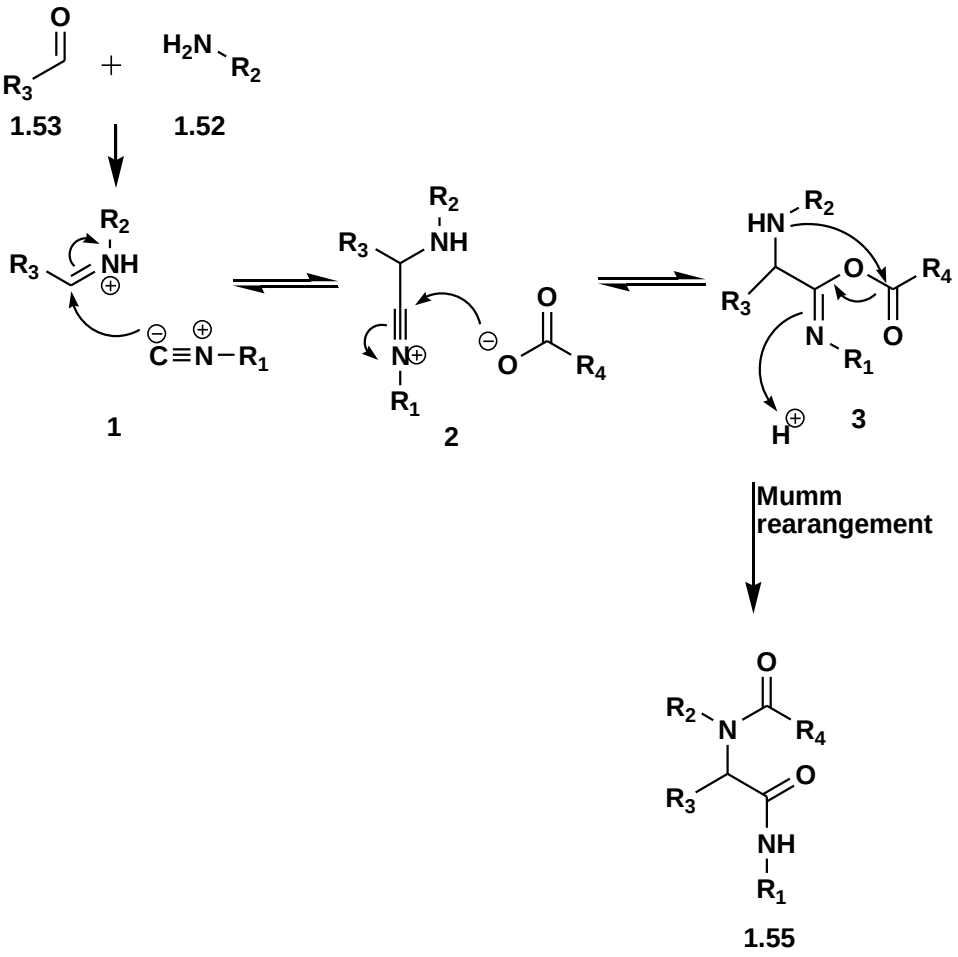
### *1.6.1 Ugi-four component reaction (U-4CR)*

The definitive Ugi reaction is a four-component reaction (U-4CR) between an isocyanide **1.51**, amine **1.52**, carbonyl compound **1.53** (aldehyde or ketone) and carboxylic acid **1.54** to give product **1.55** (Scheme 1.19).[5]



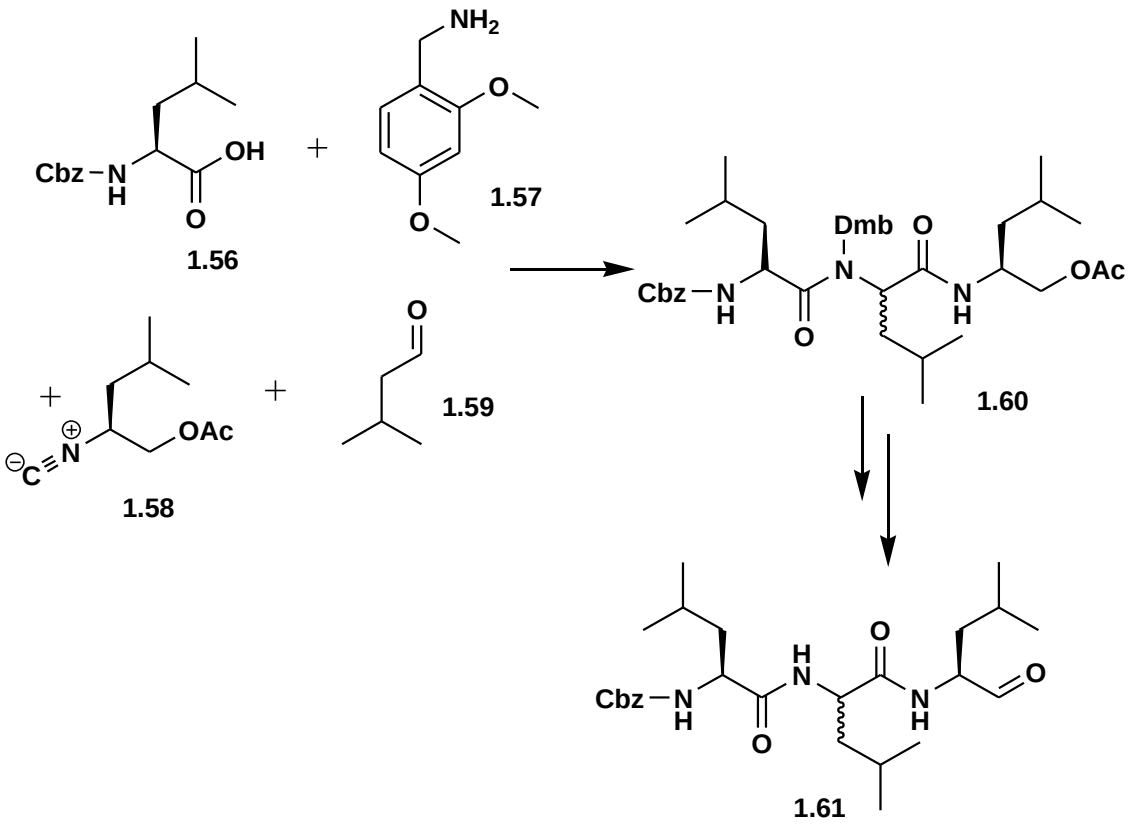

**Scheme 1.19**: The Ugi 4-CR (U-4CR)

During the Ugi-4CR a carbonyl compound **1.53** reacts with an amine **1.52** to form an imine intermediate at event **1**. The imine is activated by the carboxylic acid proton and it is then attacked by the isocyanide leading to the nitrilium intermediate which is then attacked by the conjugate base of the carboxylic acid at event **2**. This is followed by the Mumm rearangement at event **3** to give the final product **1.26** (Scheme 1.20).[14,72]



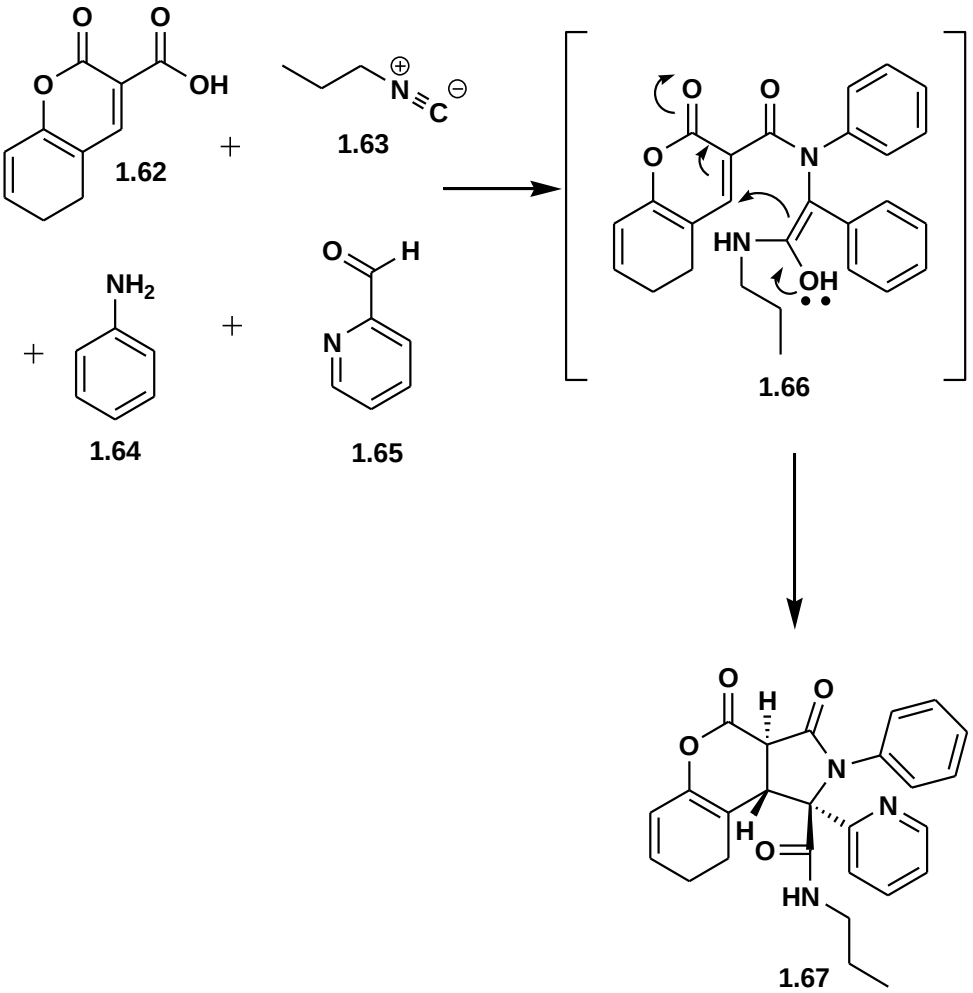

**Scheme1.20**: Nucleophilic and electrophilic reactivity of the isocyanide carbon is illustrated for the Ugi reaction.

The use of the Ugi reaction for the synthesis of a tripeptide from the reaction of amino acid **1.56**, dimethoxybenzylamine **1.57**, chiral isocyanide **1.58** and aldehyde **1.59** was reported by Mroczkiewicz and Ostaszewski.[73] The peptide aldehyde **1.61** was achieved after deprotection and oxidation of **1.60** (Scheme 1.21).



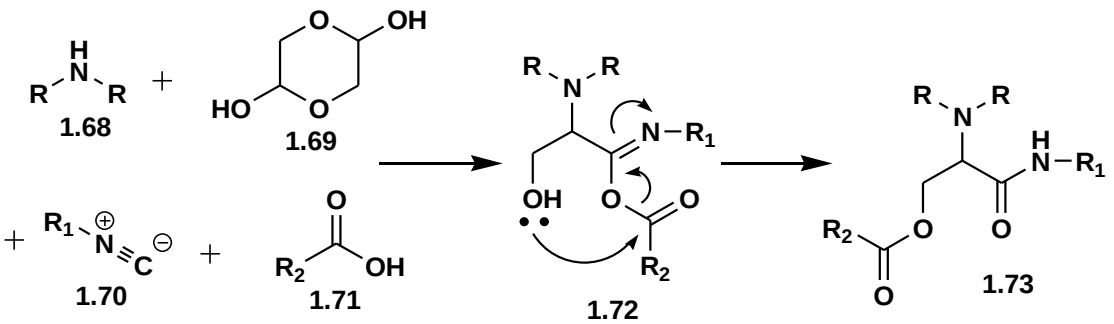

**Scheme 1.21**: Synthesis of a tripeptide using U-4CR.

Che and co-workers[74] describe the use of coumarin-3-carboxylic acid **1.62**, isocyanide **1.63**, aniline **1.64** and pyridine-2-aldehyde **1.65** in U-4CR to achieve annulated products **1.67**. The intramolecular annulation depends on the nature of the aldehyde in application: only pyridine-2- and pyridine-4-aldehydes gave rise to the annulated product, whereas the conventional U-4CR product **1.66** was obtained when benzaldehyde or pyridine-3-aldehydes were emplyoyed (Scheme 1.22).




S**cheme1.22**: Synthesis of Ugi adducts

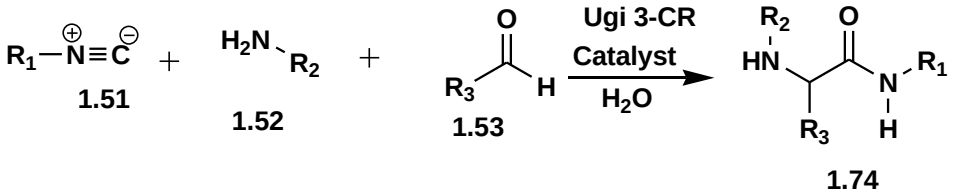
The reaction of a secondary amine **1.68**, glycolaldehyde dimer **1.69**, isocyanide **1.70** and carboxylic acid **1.71** gave rise to a novel MCR scaffold **1.73**.[75] This reaction proceeded differently from classical U-4CR intermediate **1.72**, because the secondary amine prevents the usual Mumm rearangement from occuring and the hydroxyl group from the glycolaldehyde permits O-acyl rearangement to occur, leading to a novel scaffold (Scheme 1.23).




**Scheme 1.23**: Ugi reaction using glycolaldehyde dimer

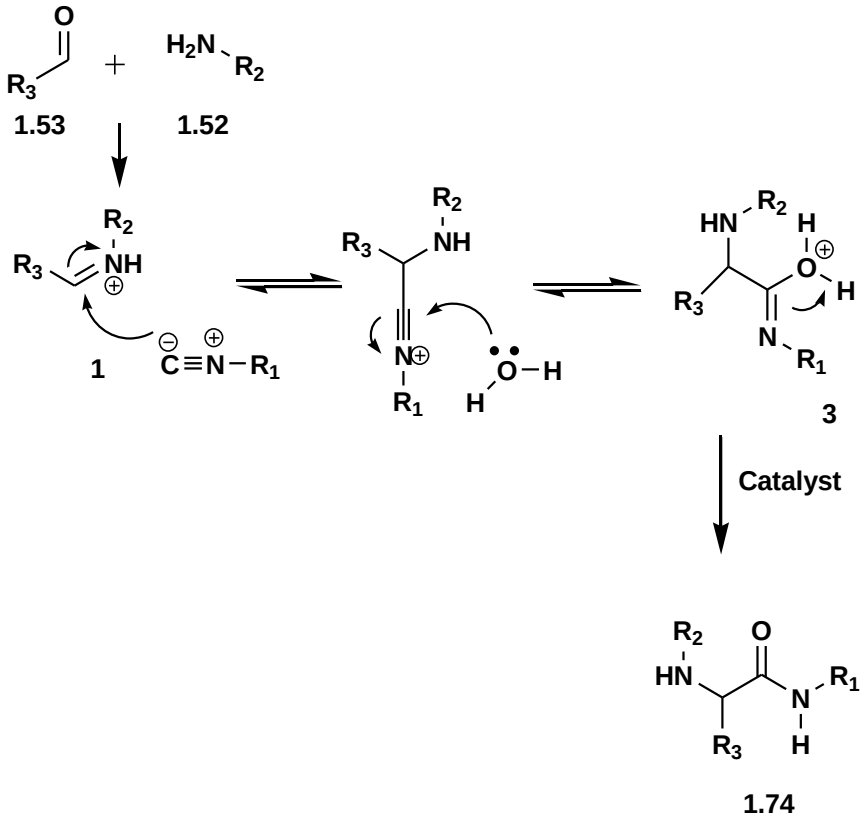
*1.6.2 Ugi-three component reaction (U-3CR)*

The U-3CR was initialy described in 1960 while investigating water as a nucleophile.[76] The reaction involved isocyanide **1.51**, primary amine **1.52** and an aldehyde **1.53** in the presence of water and a catalyst (Scheme 1.24). However, there are many Ugi-3CRs reported under different conditions to the one mentioned here (Scheme 1.24).



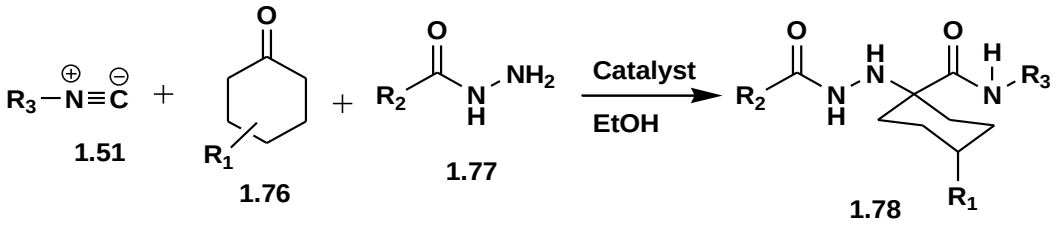

**Scheme 1.24**: The Ugi 3-CR

The mechanism is quite similar to U-4CR, however carboxylate in this case is replaced by a molecule of water that attacks the electrophilic carbon that might be activated by the catalyst, which consequently in many cases eliminates the Mumm rearangement (Scheme 1.25).[72]



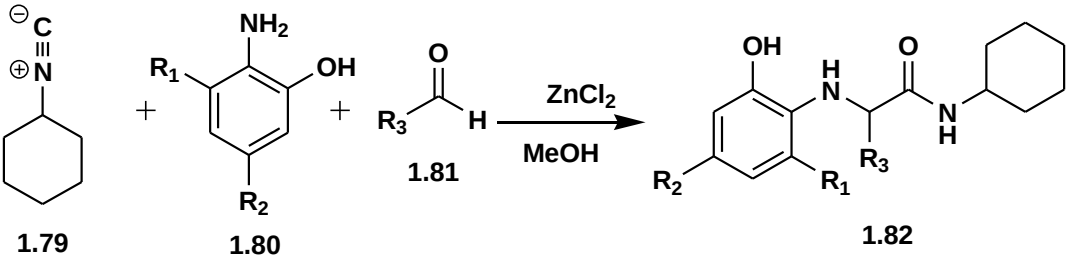

**Scheme 1.25**: Ugi 3-CR mechanism

Ramezanpour and co-workers[77] used the U-3CR for synthesis of hydrazino amides **1.78** from the reaction of isocyanides **1.51**, cyclohexanone **1.76** and hydrazides **1.77** and using ethanol as a solvent (Scheme 1.26). There were several catalysts that were employed and tested for this reaction such as *p*-toluenesulfonic acid (*p*-TsOH), $ZnCl_2$ and $ZrOCl_2$, *S*-1,1-binaphthyl-2,2-diylhydrogen phosphate, phosphorous acid ($H_3PO_4$) and camphor sulfonic acid.



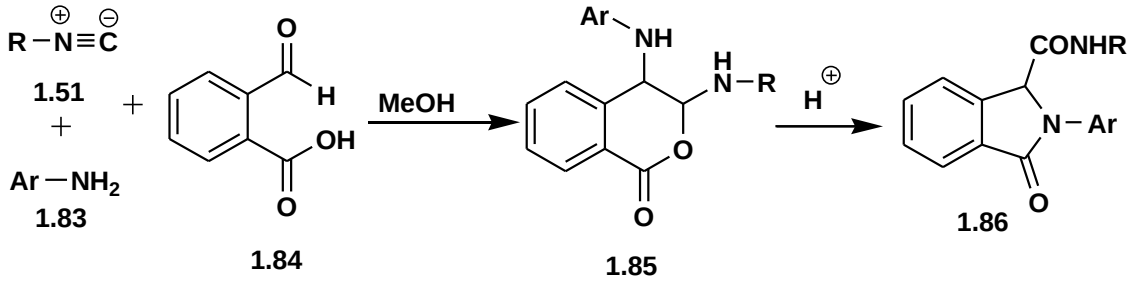

**Scheme 1.26**: Ugi-3CR for synthesis of hydrazino amides.

Shaabani and co-workers[78] describe the ability of zinc chloride to act as a catalyst for the Ugi-3CR of cyclohexyl isocyanide **1.79**, 2-aminophenols **1.80** and aldehydes **1.81** using methanol as a solvent at room temperature to achieve synthesis of *N*-cyclohexyl-2-(2-hydroxyphenylamino)acetamides **1.82** (Scheme 1.27).




**Scheme 1.27:** Ugi-3CR for synthesis of *N*-cyclohexyl-2-(2-hydroxyphenylamino)acetamides

Faggi and co-workers[79] reported the reaction of isocyanide **1.51**, aniline **1.83** and 2-formylbenzoic acid **1.84** to afford isocoumarins **1.86** (Scheme 1.28). Hoever in this case one of the reagents, formylbenzoic acid **1.84**, contains two components of carboxylic acid and an aldehyde that participate in the product formation. These types of reactions are regarded as 4 centre Ugi 3CR. interestingly, they where able to obtain the product prior to Mumm rearangement after optimising conditions and applying low nucleophilicity amines. The reactions were carried out at higher temperatures or in the presence of trace acid to afford Ugi adducts.

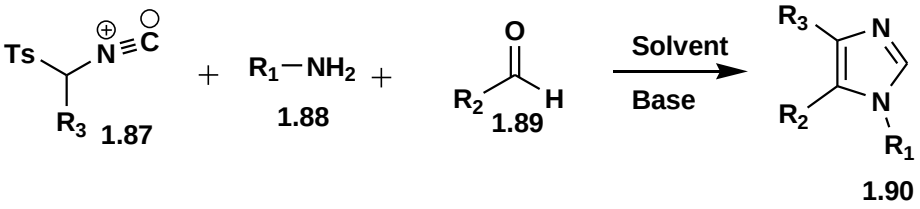



**Scheme 1.28**:4 Centre-Ugi-3CR for synthesis of isocoumarins.

These types of reactions are dominated by formation of a new stereogenic centre and the products are reported as racemic. The challenges related to enantioselective U-3CR, U-4CR and Passerini reactions include: spontaneous background reaction in appropriate solvent at room temperatures, product inhibition causes low catalyst turnover, complexity of the possible mechanism pathways and the possibility of the isocyanide coordinating to the metal catalyst.

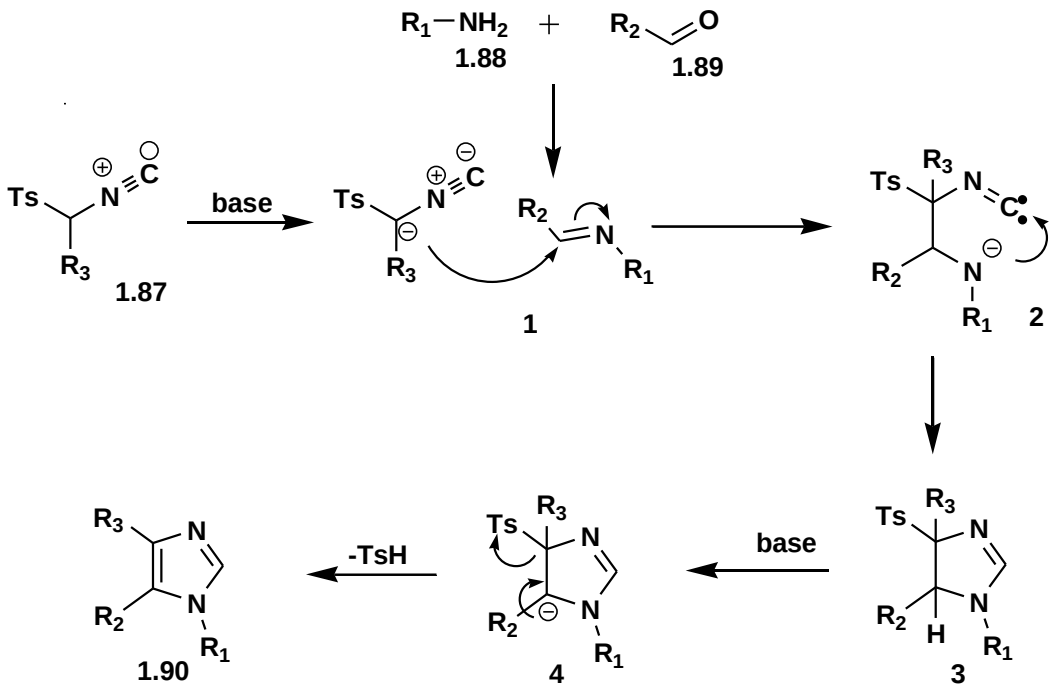
### 1.7. van Leusen reaction (V-3CR)

The van Leusen 3-CR involves reaction of α-substituted tosylmethyl isocyanide (TosMIC) **1.87**, a primary amine **1.88** and an aldehyde **1.89** to give rise to imidazole **1.90**.[80,81] The reaction occurs in the presence of solvents such as methanol, dimethoxyethane or water with base including potassium carbonate or sodium hydride as shown in Scheme 1.29.




**Scheme 1.29**: van Leusen 3-CR

The 3-CR van Leusen reaction generally proceeds by the initial reaction of the primary amine **1.88** with an aldehyde to form a Schiff base which reacts with deprotonated TosMIC at event **1** in order to react with activated TosMIC to give the intermediate shown at event **2**. Here an internal nucleophililic attack occurs leading to cyclisation to form intermediate **3** followed by elimination of *p*-toluenesulfinic acid to afford imidazole **1.90** (Scheme 1.30).[82]

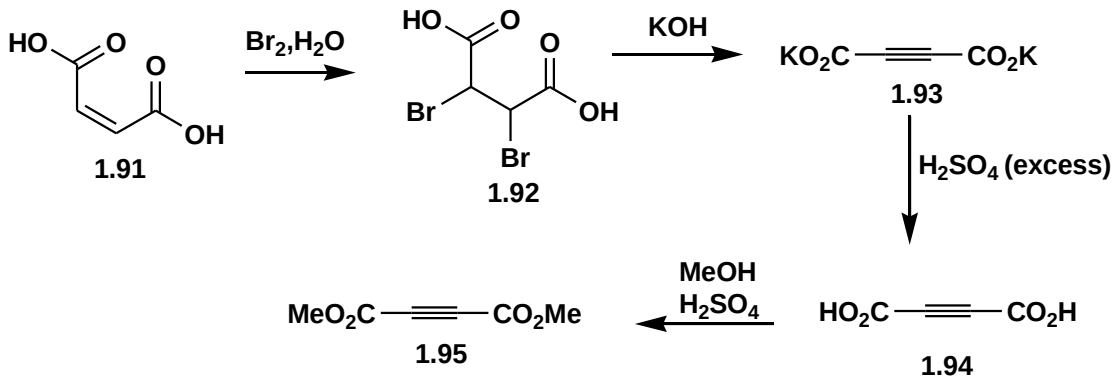



**Scheme 1.30**: van Leusen 3-CR mechanism

### 1.8 The application of dimethyl acetylenedicarboxylate in organic synthesis

Dimethyl acetylenedicarboxylate (DMAD) is an electron deficient alkyne diester which exists as a liquid at room temperature and is highly electrophilic. DMAD versatility makes it useful in cycloaddition reactions as a dienophile and a dipolarophile. In addition, DMAD is also used to form carbon-carbon bonds and in multicomponent reactions for developing heterocyclic scaffolds.[83] In line with the essential discovery of DMAD reactions with heterocyclic compounds by Diels and co-workers[84] and Acheson and Elmore,[85] the utility of DMAD in organic synthesis has gained tremendous attention.[86,87] The common fundamental properties and applications of DMAD in organic synthesis includes Michael reactions, Diels-Alder, 1,3-dipolar and [2+2] cycloadditions and in MCRs.

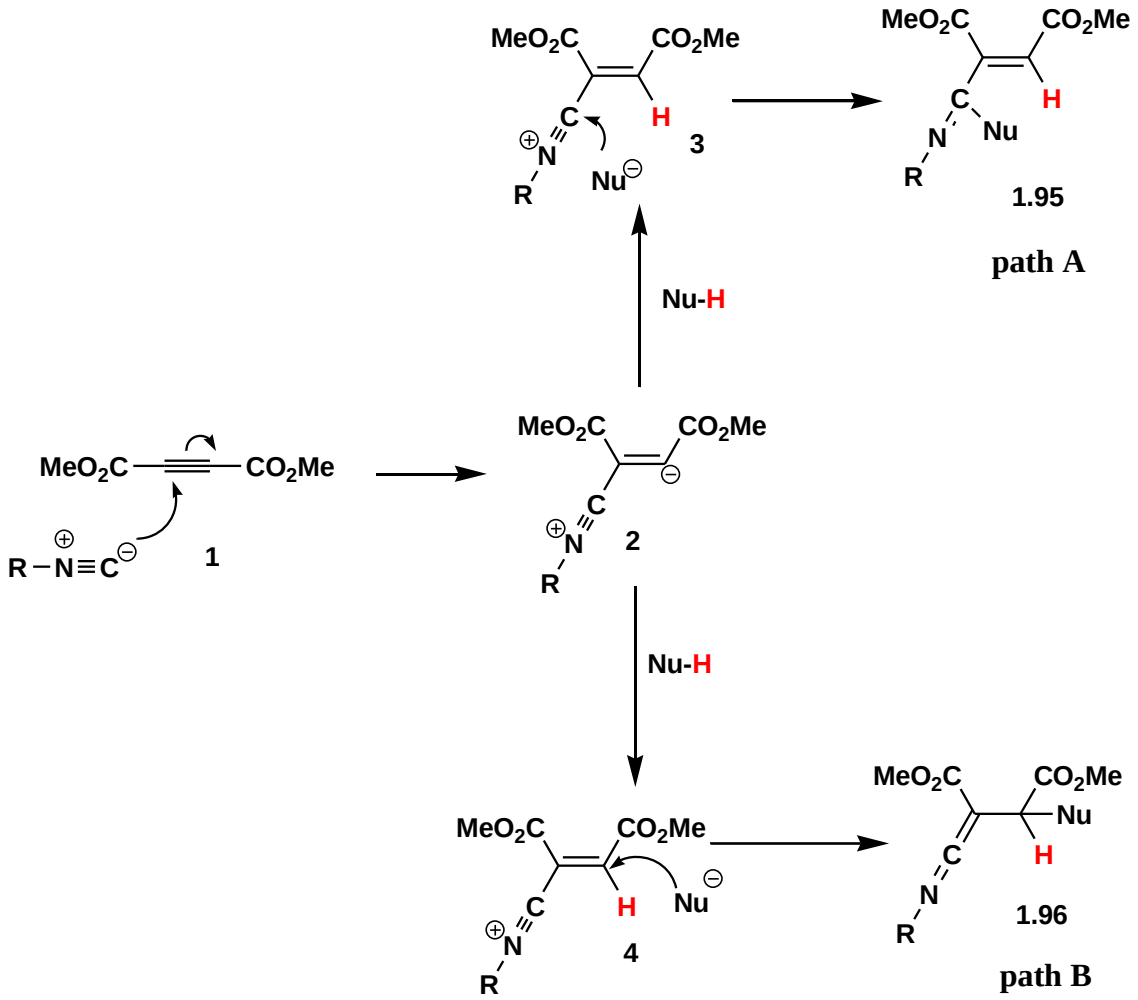
DMAD is one of the economically affordable and accessible reagents. The preparation of DMAD was originally reported by Bandrowski[88] in 1877, after brominating maleic acid **1.91** to form intermediate **1.92** which is dehydrohalogenated by potassium hydroxide resulting in **1.93** which was treated with excess sulfuric acid to yield compound **1.94**. Compound **1.94** was then esterified with methanol and sulfuric acid to afford DMAD **1.95** (Scheme 1.31).




**Scheme 1.31**: Preparation of DMAD.

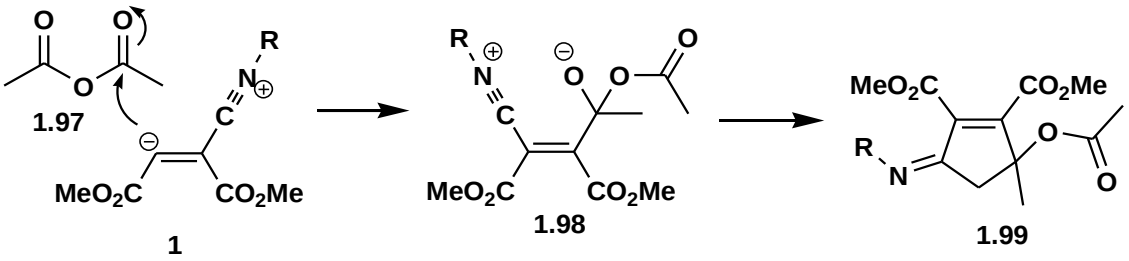
*1.8.1 DMAD and isocyanides in multicomponent reactions*

Isocyanide-based multicomponent reactions (IMCRs) are considered as one of the special subclasses in chemical synthesis, which are extremely versatile and interesting. IMCRs possess considerable potential that lies in the diversity of bond-formations and high substrate scope. This subclass comprises of interesting chemistry, which includes a nucleophile and an electrophile reacting at the same atom, leading to formation of adducts. The reaction of isocyanides with DMAD was first described by Winterfeldt and co-workers[89] as an exceptional reaction that required more attention. The addition of neutral nucleophiles to electrophilic acceptors generates a transient zwitterion intermediate. One of the known examples of DMAD in MCRs, arises from the reaction of an isocyanide with DMAD (event **1**, Scheme 1.32) leading to a zwitterion adduct (event **2**) which might be trapped in different ways to give multiple reaction possibilities.[15] One possibility is that the zwitterion reacts with a nucleophile possessing an acidic proton. Under these conditions, the two possible pathways for the zwitterion (event **2**) to react are shown in Scheme 1.32. The first step that occurs is deprotonation of the nucleophile containing an acidic proton such as C-H, N-H, O-H or SH. Subsequently, the anionic nucleophile may possibly attack the nitrilium ion *via* a 1,2 route (path A) or a 1,4 route (path B) to yield an imine **1.95** or ketenimine **1.96**, respectively, as shown in Scheme 1.31.




**Scheme 1.32**: Possible zwitterion reaction pathways with nucleophile.

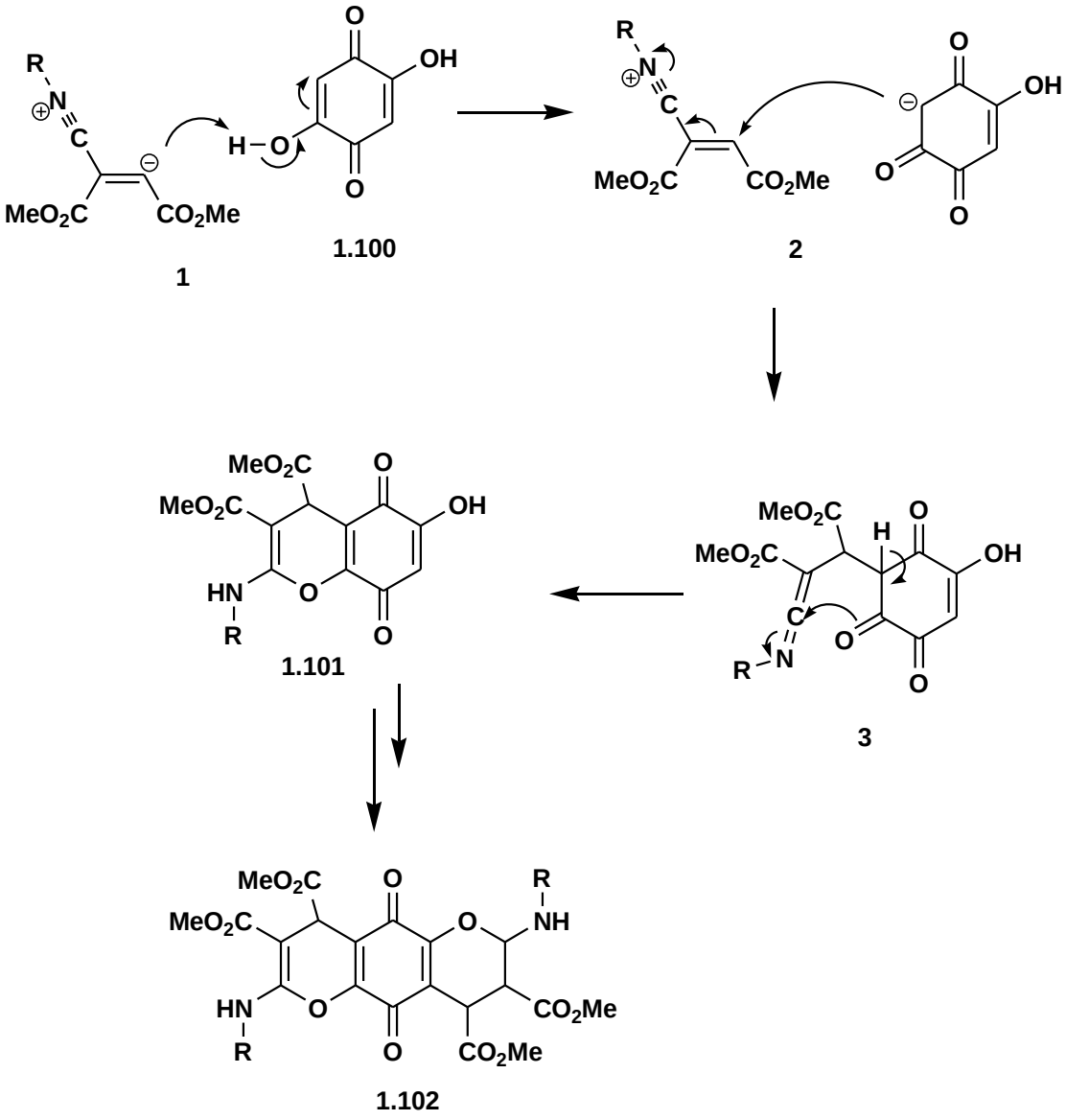
The second possible reaction of a zwitterion generated from an isocyanide and DMAD involves an electrophile. During this development event the electrophile **1.97** acts as a dipolarophile, where the zwitterion at event **1** initially attack the electrophilic centre giving rise to a nucleophile which subsequently attacks the nitrilium ion. One of the examples reported by Shabaani and co-workers[90] is represented in Scheme 1.33.




**Scheme 1.33**: Zwitterion reaction with an electrophile.

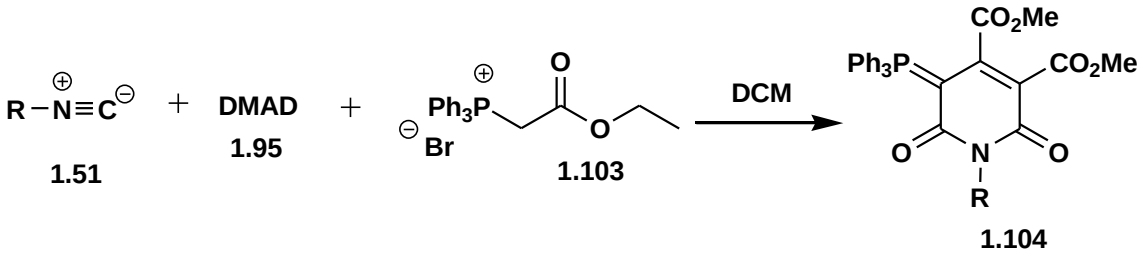
*1.8.1.1 Synthesis of heterocyclic scaffolds using zwitterions and nucleophiles containing acidic protons.*

Shaabani and co-workers[91] reported the synthesis of chromene derivatives using a zwitterion adduct formed by isocyanides and DMAD to react with 2,5-dihydroxycyclohexa-2,5-diene-1,4-dione **1.100** containing an acid proton (C-H) as shown in Scheme 1.34. The proposed mechanism proceeds by the removal of a proton from compound **1.100** at event **1** followed by 1,4-nucleophilic attack by a carbon nucleophile at event **2** to give the ketenimine. The intramolecular attack by oxygen on the ketenimine at event **3** leads to the formation of chromene **1.101**, in instances where there is excess isocyanide and DMAD the same reaction proceeds on the second hydroxyl group to afford compound **1.102**.



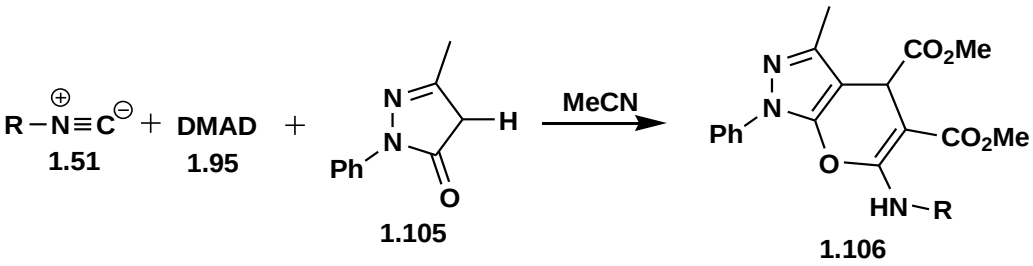

**Scheme 1.34**: Synthesis of chromene derivatives

Shaabani and co-workers[92] reported the one-pot three-component reaction of alkyl isocyanide **1.51** with DMAD **1.95** in the presence of triphenylphosphonium bromide **1.103** which contains an acidic C-H using dichloromethane as a solvent at room temperature. The resulting six-membered heterocyclic compounds **1.104** were obtained in a yield range of 51% - 63% as shown in Scheme 1.35.



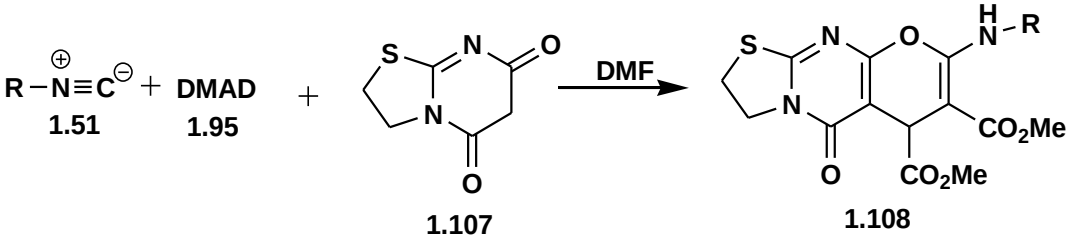

**Scheme 1.35**: Synthesis of 6-membered heterocyclic compounds from triphenylphosphonium bromide.

The reaction of isocyanide **1.51** with DMAD **1.95** in the presence of compound **1.105** consisting of an acidic proton (C-H) using acetonitrile as a solvent at room temperature gave rise to pyrano[2,3-*c*]pyrazole derivatives[93] (Scheme 1.36).



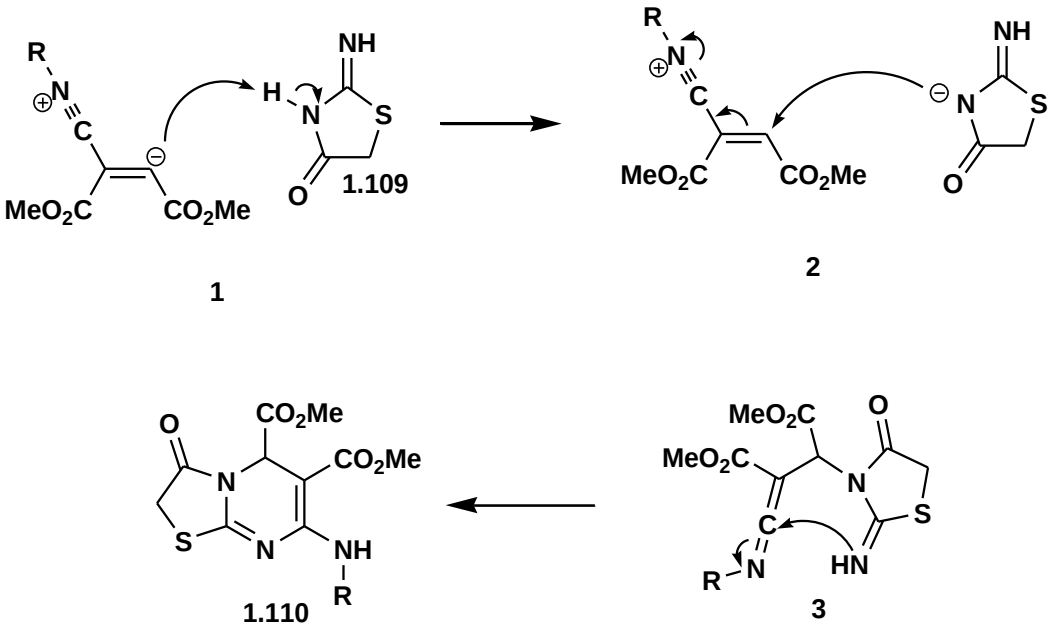

**Scheme 1.36:** Synthesis of pyrano[2,3-*c*]pyrazole derivatives

Zangouei and co-workers[94] reported a facile one-pot synthesis method using isocyanide **1.51**, DMAD **1.95** and 2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-5,7(6*H*)-dione **1.107** which contains an acidic C-H to afford pyranothiazolopyrimidines **1.108** using *N*,*N*-dimethylformamide as solvent at 100 °C (Scheme 1.37).



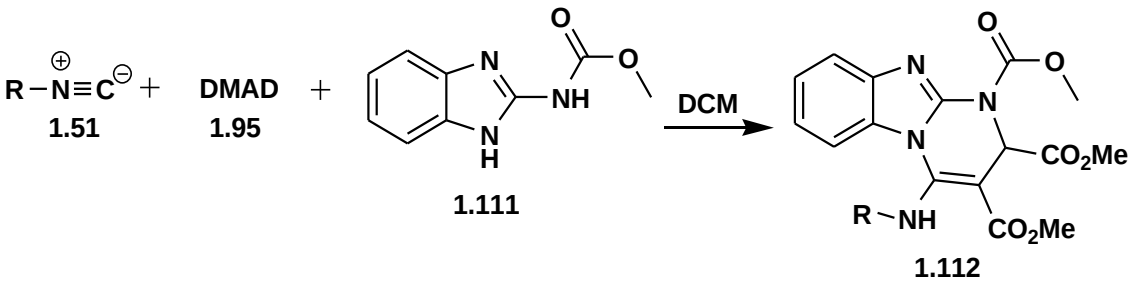
**Scheme 1.37**: Synthesis pyranothiazolopyrimidine derivatives.

Esmaeili and co-workers[95] reported the reaction of isocyanide **1.51** and DMAD **1.95** with 2-imino-1,3-thiazolidin-4-one **1.109** containing an acidic N-H proton and the internal nucleophile to react with the ketenimine as shown at event **3**, to afford 3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidines **1.110** (Scheme 1.38).



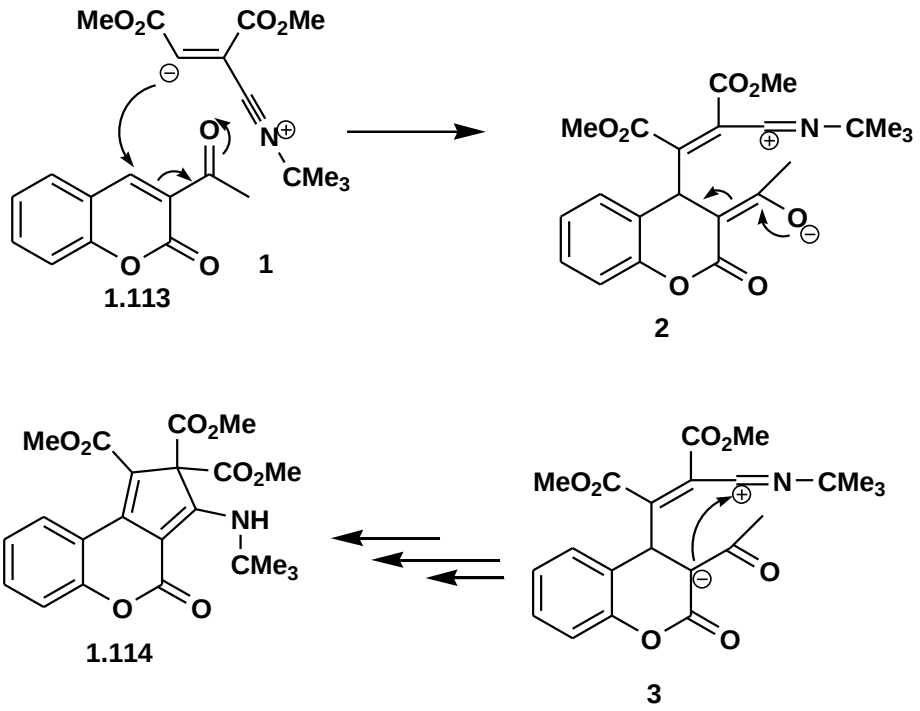

**Scheme 1.38:** 3-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine derivatives.

Hassanabadi and co-workers[96] reported the reaction of isocyanide **1.51** and DMAD **1.95** in the presence of benzimidazole carbamate **1.111** which contains an acidic proton (N-H) and internal nucleophile to afford 2*H*-pyrimido[1,2-*a*]benzimidazoles **1.112** (Scheme 1.39)




**Scheme 1.39:** Synthesis of 2*H*-pyrimido[1,2-*a*]benzimidazoles derivative.

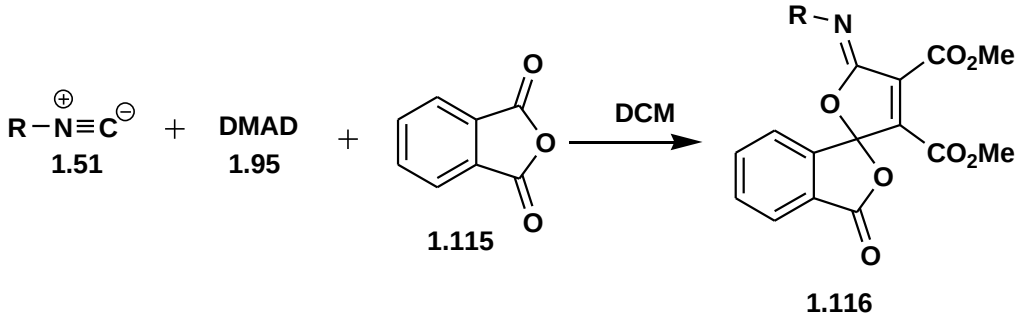
Ghandi and co-workers[97] reported the reaction of 3-acetyl-2*H*chromen-2-ones **1.113** with a zwitterion adduct to obtain cyclopentadiene-fused chromanones **1.114** in moderate to good yield under reflux using toluene as solvent (Scheme 1.40).




**Scheme 1.40:** Synthesis of cyclopentadiene-fused chromanones derivative.

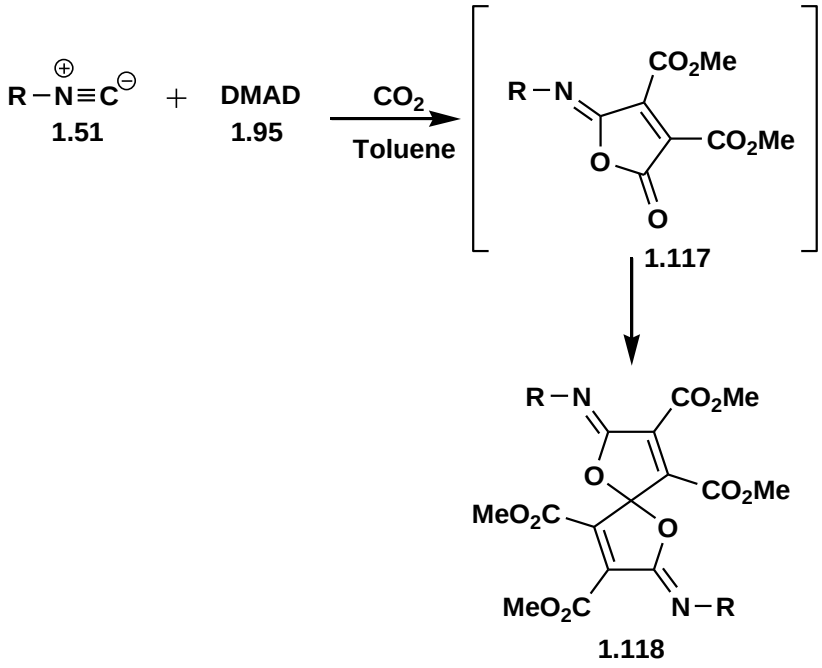
*1.8.1.2 Synthesis of heterocyclic scaffods using zwitterions and electrophiles*

The reaction of isocyanide **1.51** and DMAD **1.95** with phthalic anhydride **1.115** at room temperature using dichloromethane as a solvent gave rise to benzo-fused spirolactones **1.116** as shown in Scheme 1.41. [90]



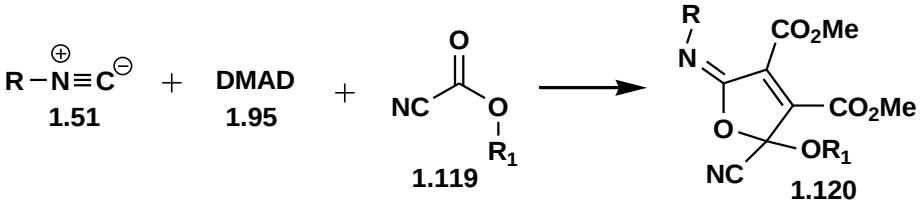

**Scheme 1.41:** Synthesis of benzo-fused spirolactones derivatives.

Zhao and co-workers[98] reported the reaction of two molecules of isocyanide **1.51** and DMAD **1.95** in the presence of carbon dioxide to afford dioxospiro compounds **1.118** using toluene as a solvent at 80 ºC (Scheme 1.42).




**Scheme 1.42:** Synthesis of dioxospiro derivatives.

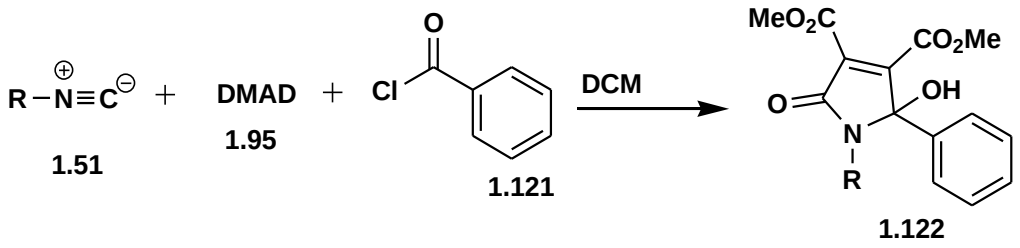
Yavari and co-workers[99] reported the reaction of isocyanide **1.51** and DMAD **1.95** with alkyl cyanoformates **1.119** to obtain dialkyl 2-cyano-5-alkyl(aryl)imino-2-alkoxy-2,5-dihydrofuran-3,4-dicarboxylates **1.120** under solvent-free conditions at room temperature as shown in Scheme 1.43.




**Scheme 1.43:** Synthesis of dialkyl 2-cyano-5-alkyl(aryl)imino-2-alkoxy-2,5-dihydrofuran-3,4-dicarboxylates derivatives

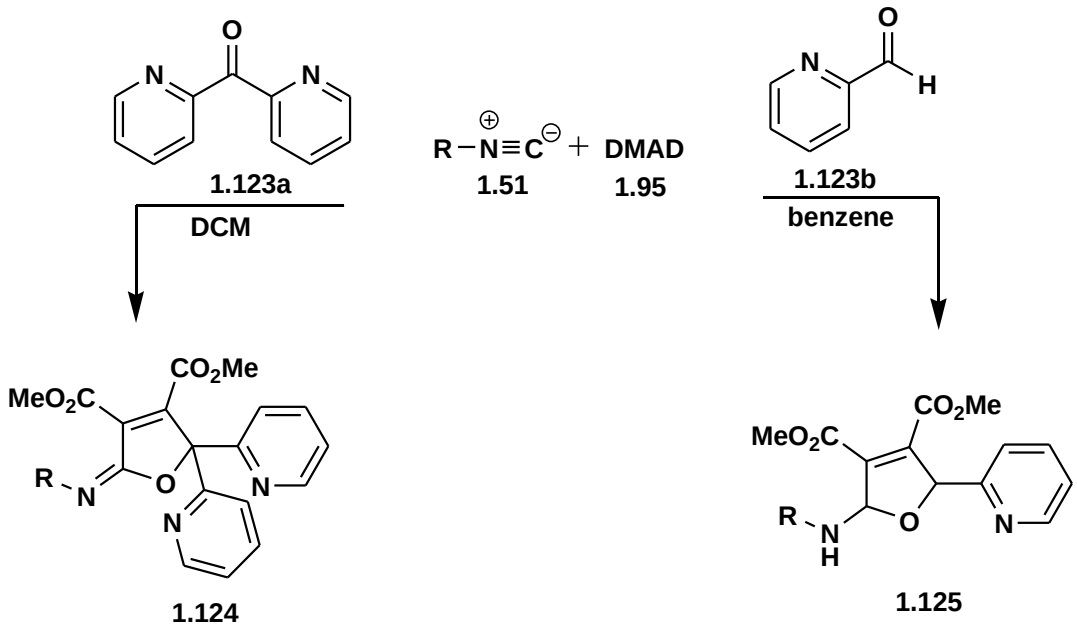
The use of benzoyl chloride **1.121** as an electrophile for synthesis of heterocyclic compounds by employing a zwitterion adduct arising from the reaction of DMAD and alkyl isocyanide

gave rise to five-membered heterocyclic rings **1.122** (Scheme 1.44) as reported by Yavari and co-workers.[100] The heterocyclic products **1.122** were achieved at room temperature using dichloromethane as a solvent in a percentage yield range of 75% -82%.



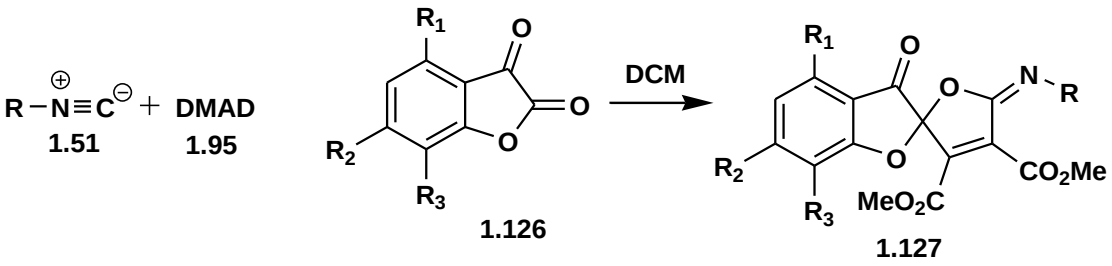

**Scheme 1.44**: Synthesis of 5-membered heterocyclic compounds.

Hazeri and co-workers[101] used pyridine-containing carbonyl compounds **1.123a** and **1.123b** as electrophiles in order to react with the zwitterion adduct generated from DMAD **1.95** and alkyl isocyanides **1.51** to obtain stable 5-membered heterocyclic compounds **1.124** (90% - 95%) and **1.125** (90% - 95%) in excellent yields (Scheme 1.45).



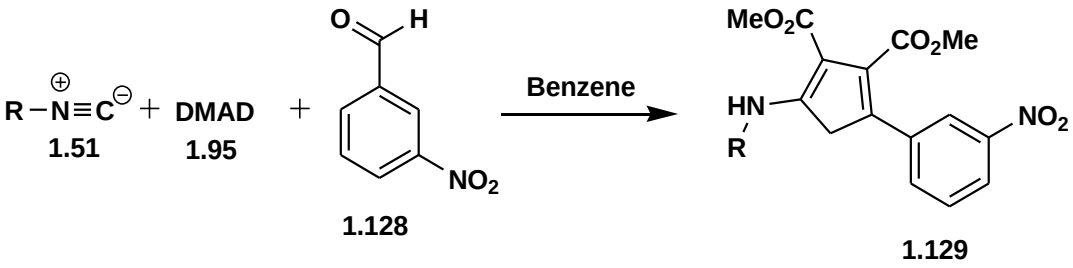

**Scheme 1.45**: Synthesis of 5-membered heterocyclic compounds from pyridines.

Esmaeili and Vesalipoor[102] reported the reaction of alkyl isocyanides **1.51** and DMAD **1.95** with benzofuran-2,3-dione derivatives **1.126** to obtain γ-spiroiminolactones **1.127** in good yield under reflux using dichloromethane as solvent (Scheme 1.46).




**Scheme 1.46**: Synthesis of γ-spiroiminolactones

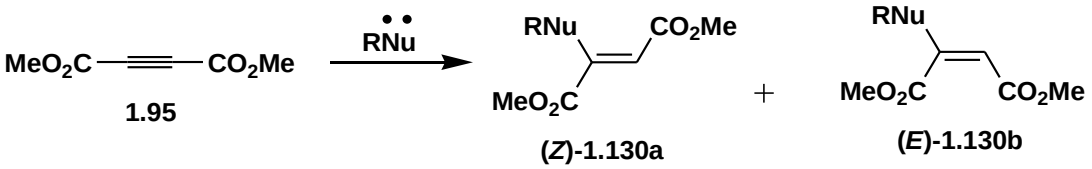
Nair and co-workers[103] reported the reaction of alkyl isocyanides **1.51** and DMAD **1.95** with aldehydes **1.128** to obtain 2-aminofurans **1.129** in good yields at 80 ºC using benzene as a solvent (Scheme 1.47).




**Scheme 1.47**: Synthesis of 2-aminofurans

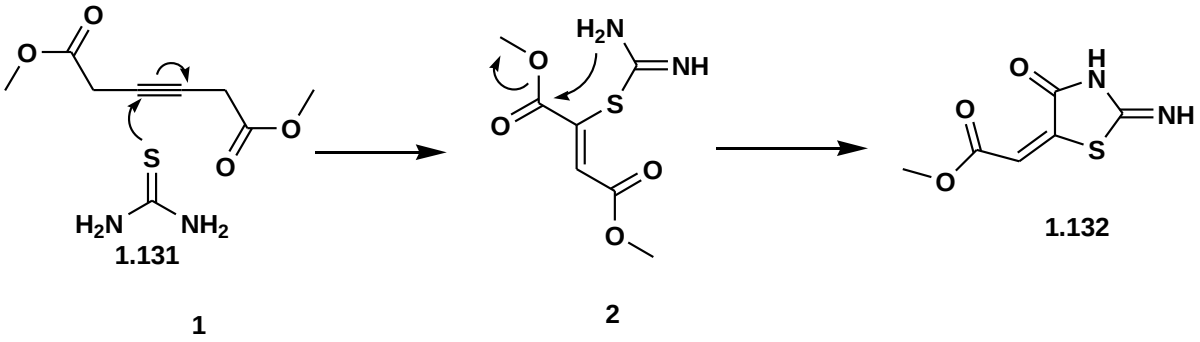
*1.8.2 DMAD in Michael reactions*

DMAD is a strong electrophile that is considered a powerful Michael acceptor which can react with various nucleophiles (Scheme 1.48). The commonly known nucleophiles that react with DMAD smoothly include sulfur, nitrogen, oxygen, carbon, sulfur and phosphorus nucleophiles. There are many reactions which apply DMAD as cyclising agent to achieve heterocyclic scaffolds such as pyrimidines,[104] thiazines[105] and thiazoles.[106] In these reactions DMAD initially acts as a Michael acceptor.



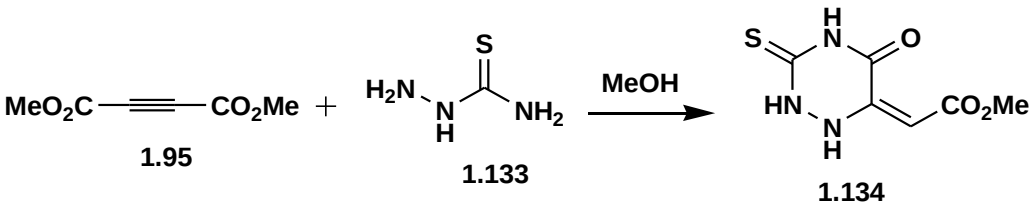

**Scheme 1.48**: DMAD as a Michael acceptor

The reaction between DMAD **1.95** and thiourea **1.131** using methanol as a solvent gives rise to thiazole-based product **1.132** as represented in Scheme 1.49. Generally the reaction proceeds by initial attack of sulfur on one of the alkyne carbon atoms of DMAD at event **1** to form an intermediate at event **2** leading to formation of 1,3-thiazolidin-4-ones **1.132**. Compound **1.132** was reported by Hendrickson and co-workers[107] in 91% yield using methanol as solvent while Choudhary and Peddinti[108] prepared **1.132** in water in 100% yield (Scheme 1.49).



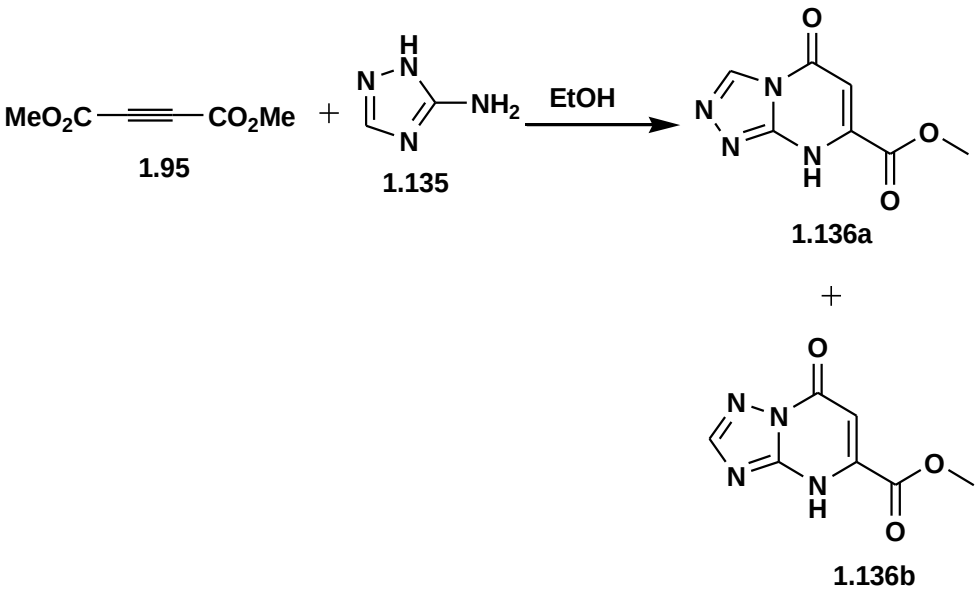

**Scheme 1.49**: DMAD as a Michael acceptor with thiourea

When DMAD **1.95** was treated with thiosemicarbazide **1.133** under microwave irradiation in the presence of methanol as a solvent or in a solvent-free system, the reaction gave rise to thiazine derivative **1.134** (Scheme 1.50) as reported by Heravi and co-workers.[109]



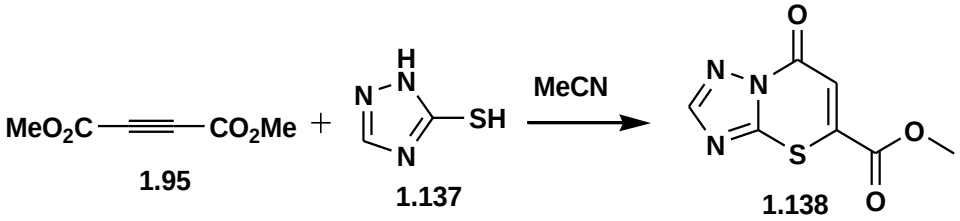

**Scheme 1.50:** DMAD as a Michael acceptor with thiosemicarbazide.

Reimlinger and co-worker[104] reported the reaction of DMAD **1.95** with 1*H*-1,2,4-triazol-5-amine **1.135** under ethanol reflux to achieve two isomeric products of pyrimidines **1.136a** and **1.136b** in a yield of 29% and 10%, respectively (Scheme 1.51).




**Scheme 1.51**: DMAD as a Michael acceptor with 1*H*-1,2,4-triazol-5-amine

Shah and co-workers[105] reported the reaction of DMAD **1.95** with 1*H*-1,2,4-triazole-5-thiol **1.137** using acetonitrile as solvent at room temperature to achieve 74% of thiazine **1.138** (Scheme 1.52).

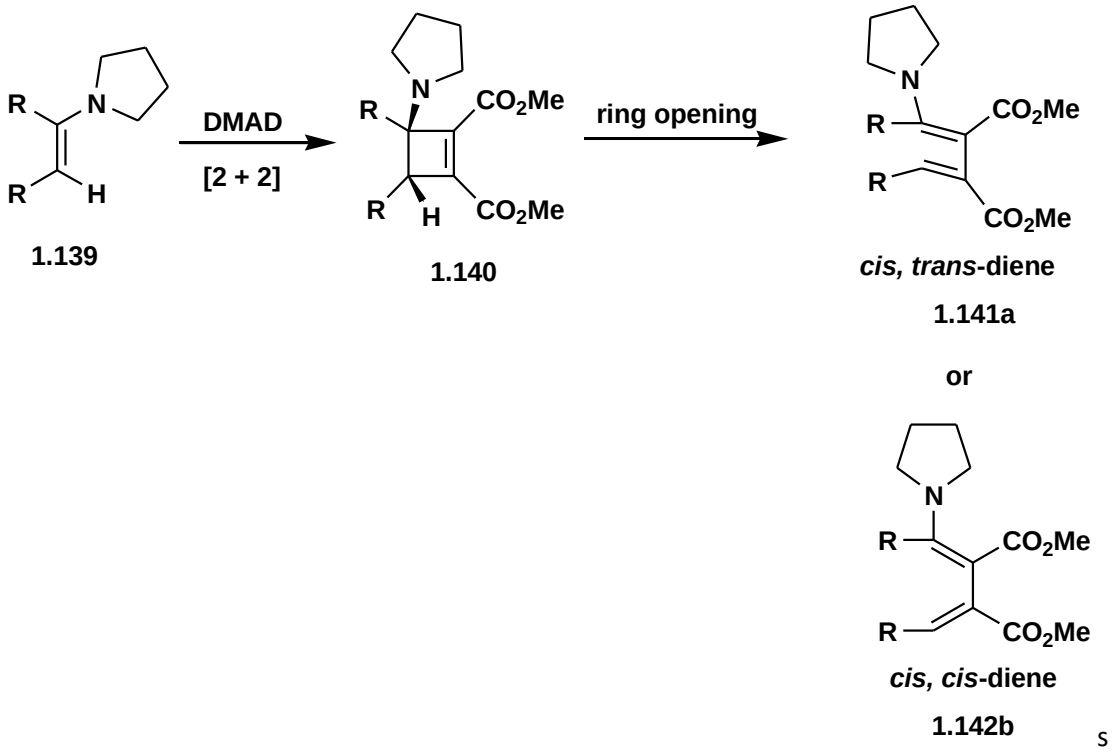



**Scheme 1.52**: DMAD as a Michael acceptor towards 1*H*-1,2,4-triazol-5-amine

*1.8.3 DMAD in cycloaddition reactions*

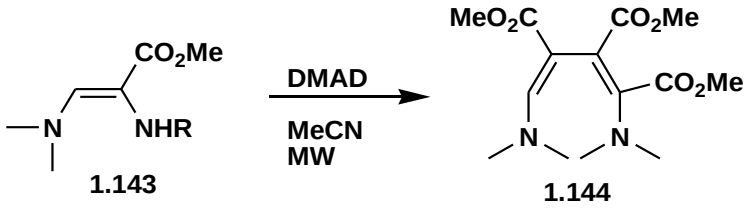
As mentioned earlier DMAD is extensively used as a dienophile in cycloaddition reactions. Importantly DMAD is used as a standard in Diels-Alder reactions to check the efficiency of various dienes and 1,3-dipolar cycloaddition reactions. DMAD is known to undergo [2 + 2]

cycloaddition reactions[110] and 1,3-dipolar cycloaddition with diazoalkanes, nitrile oxide and carbonyl ylides.[111] The reaction of diene **1.139** with DMAD **1.95** *via* [2 + 2] cycloaddition gives rise to product **1.140** which subsequently undergoes ring opening to give product **1.141** (Scheme 1.53). This is one of the valid approaches for constructing π-conjugated systems.




**Scheme 1.53**: DMAD in [2 + 2] cycloaddition reaction.

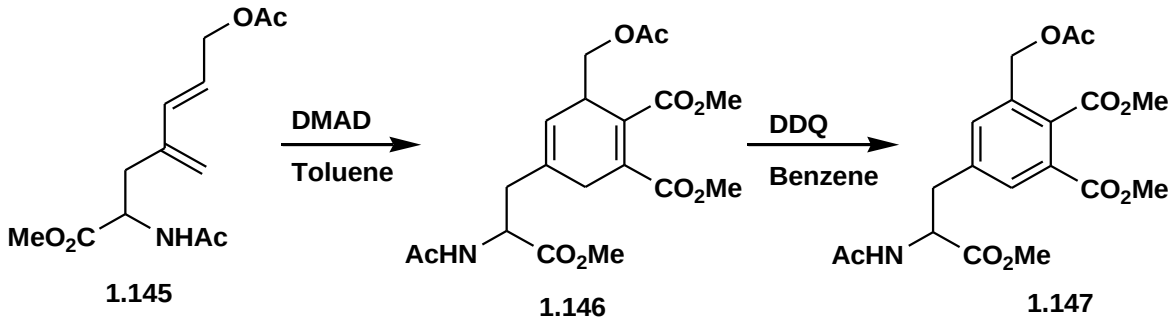
Uršič and co-workers reported the synthesis of butadienes **1.144** using microwave-assisted regiospecific [2 + 2] cycloadditions of DMAD and diene derivatives **1.143** (Scheme 1.54).




**Scheme 1.54**: DMAD in [2 + 2] cycloaddition under MW conditions.

One of the facile approaches for the construction of aromatic heterocyclic systems relies on the [4 + 2] cycloaddition reaction of DMAD and the 1,3-dienes, followed by an oxidation. As an examples Kotha and co-workers[112] reported the Diels-Alder reaction of the diene **1.145** with

DMAD leading to the formation of product **1.146** which is subsequently oxidised to give rise to cycloadduct **1.147** (Scheme 1.55).




**Scheme 1.55**: DMAD in [4 + 2] cycloaddition reaction.

### 1.9. Multicomponent reactions in medicinal chemistry

The use of MCRs towards production of bioactive compounds in medicinal chemistry is growing. The advantage of MCRs is their ability to rapidly generate molecular diversity and structural complexity. The products generated from MCRs include biologically active compounds[113] and MCRs allow design of compounds that explore different part of chemical space.[114] Figure 1.3 shows some of the MCR products that were discovered as bioactive compounds in medicinal chemistry. The use of the U-3CR for the synthesis of the drug Lidocaine (Xylocaine) (Figure 1.3a), which is used to induce anaesthesia, is one of the early applications of MCRs in drug discovery.[5] The synthesis proceeds by reaction of formaldehyde with diethylamine in the presence of 2,6-dimethylphenylisocyanide. The synthesis of phenylglycinamides and mandelamides, classes of compounds that exhibit fungicidal activity, was reported by Lamberth and co-workers.[115] The synthesis involves a two step sequence starting with a Passerini-3CR to afford an α-acyloxycarboxamide. The second step proceeds by alkylation with propargyl bromide to give rise to mandipropamid. Mandipropamid (Figure 1.3b) was found to possess excellent activity against phytopathogens such as *Phytophthora infestans* (potato and tomato late blight) and *Plasmopara viticola* (grape downy mildew). The van Leusen-3CR that involves the reaction of tosylmethyl isocyanide (TosMIC) with a primary amine in the presence of aldehyde using a base was used to give rise to 1,4,5-trisubstituted imidazoles as p38 MAP kinase inhibitors (Figure 1.3c).[81] Nifedipine (Procardia) (Figure 1.3d), which functions as a calcium channel blocker, was synthesised using the Hantzsch-3CR from 2-nitrobenzaldehyde with methyl acetoacetate in the presence of ammonia.[116] In addition, some of the other MCRs such as the Staudinger-3CR and Pauson-Khand-3CR have also produced

useful bioactive compounds for medicinal purposes. The Staudinger-3CR is used for the synthesis of Ezetimibe (Zetia) (Figure 1.3e) which functions to selectively inhibit the intestinal absorption of cholesterol.[117] The Pauson-Khand-3CR was used to produce Prostaglandin B1 (Figure 1.3f) which exhibits antioxidant and ionophoric activities.[118]

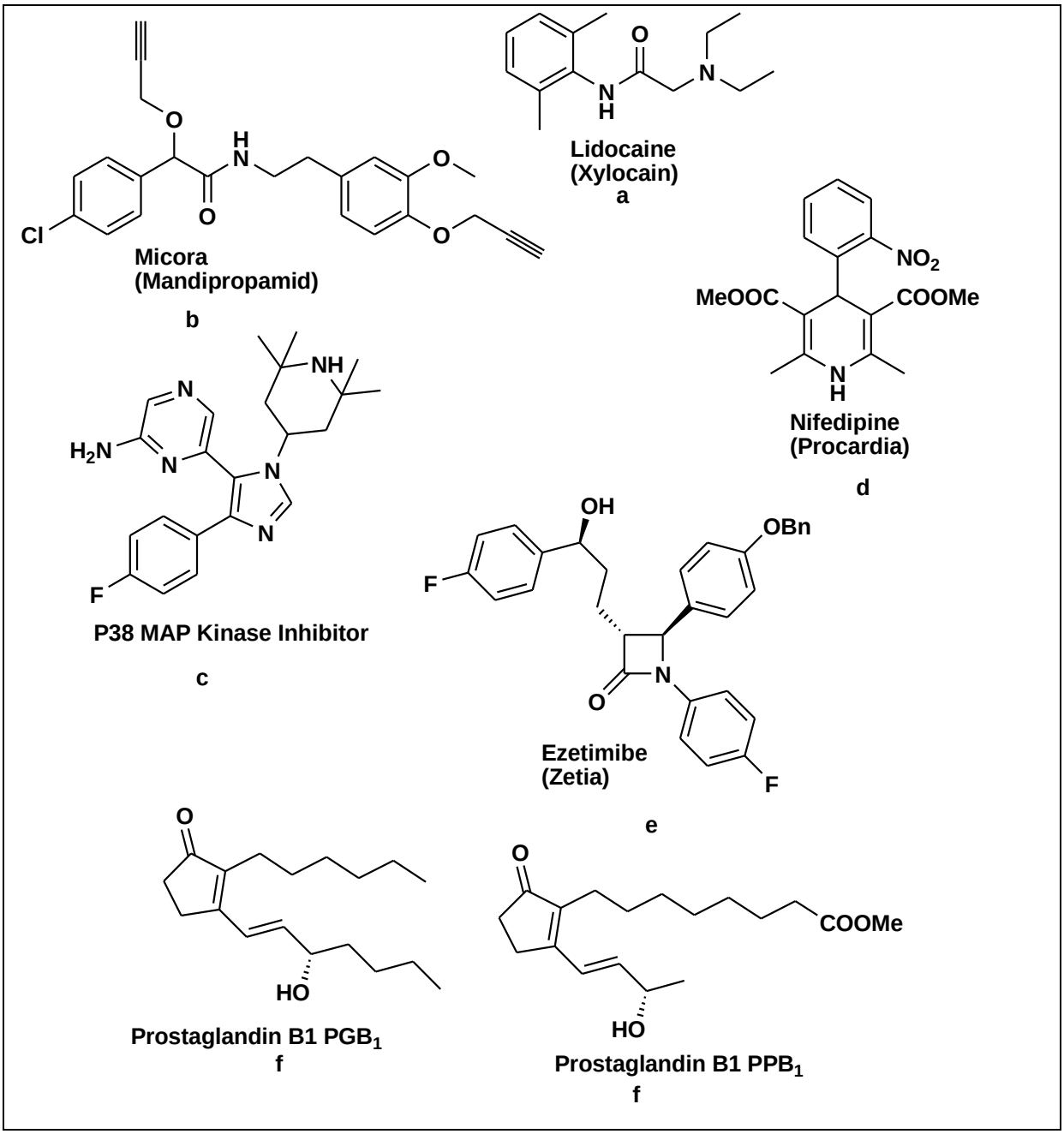
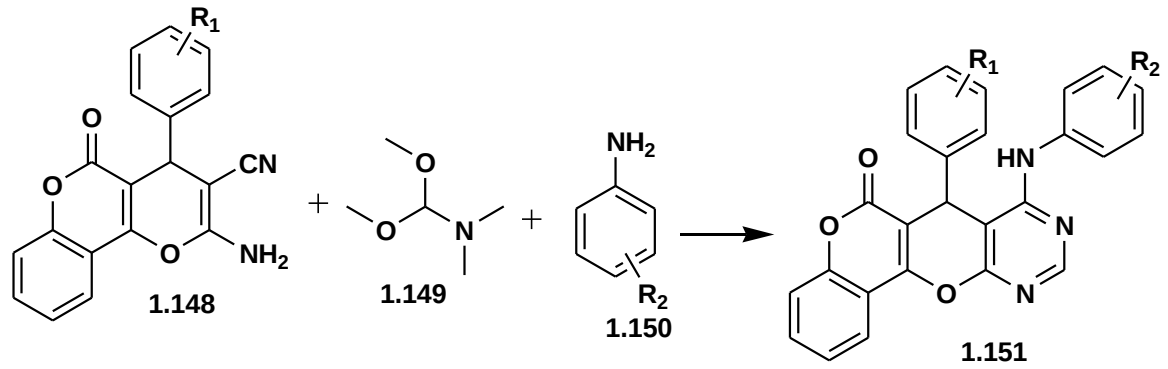



**Figure 1.3**: Biologically active MCR products (a) Lidocaine (Xylocain) (b) Micora (Mandipropamid), (c) p38 MAP Kinase Inhibitor, (d) Nifedioine (Procardia), (e) Ezetimibe (Zetia) and (f) Prostaglandin B1 ($PGB_1$ and $PPB_1$)

Suresh and co-workers[119] synthesised chromeno-pyridine derivatives which exhibited promising antibacterial activities (Scheme 1.56). The reaction proceeds by mixing 2-amino-5-

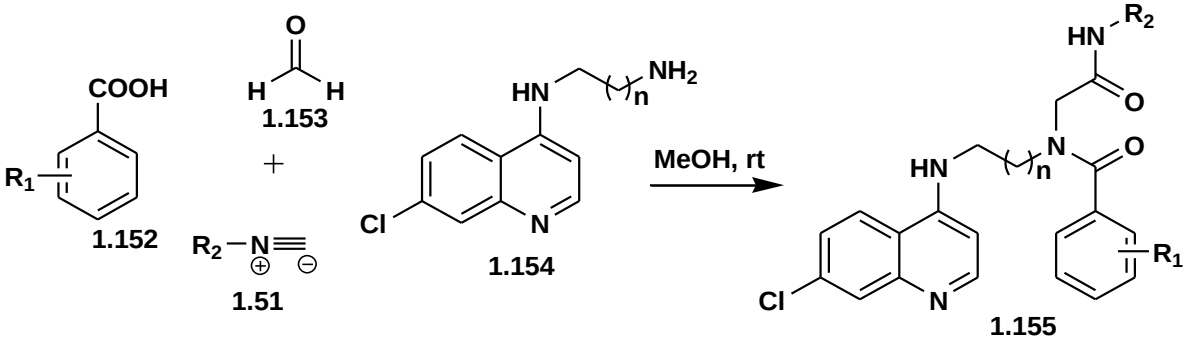
oxo-4-phenyl-4,5-dihydropyrano[3,2-*c*]chromene-3-carbontrile derivatives **1.148** and *N*,*N*-dimethylformamide dimethyl acetal **1.149** in the presence of ionic liquid [Bmim]$HSO_4$ followed by addition of aniline **1.150** to give rise to chromeno-pyrimidine derivatives **1.151** as shown on Scheme 1.56. The antibacterial activity *in vitro* included Gram-positive and Gram-negative bacterial strains such as *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella planticola*.




**$R_1$, $R_2$ = alkyl, $NO_2$, Halide, methoxy**

**Scheme 1.56**: One-pot three component reaction to chromeno-pyrimidine derivatives.

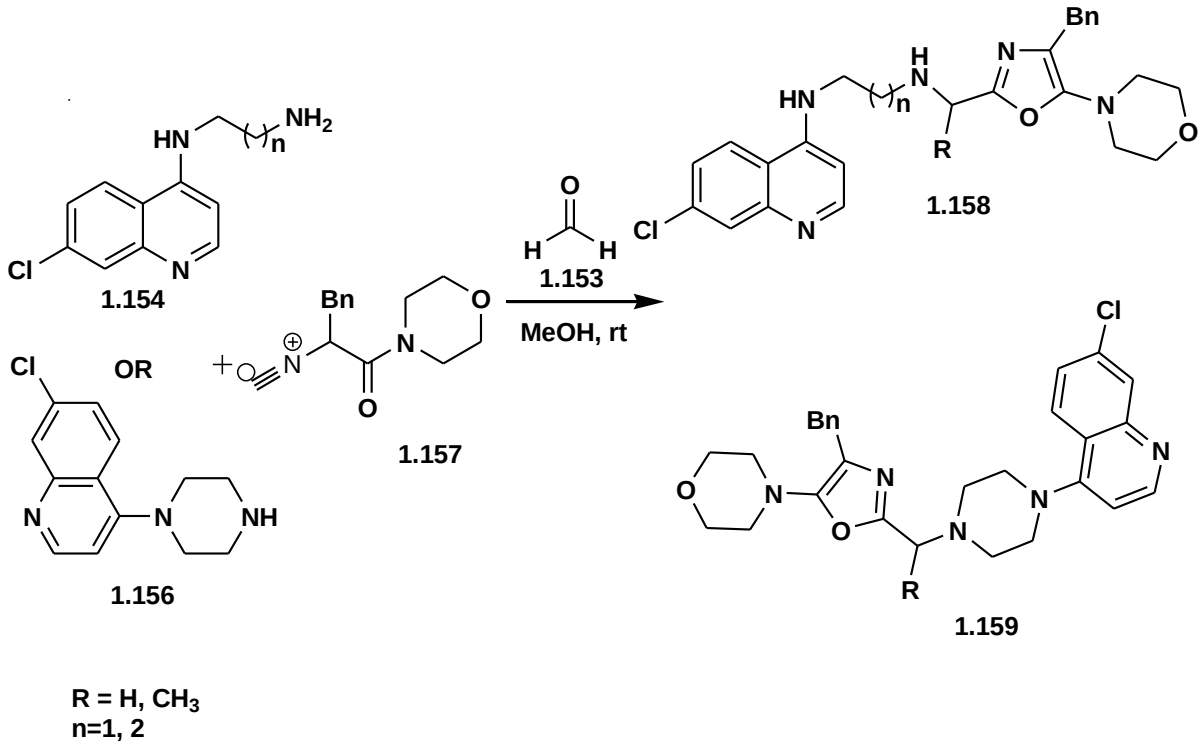
Musonda and co-workers[120] in 2004 reported new 4-aminoquinoline Ugi adducts from the reaction of excess isocyanide **1.51** with carboxylic acid **1.152** in the presence of formaldehyde **1.153** amine **1.154** to give rise to a new class of 4-aminoquinoline compounds **1.155** (Scheme 1.57). This class of compounds **1.155** exhibited significant antimalarial activity against three strains of the malaria parasite *P. falciparum*: CQ-sensitive D10, and CQ-resistant K1 and W2.




**$R_1$, $R_2$, =alkyl, OH**
**n=1, 2**

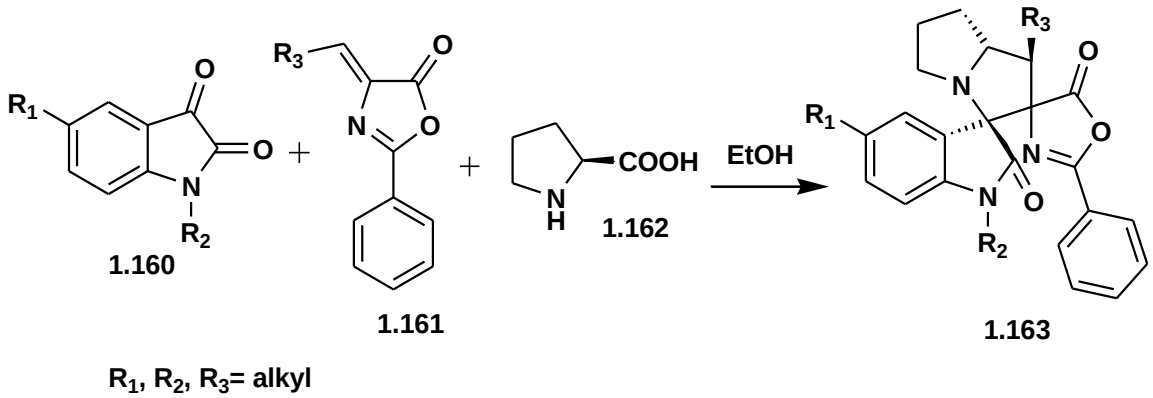
**Scheme 1.57**: Synthesis of new adducts using the U-4CR.

In 2007, Musonda and co-workers[121] used formaldehyde **1.153** with amines **1.154** or **1.156** in the presence isocyanoacetamide **1.157** in a one pot-three component reaction to afford aminoxazole 4-aminoquinolines **1.158** or **1.159** (Scheme 1.58). These compounds **1.158** and **1.159** showed good antiplasmodial activity against two strains of *P. falciparum*: the chloroquine-sensitive 3D7 strain and chloroquine-resistant K1 strain.



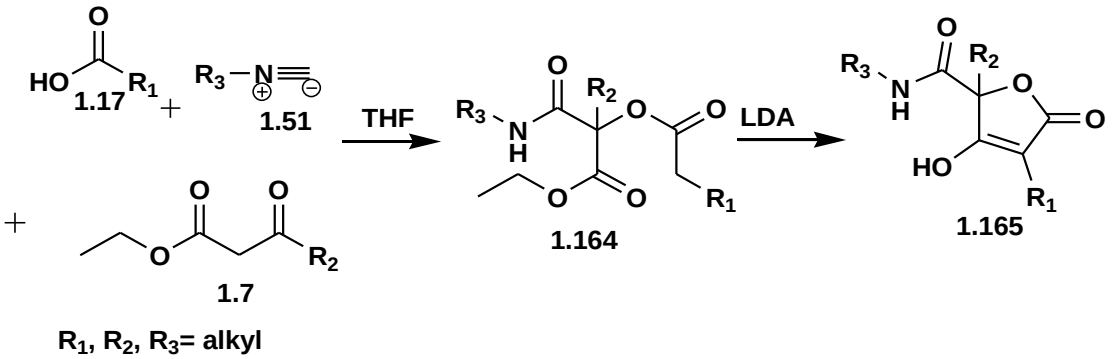

**Scheme 1.58**: Synthesis of new aminoxazole 4-aminoquinolines using 3-CR.

Dong and co-workers[122] reported the synthesis of oxazolone-grafted spirooxindoles as shown in Scheme 1.59 which gave good anti-cancer activities (HCT116 human colon cancer cells and HepG2 hepatocellular carcinoma cell)). The compounds were synthesised from the reaction of isatin **1.160** with 5-methyleneoxazolidine-2,4-diones derivatives **1.161** in the presence of amino acids **1.162** to afford novel spirooxindole compounds **1.163**. (Scheme 1.59)




**Scheme 1.59**: Synthesis of new oxazolone-grafted spirooxindoles

The reaction of α-ketoester **1.7** and acetic acid derivative **1.17** in the presence of an isocyanide **1.19** at room temperature using THF as a solvent gave rise to a Passerini (P-3CR) intermediate **1.164** which was treated with lithium diisopropylamide (LDA) at low temperature under inert nitrogen conditions to afford novel non-peptidic inhibitors **1.165** of HIV-1 protease[123] as shown in Scheme 1.60.




**Scheme 1.60**: Synthesis of novel non-peptidic inhibitors *via* P-3CR.

The scope of MCRs in medicinal chemistry continues to grow due to good practices such as use of safe environmentally friendly conditions where the necessity of waste treatment and multistep experimental work-up procedures are avoided and cheap commercially accessible reactants can be used.

### 1.10 Multicomponent reactions in green chemistry practice

Green sustainable chemistry practices ensuring a safe environment in laboratories and use of efficient economical chemical processes is highly recommended for synthesis and preparations

of desired products. Using MCRs for the synthesis of pharmaceutical and industrial products is considered as one of the recognisable options for increasing "greenness". MCRs present an excellent opportunity for the realisation of a safe environment and friendly practices by green production processes. The best known recommended practices and conditions for a green production process include: the use of green solvents, operating at mild temperatures, applying biocatalysts and the practice of catalyst-free reactions.

### *1.10.1 The Principles of Green Chemistry*

The standard guidelines for green chemistry practice and sustainable production are the Twelve Principles of Green Chemistry defined by Anastas and Warner in 1998,[124]acknowledged and redefined by Nigist and co-workers[125] and Anastas and Eghbali.[126] The following green principles definitions and signs were directly adopted from the Sigma-Aldrich website.[48]

1. **Prevention:** Prevention of waste is better than treatment of waste

**2. Atom Economy:** All materials used during the synthesis or preparation should be incoporated into the final product.

**3. Less Hazardous Chemical Syntheses**: Synthetic method should be designed to possess little or no toxicity to human health and the environment.

**4. Designing Safer Chemicals** Production of chemicals should be safe and less harmful.

**5. Safer Solvents and Auxiliaries:** The use of safe solvents such as water and acetone is recommended.

**6. Design for Energy Efficiency:** During chemical processes energy requirements should be minimised by using conditions such as ambient temperature and pressure during synthesis.

**7. Use of Renewable Feedstocks:** Renewable materials are recommended rather than depleting materials during applications.

**8. Reduce Derivatives:** Derivatisation such as extra protection steps should be avoided to prevent additional waste from new materials during chemical production.

**9. Catalysis:** The use of safe catalytic reagents and recyclable catalysts is recommended.

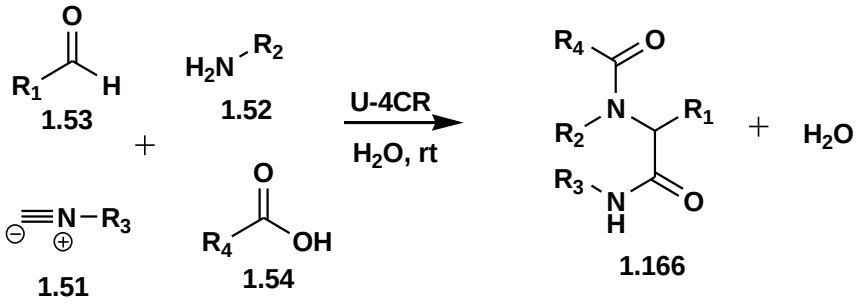
**10. Design for Degradation:** The prepared or synthesised chemical products should be designed in a way to be degradable at the end of use and avoid products that persist in the environment.

**11. Real-time analysis for Pollution Prevention:** Development of safe and sustainable methods for production is recommended to avoid formation of hazardous substances.

**12. Inherently Safer Chemistry for Accident Prevention:** Safe and non-explosive substances should be chosen in chemical processes to minimize the possibility of chemical accidents, explosions and fires.

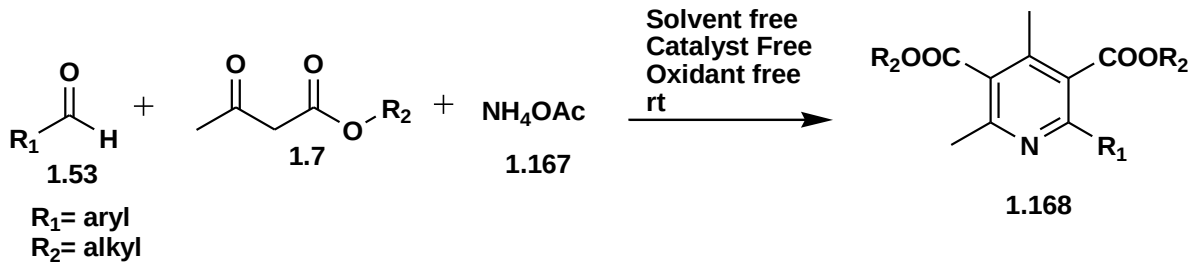
*1.10.2 Examples of Green Chemistry in practice*

Ingold and co-workers[127] reported green production of an Ugi-4CR product from the reaction of *tert*-butyl isocyanide **1.51** and aniline **1.52** in the presence of benzaldehyde **1.53** and benzoic acid **1.54** at room temperature using water as a solvent to afford compound **1.166** (Scheme 1.61).




**Scheme 1.61**: Green production of Ugi-4CR adduct.

Shen and co-workers[128] discovered a green production process after reacting ethyl acetoacetate **1.7** and benzaldehyde **1.53** in the presence of ammonium acetate **1.167** to obtain unsymmetric 2-arylpyridine **1.168** under solvent-free, heat-free, catalyst-free and oxidant-free conditions at room temperature (Scheme 1.62).



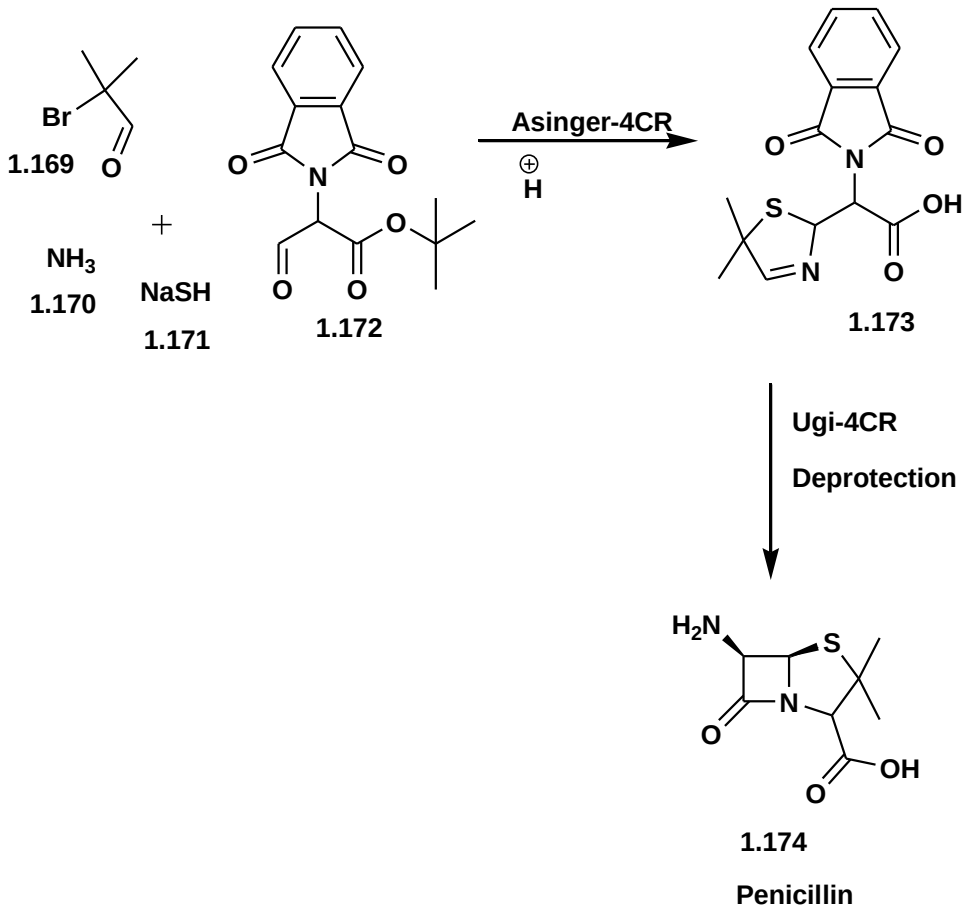

**Scheme 1.62:** Green production of unsymmetric 2-arylpyridines.

There are other recommended and used catalysts in green production processes such as: biocatalysts (lipase enzymes) from wheat germ for resolution of MCR adducts,[129] nanocatalysts,[130] and photocatalysts such as zirconium-based metal-organic frameworks catalysts.[131]

Waste/ product ratios from production in the pharmaceutical industry are higher than those found for the industrial sectors and hence waste treatment remains a major concern in the pharmaceutical industry.[132]

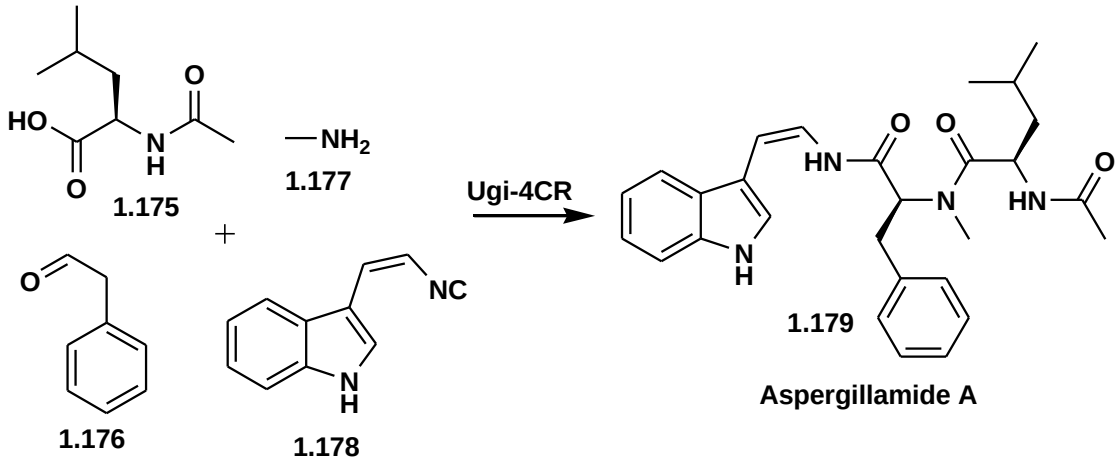
## 1.11 The use MCRs for synthesis of natural products

The synthesis of natural products and their analogues using MCRs is well exploited and there are several examples in literature.[133] In 1980 Ugi reported the synthesis of complex antibiotic penicillin using two MCR approaches: the Asinger reaction and U-4CR.[134] The reaction proceeded by initial Asinger 4-CR which gave rise to intermediate **1.173** followed by U-4CR and deprotection to afford penicillin **1.174**[135] as shown in Scheme 1.63.



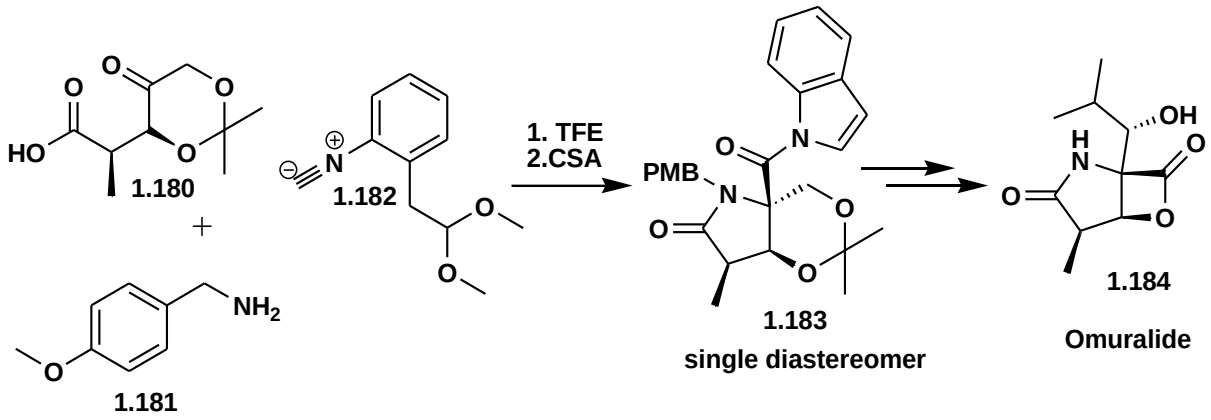

**Scheme 1.63:** Synthesis of Penicillin using Asinger 4CR and the Ugi 4CR.

The synthesis of aspergillamide A using an MCR approach was reported by Beck and co-workers[136] using a one step U-4CR of *N*-acetylleucine **1.175**, phenylacetaldehyde **1.176** methylamine **1.177**, and (*E*/*Z*)-3-(2-isocyanoethenyl)indole **1.179** as shown in Scheme 1.64.



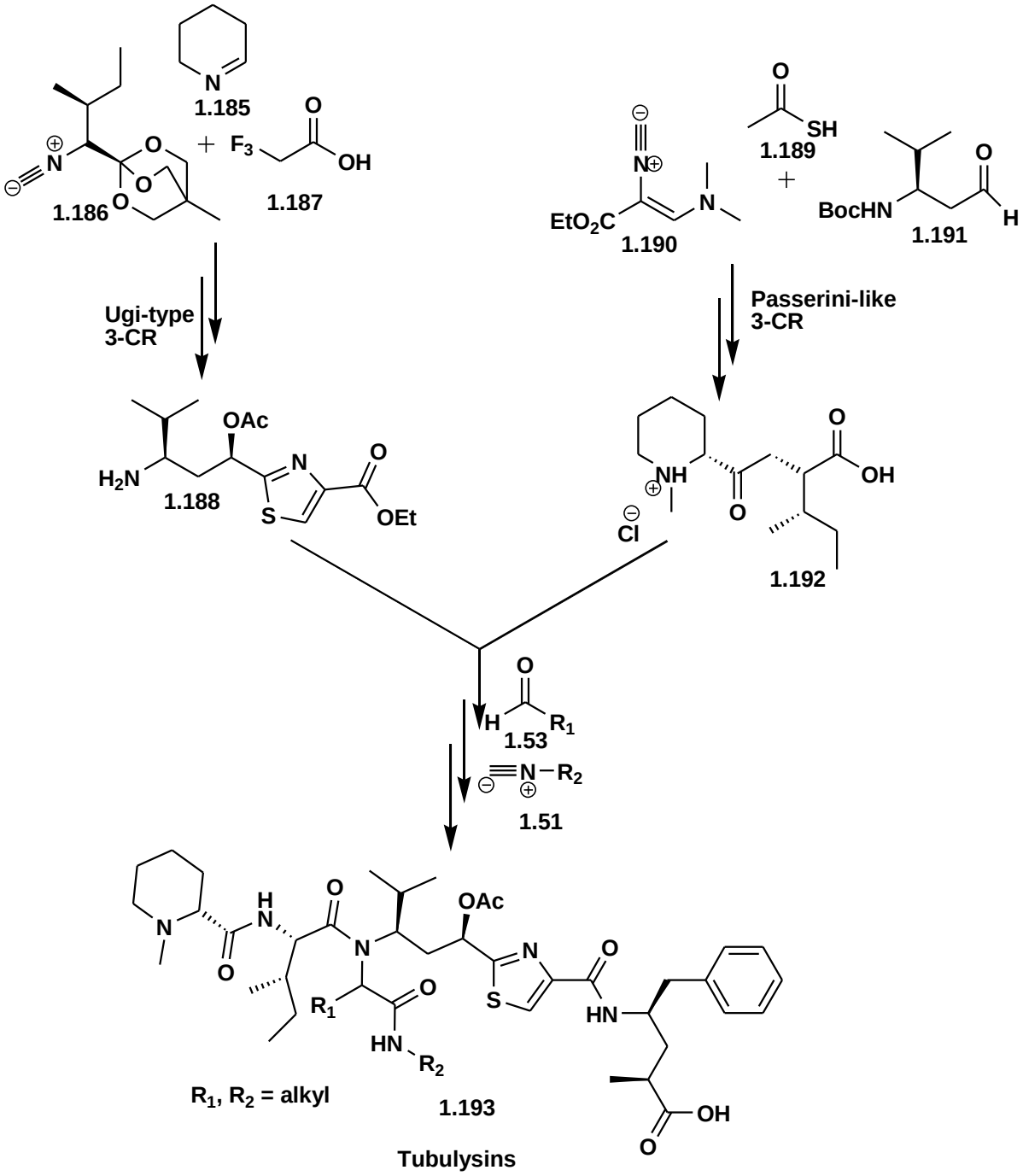

**Scheme 1.64:** Synthesis of aspergillamide A using the Ugi 4CR.

An MCR approach for the synthesis of proteasome inhibitor omuralide was reported by Gilley and co-workers[137] using an intramolecular Ugi reaction. The reaction involves ketocarboxylic acid **1.180**, *p*-methoxybenzylamine **1.181** and 1-isocyano-2-(2,2-dimethoxyethyl)benzene **1.182** to form an indole acyl intermediate **1.183** which subsequently led to the final natural product of omuradile **1.184** (Scheme 1.65).



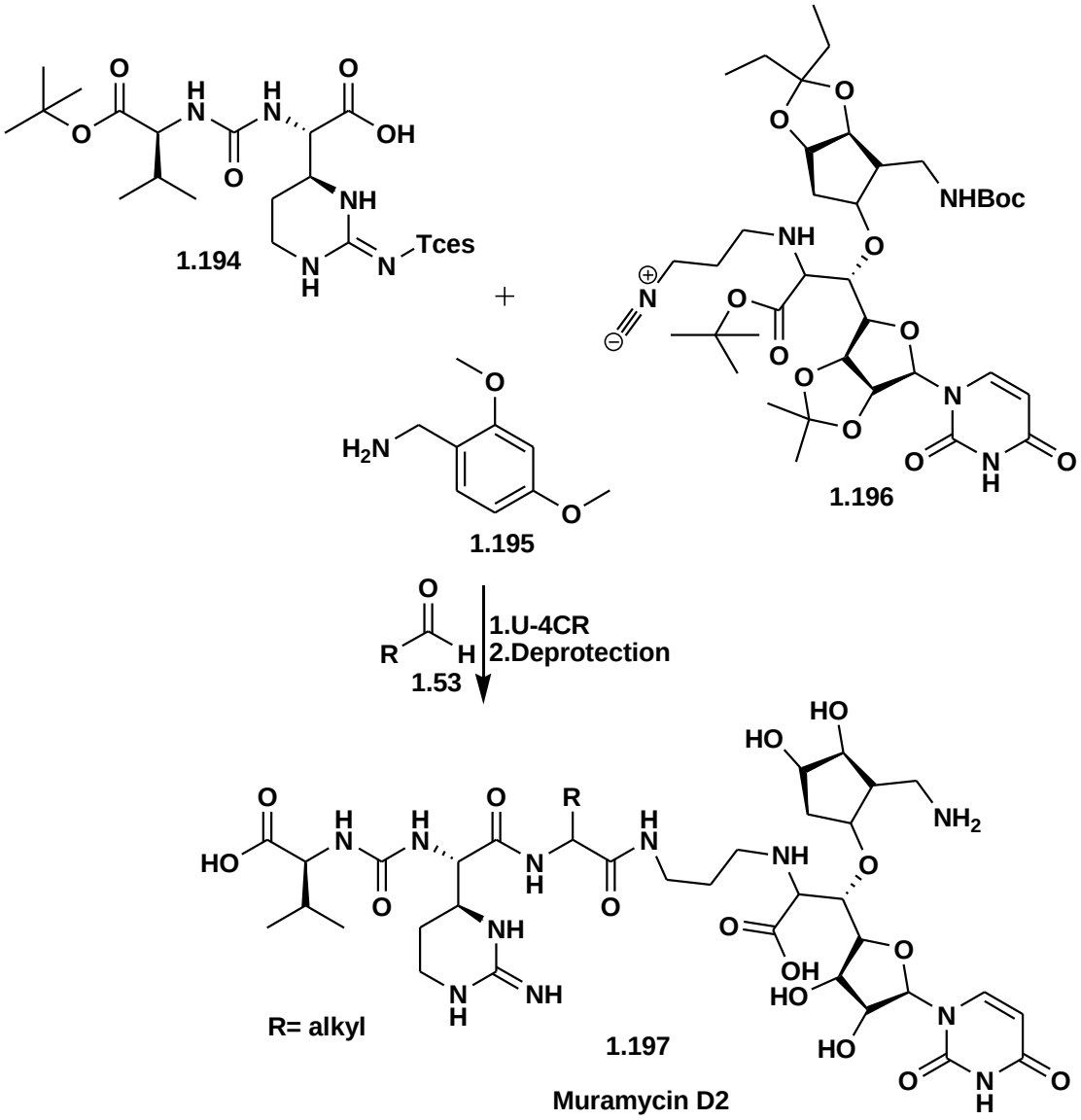

**Scheme 1.65:** Synthesis of omuralide using intramolecular Ugi reaction.

Pando and co-workers[138] reported the synthesis of natural product analogues of tubugis which exhibit anticancer activities. The reactions were achieved by union of two MCRs: a Passerini-like 3-CR and an Ugi-3CR to achieve intermediates **1.188** [139] and **1.192** [140] which were treated with an isocyanide **1.51** and appropriate aldehyde **1.53** to afford the final product tubulysins **1.193** *via* U-4CR as shown in Scheme 1.66.




**Scheme 1.66:** Synthesis of tubulysins using Ugi 4CR.

Tanino and co-workers[141] reported an Ugi-4CR approach for the synthesis of natural muramycin D2 and its epimer from the reaction of aldehyde **1.53** with carboxylic acid **1.194** in the presence of 2,4-dimethoxybenzylamine **1.195** and highly functionalised isocyanide **1.196** (Scheme 1.67). This method is one of the most elegant approaches for the synthesis of a natural product *via* an MCR.




**Scheme 1.67:** Synthesis of muramycin D2 using Ugi 4CR.

MCRs are very useful in the preparation of natural products and development of drugs in the pharmaceutical industry. Some of the several natural products prepared using MCRs in clinical studies are shown in Figure 1.4.

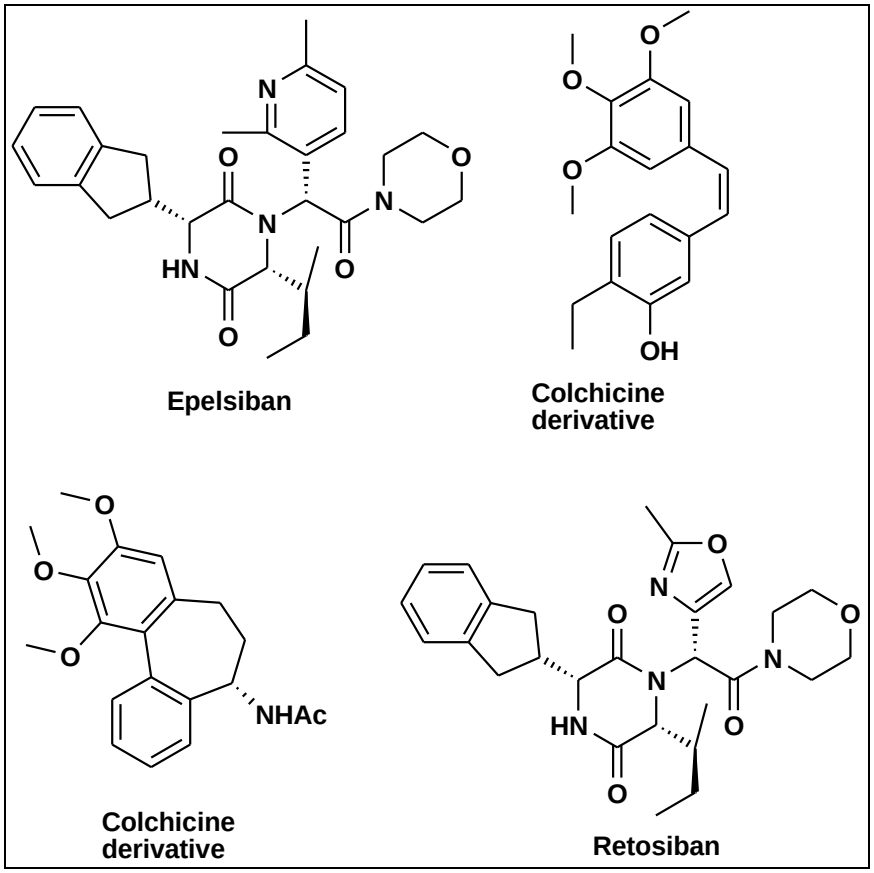



Figure 1.4: Clinical candidates in cancer studies (Colchicine derivatives) and clinical candidate in neonatal morbidity studies (Epelsiban and Retosiban).

### 1.12 Research background

An ongoing interest in our laboratories is the identification of small molecules able to disrupt protein-protein interactions between HIV-1 integrase and Lens Epithelium-Derived Growth Factor (HIV-1-IN-LEDGF/p75).[142,143] The protein-protein interactions between the HIV-1 IN and LEDGF are discussed in detail in Chapter 2. One of the initial strategies used in this project involved preliminary computational modeling (virtual screening) studies using Schrödinger Maestro 11.2 software which were conducted as a starting point to identify possible heterocyclic scaffolds able to disrupt this interaction. The virtual screening was used to select heterocyclic scaffolds that could then be used as a template for the design and synthesis of small molecule libraries that would lead to the identification of molecules able to disrupt the interaction between HIV-IN and LEDGEF/p75. The screening was done using both physical and online ChemBridge and National Institutes of Health (NIH) Clinical Collection libraries available at Mintek.

Some of the promising virtual screening docking results from the ChemBridge and NIH libraries consisted of fused-ring systems comprising five-six membered pyrimidine analogues, six-six membered quinoxaline and quinolone analogues and six-seven membered diazepine analogues (Figure 1.5).

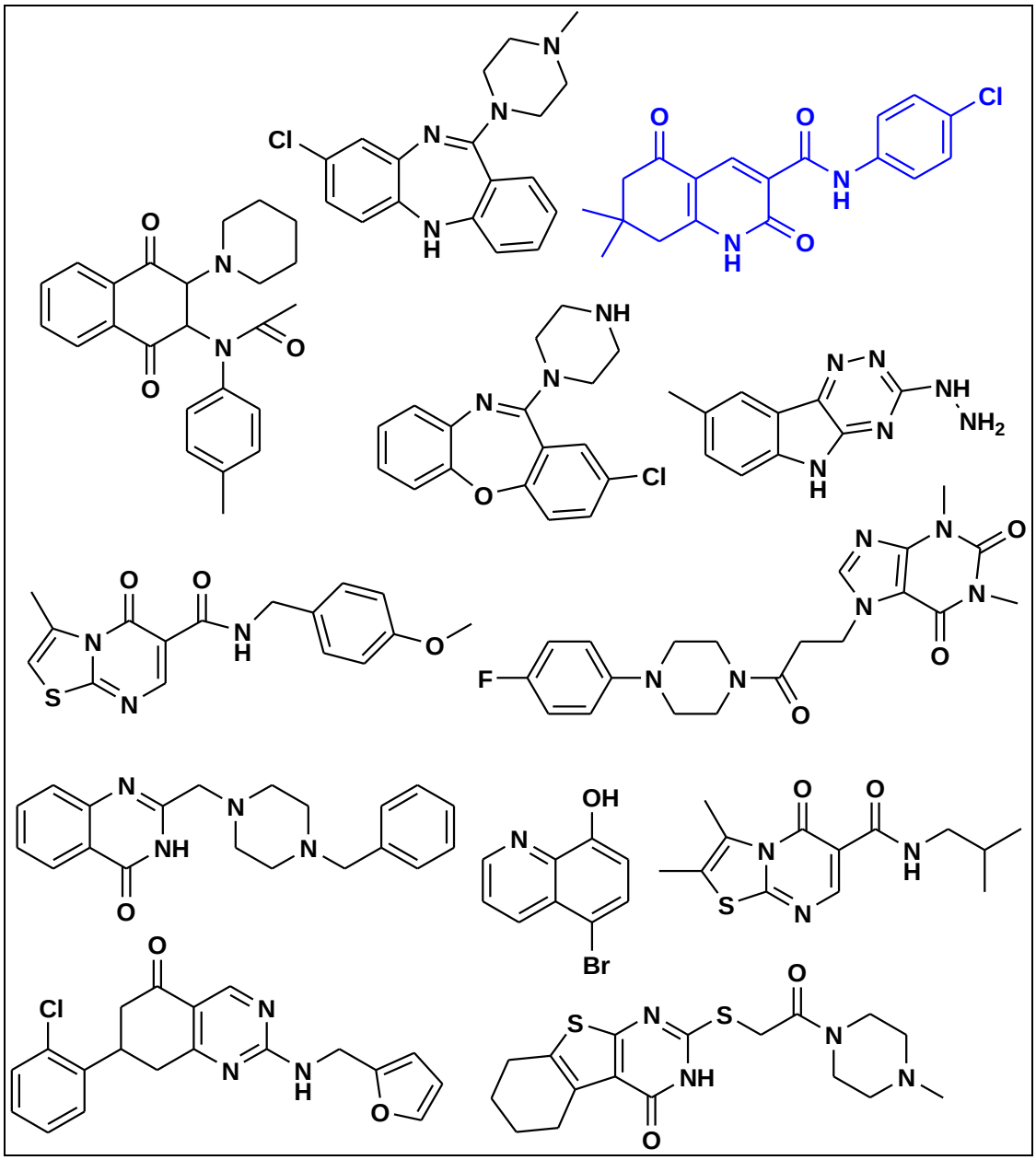


**Figure 1.5**: ChemBridge and NIH compounds showing promising inhibition of HIV-1-IN-LEDGF/p75 interactions from virtual screening.

The ChemBridge library was further subjected to high throughput screening to find possible inhibitors of the HIV-1-IN-LEDGF/p75 interaction. The compounds were pooled and screened

through a pre-developed HIV-1 Alphascreen assay[144] to identify compounds that were able to directly disrupt the protein-protein interaction of HIV-1 IN and LEDGF 2. A total of 20 000 compounds were screened and fewer than 300 compounds showed inhibition above 50% at a single dose of 10 μM in the pooled assay. Some of the chosen compounds with promising inhibition are represented in Figure 1.6. From Figure1.5 and 1.6 it is clear that the compounds of interest for disruption of the HIV-1 IN and LEDGF are in large part nitrogen and sulfur-based heterocycles, many of which contain fused six-six or six-five rings. Although not much is known about the structure-activity relationships for inhibitors of this protein-protein interaction, modelling and screening gave us a good starting point for the design of compounds for synthesis in this project.

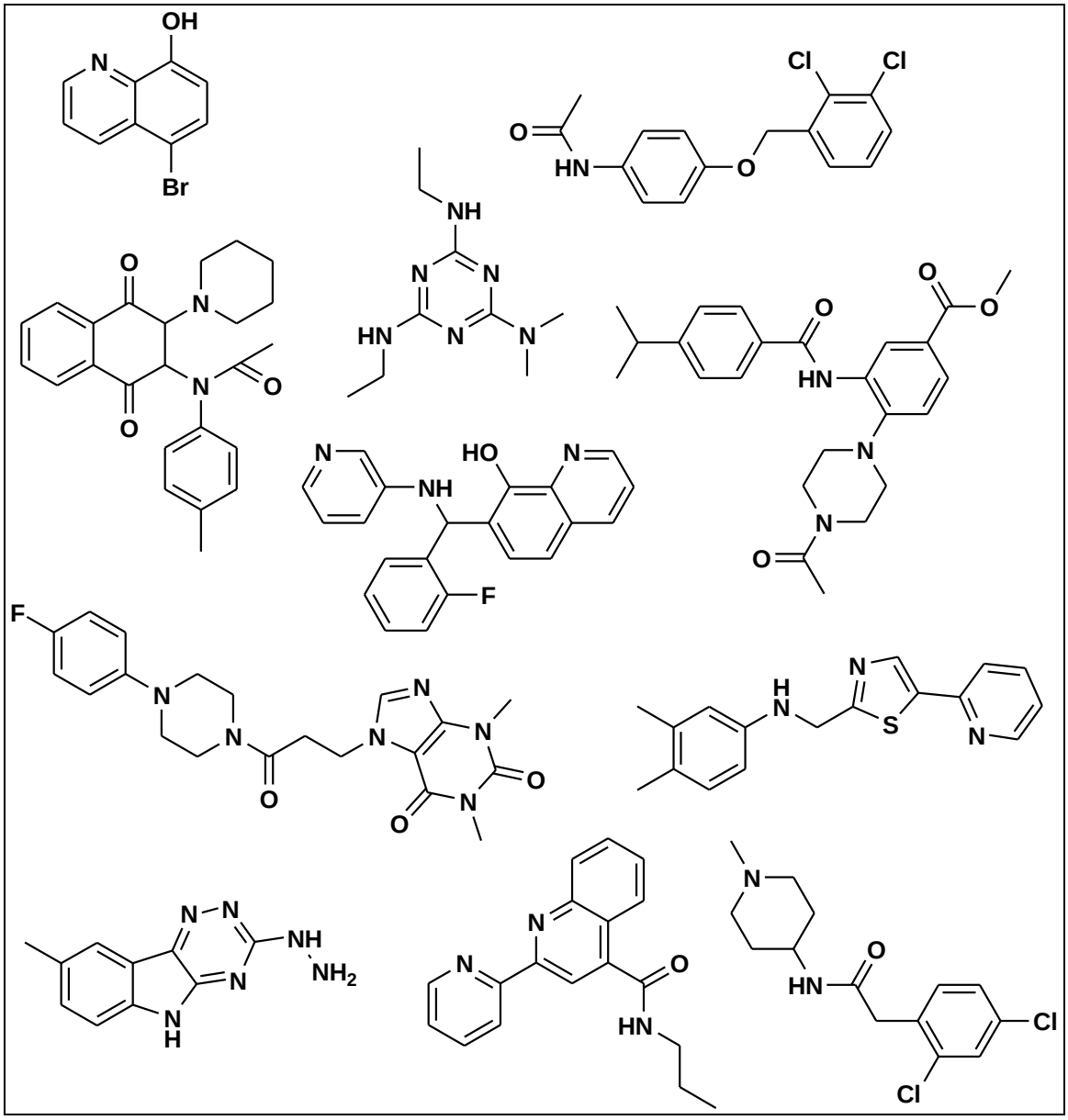



**Figure 1.6**: ChemBridge compounds showing more than 50% inhibition of HIV-1-IN-LEDGF/p75 interactions from the Alphascreen assay.

The virtual screening and Alphascreen assay data obtained was considered in conjunction with experimental synthetic design of heterocyclic scaffolds. The accessibility and/or feasibility of the synthetic routes envisaged was also considered based on chemical reagent availability. The preliminary results, together with the versatility of MCRs led us to the idea to use an MCR approach for the synthesis of fused pyrimidines, thiazines, quinoxalines, and diazepine scaffolds. In addition, one of the potential ChemBridge ligands, (*N*-(4-chlorophenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide) highlighted in blue

colour was directly chosen for its ease of synthesis based on availability of reagents and possible synthetic route to generate a family of compounds (Figure 1.5).

### *1.12.1 Project strategy and aims*

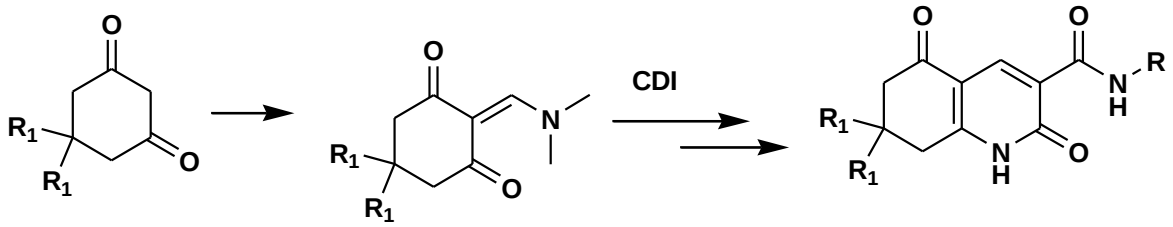
MCRs are considered a facile approach to the synthesis of complex molecules that have many advantages over the traditionally known one and two component reactions in synthetic chemistry. These advantages include a reduction in the number of sequential multiple reactions required, as well as better overall percentage yields.[14] Using MCRs for the synthesis of heterocyclic frameworks in drug discovery and other products continues to receive attention due the possibility of developing diverse and structurally complex compound libraries. The other factors that MCRs promote include: commercially cheaper and accessible reactants and atom economy, easy experimental work-up with better yields, safe green production processes by avoiding the use of hazardous solvents, catalysts and generation of heavy metal waste in the laboratories.

The aim of this project was to develop synthetic methods for possibly bio-active heterocyclic scaffolds *via* 3 component reactions (3-CR) from 5 membered-rings (thiazole, triazolo, oxazolo, pyrrolidine derivatives) and 6 membered-rings (thiazine derivatives). The other factor considered was to practice green chemistry principles where applicable, by applying catalyst-free conversions, mild reaction conditions (room temperature) and the safe use of recommended green solvents such as acetone, ethanol and isopropanol, taking into account the possibility to involve biocatalysts (lipases) as the next step for the hydrolysis and resolution of some of the synthesised scaffolds.

### *1.12.2 Synthetic approach via MCRs*

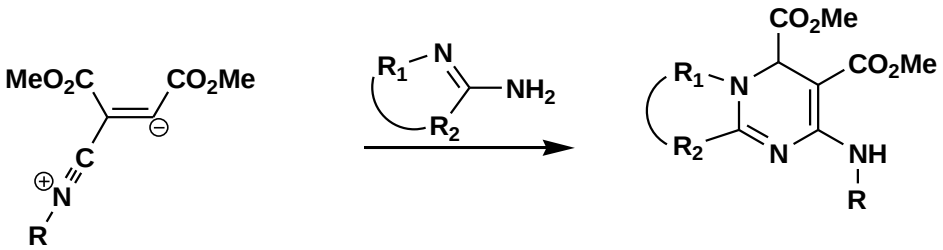
This study focuses on using the reaction of an isocyanide and an electron deficient alkyne to give rise to a zwitterion intermediate that can then be reacted further in order to build fused heterocyclic scaffolds. The study is also concerned with the reactivity of the zwitterion adduct towards nucleophiles containing an acidic proton. In addition, we were also concerned with the reactivity of dialkyl acetylenedicarboxylate towards various nucleophiles.

The initial preliminary computational modelling studies led us to the synthesis of the hexahydroquinoline scaffold. This approach was done by using carbonyldiimidazole (CDI) as a coupling agent of primary amines and hexahydroquinoline acids to give rise to hexahydroquinoline derivatives (Scheme 1.68).



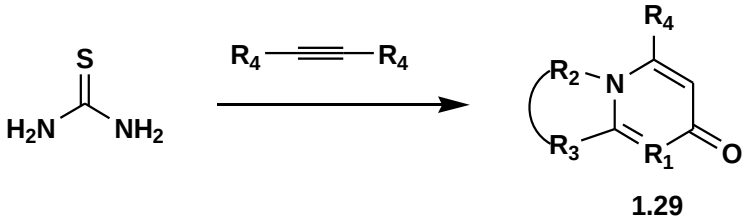

**Scheme 1.68**

The first MCR approach employed a zwitterion adduct formed from the reaction of an isocyanide and DMAD/ DEtAD as an intermediate to react with nucleophilic 5-or 6-membered-ring substrates possessing an acidic proton, as shown in Scheme 1.69. These reactions gave rise to the fused pyrimidine- and thiazine- based compounds containing at least one stereogenic centre.




**Scheme 1.69**

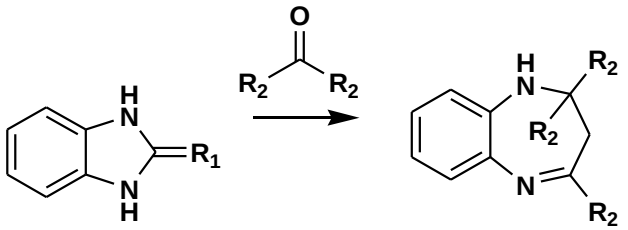
The second MCR approach was based on developing an alternative route to the synthesis of pyrimidine derivatives (bearing no stereogenic centres) from a Michael reaction (2-CR) to a one-pot 3-CR. The approach incorporated thiourea, α-haloketones and DMAD/DEtAD in the reaction to produce pyrimidine scaffolds (Scheme 1.70).




**1.29**

**Scheme 1.70**

The third MCR approach came after we aimed to further react the zwitterion adduct with benzimidazole derivatives which led unexpectedly to benzodiazepine as a product. The DMAD/DEtAD played no role in the reaction and were just mere spectators, while acetone, isocyanide and benzimidazole derivatives were part of the 3-CR to form benzodiazapines (Scheme 1.71).




**Scheme 1.71**

*1.12.3 Outline:*

The study is presented with the the following outline:

**In chapter one**, introduction and literature review.

**In Chapter two,** we discuss the synthesis of hexahydroquinolines using CDI coupling agent for the reaction of carboxylic acids (hexahydroquinolines) and primary amines (anilines). This class of compounds was adopted from the ChemBridge DIVERset screening library of compounds, available at Mintek.

**In Chapter three**, we discuss the synthesis of highly functionalised pyrimidines *via* one pot 3-CR from isocyanides, DMAD/DEtAD and 5-membered ring substrates.

**In Chapter four**, we present an efficient and facile one pot 3-CR for the synthesis of novel thiazine-based heterocyclic compounds *via* the zwitterion adduct intermediate.

**In Chapter five**, we report an alternative one pot 3-CR route for synthesis of pyrimidine derivatives using thiourea, α-haloketones and DMAD/DEtAD. This method showed a significant yield improvement on other methods and easy experimental work-up compared to the traditionally known multistep reactions.

**In Chapter six,** we discuss the synthesis of diazepine- and quinoxaline-based derivatives *via* one pot 3-CR using acetone/ketones, isocyanides and benzimidazole. We further generated diazepine scaffold products from DMAD/DEtAD/DTAD and benzimidazole or 1,2-phenylenediamine derivatives.

**In Chapter seven,** a summary, conclusions and future work are discussed.

**In Chapter eight,** experimental procedures are provided.

**Appendix,** presentation of selected data.

# CHAPTER 2

# Synthesis of highly functionalised hexahydroquinolines using CDI as a mediator

## 2.1. Introduction

Computational modeling was used as one of the strategies to identify possibly bioactive scaffolds in continuation of our laboratory's research aiming to identify small molecules with the capability to disrupt protein-protein interactions between HIV-1 integrase and Lens Epithelium-Derived Growth Factor (HIV-1-IN-LEDGF/p75). The screening was done on ChemBridge and National Institutes of Health (NIH) Clinical Collection libraries using Schrödinger Maestro 11.2 software. The NIH Clinical Collection library consists of 727 drug-like compounds, and are available for re-purposing and ChemBridge Diverse Collection library of 20000 diverse and unique drug-like structures. The references used were Mut101, an allosteric inhibitor of the HIV-1-IN-LEDGF/p75 interaction and CX05168, an inhibitor of the HIV-1-IN-LEDGF/p75 interaction and a modulator of IN multimerization. Mut101 in application shows great capabilities of anti-HIV-1 activity at the integration and post-integration steps during the viral replication cycle. Mut101 binds a unique target on IN and secondly it is capable of blocking HIV-1 replication in virus-producer cells in post-integration as an active antiretroviral agent.[145] CX05168 was designed from a series of 2-(quinolin-3-yl)acetic acid derivatives developed as inhibitors of HIV-1-IN-LEDGF/p75 interaction, and HIV-1 replication by blocking the integration step and modulating IN Multimerization. [146,147] The virtual screening, represented in Figure 2.1, involved protein preparation, ligand preparations, and docking.

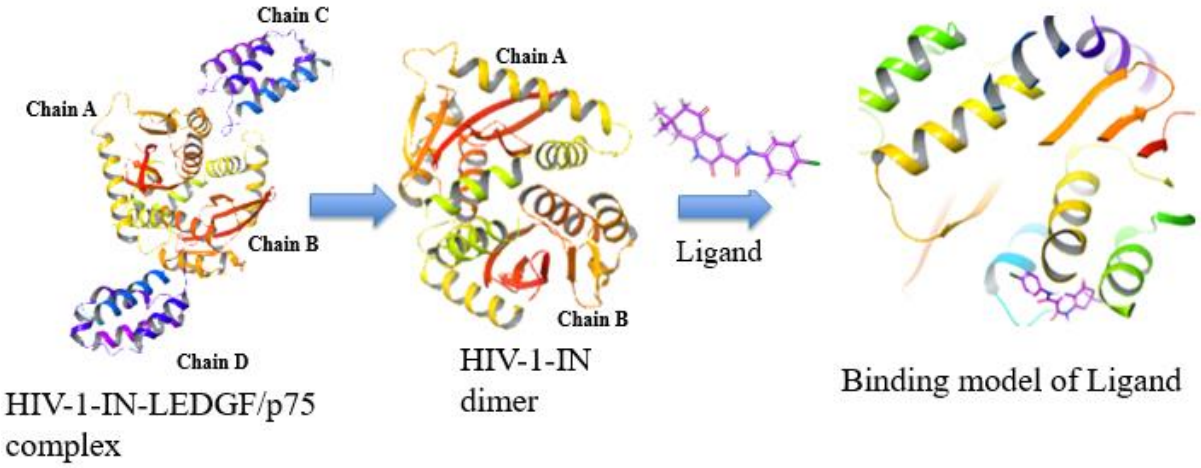



**Figure: 2.1**: Virtual screening of ChemBridge and NIH libraries.

## 2.2 Virtual screening of HIV-1 Integrase in Complex with LEDGF/p75 Binding Site Inhibitors

### *2.2.1 HIV-1 integrase and LEDGF/p75 complex*

In terms of antiretroviral therapy for treatment of HIV infection, long term side effects such as toxicity and drug-resistant viral variants remain major challenges that lead to the continuous search for new drugs.[148] HIV-IN has been discovered to interact with LEDGF/p75 as a binding partner, thus the LEDGF/p75-IN interaction has been classified as a valuable target for developing the next generation of antiretroviral drugs.[149] The crystal structure of the dimer of the HIV-1 IN integrase binding domain (IBD) showed that the LEDGF/p75 amino acid residues Lys364, Ile365, Asp366, Phe406 and Val408 mediate the interaction with HIV- IN.[150,151] This amino acid network interacts with HIV-IN in the inter-helical loop region of the structure. During the integration the HIV dimer directly contacts the LEDG/p75 through two regions of the catalytic core domain (CCD) which are the Trp131, Trp132 and the Ile161 to Glu170 regions and these generate an interface produced by two subunits of the IN-core dimer.[150] The interaction of the two subunits forms the hydrophobic pocket formed by Leu102, Ala128, Ala129 and Trp132 from one IN subunit and Thr174 and Met178 of the second unit and the Ile365 from the LEDGF/p75 side chain contacts the hydrophobic pocket formed.[150,152] The Ile364 further generates a hydrogen-bond with the backbone carbonyl group of IN Gln168 and the Asp 366 from LEDGF/p75 forms a hydrogen-bond with Glu170.[150] The amino acids which

are present in LEDGF/p75 and participate during the interaction and are in contact with the HIV-IBD were also confirmed by mutagenesis studies which included Ala128, His170, Thr174, Trp132 and Gln170.[153,150] The mutation of these residues on integrase renders the protein inadequate or deficient for LEDGF/p75 interaction. The crystal structure of the HIV-IN of the core catalytic domain as part of the IBD was achieved and showed that a pair of LEDGF/p75-IBD molecules could bind at the similar-matching target of the HIV-IN catalytic domain dimer.[150] Previous endeavours to determine whether LEDGF/p75 was vital and efficient for HIV replication used RNAi knockout techniques in human cells.[154,155] The findings also supplemented the idea that LEDGF/p75 acted as a tethering factor, binding to HIV and chromatin to direct and promote viral replication to host cells and active genes. The overexpression of the IBD of LEDGF/p75 in human cells, supported by additional studies in human cell lines, provided an evidence based concept that the interaction of LEDGF/p75-IN can be a suitable and druggable target for anti-HIV therapy.

### *2.2.2 Ligand preparation*

The ChemBridge and NIH ligands were prepared from 2-dimensional to 3-dimensional versions prior to screening by the "LigPrep" protocol for generation of possible combinations of stereoisomers and conformers using Schrödinger Maestro 11.2 as shown in Figure 2.2.

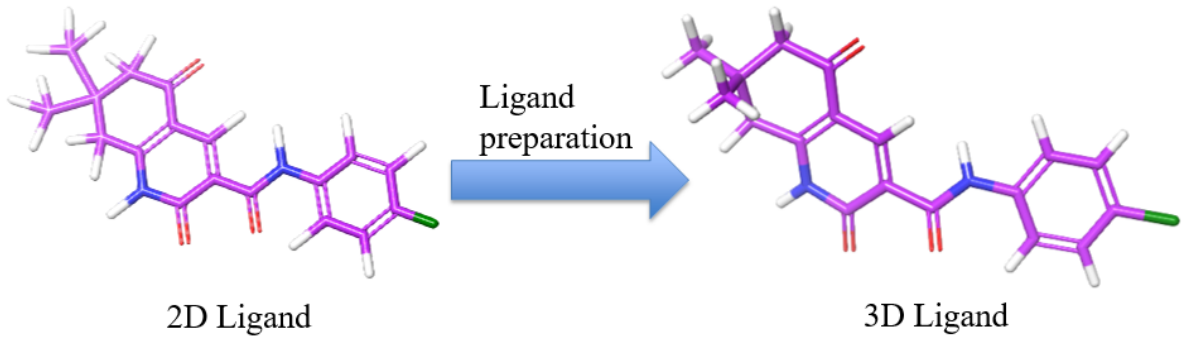



**Figure 2.2**: Ligand preparation using Maestro 11.2.

### *2.2.4 Preparation of HIV-1 IN protein*

The HIV-1-IN-LEDGF/p75 structure used for modelling was obtained from the Protein Data Bank (PDB) as the X-ray crystal structure code 2B4J.[149] Using "Protein Preparation Wizard" from Schrödinger Maestro 11.2 chain C and D (LEDGF/p75) were removed to obtain HIV-IN

(Chain A and B). Water molecules and hydrogens were removed and locations of new assigned hydrogens were minimized using the OPLS force field.[156] The missing residues and loop regions of chain A and B were constructed and the cap terminals were considered.[157] The Receptor Grid Generation process was followed to modify and detect the binding site for docking purposes (Figure 2.3).[158]

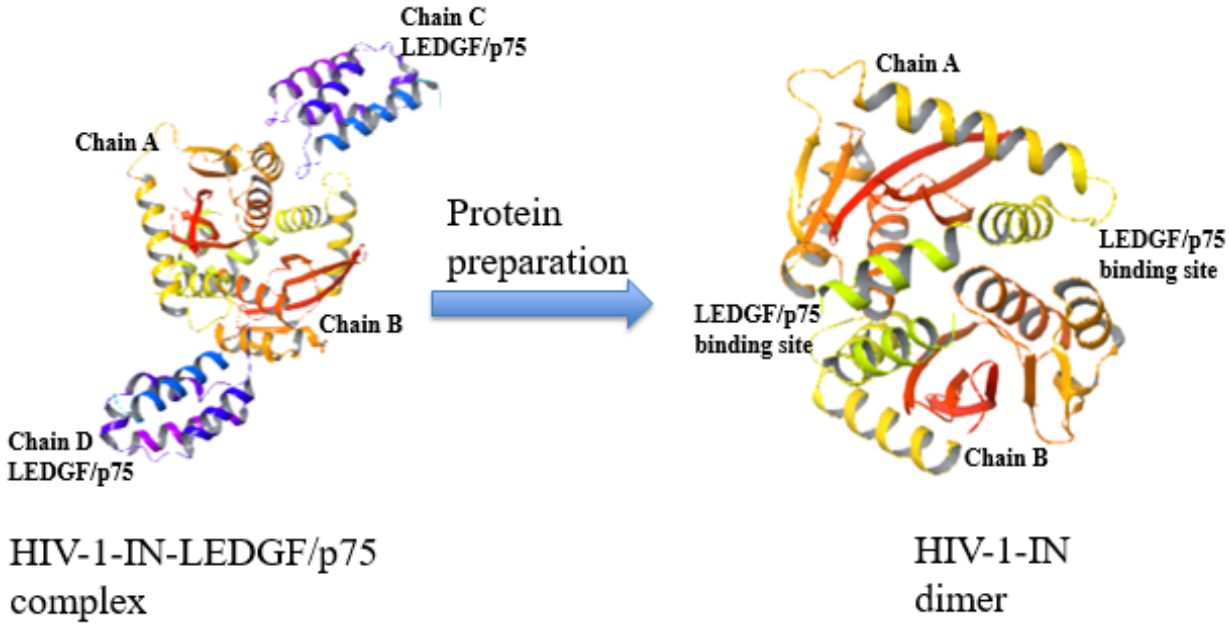



**Figure 2.3**: Protein-protein preparation using Maestro 11.2.

*2.2.5 Molecular docking of compounds into the HIV IN binding site*

The molecular docking studies were performed to estimate the binding affinity of the compounds (ligand) with the receptor (HIV-IN) dimer. The compounds, with references Mut101 and CX05168 were positioned in the same binding sites of the HIV-1-IN where LEDGF binds using the "ligand docking" protocol using standard precision (SP) followed by extra precision (XP) from the Schrödinger Maestro 11.2 suite. During docking the program takes ligand-receptor poses as input and provides rankings or the binding affinity of the pose. These poses gave ligand docking scores, glide scores and glide emodel scores and then their binding energy was calculated using the MM-GBSA method. The HIV-IN dimer interacts with LEDGF/p75 using a hydrogen-bond network at the integrase-binding domain (IBD) from the specific backbone conformation of amino acid residues 168-171 and the hydrophobic patch on the side chain residues of LEDGF/p75 from Ile365, Phe406, and Val408.[159] The CX05168 carboxylic acid group interacts with HIV-IN residue Glu170 through a hydrogen-bond and

oxygen interaction from the carbonyl group with His171 and Thr174 as shown in Figure 2.4 and Figure 2.5.

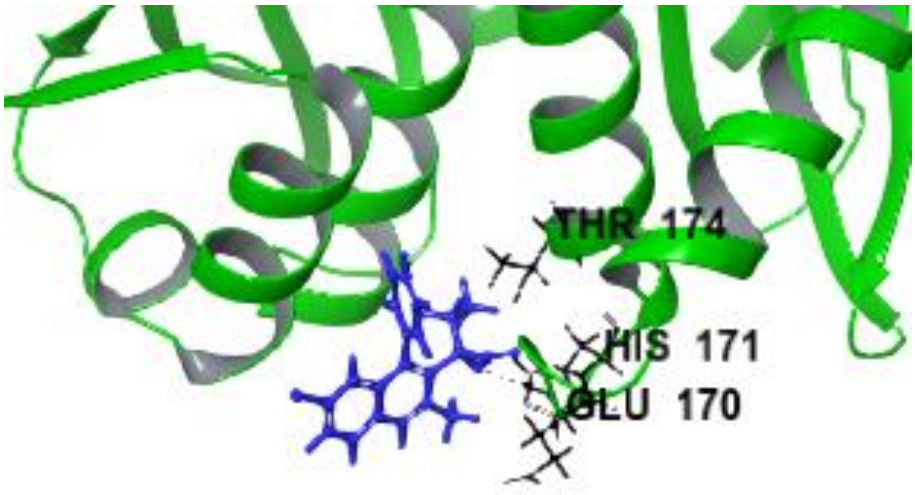



**Figure: 2.4**: 3-Dimensional binding mode of CX05168 in the LEDGF/p75 binding site of HIV-1 IN

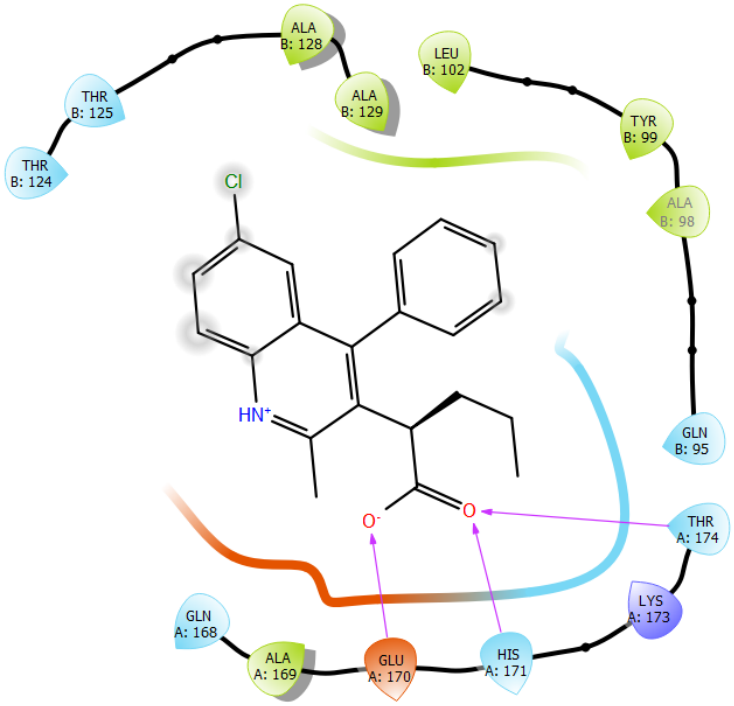



**Figure: 2.5:** 2-Dimensional binding mode of CX05168 in the LEDGF/p75 binding site of HIV-1 IN

The other reference compound used, Mut101, contains a carboxylic acid group that interacts with HIV-IN residues His171 and Thr174 through a hydrogen-bond, while Glu170 interacts *via* oxygen of the carbonyl group (Figure 2.6 and Figure 2.7)

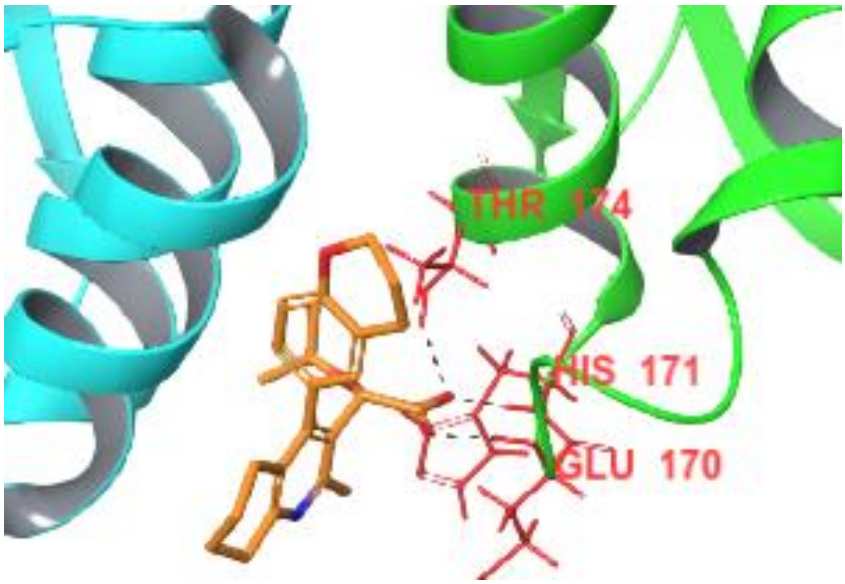



**Figure: 2.6:** 3-Dimensional binding mode of Mut101in the LEDGF/p75 binding site of HIV-1 IN

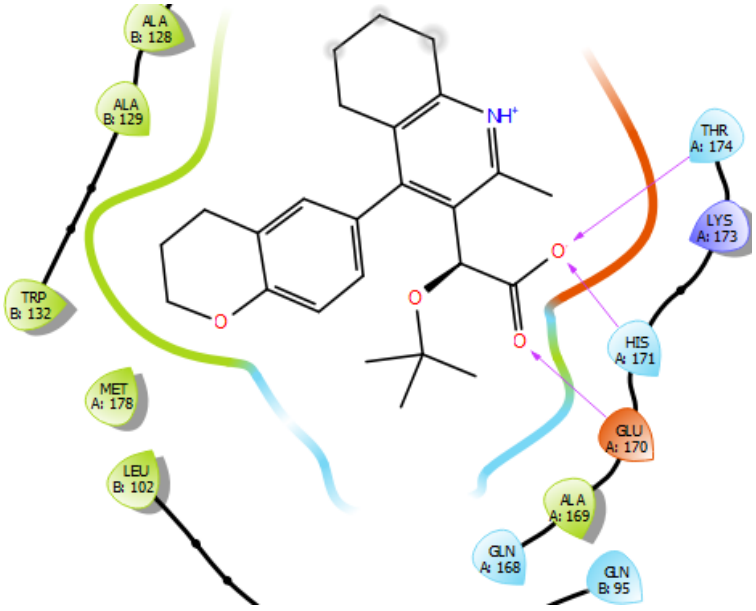



**Figure: 2.7:** 2-Dimensional binding mode of Mut101 in the LEDGF/p75 binding site of HIV-1 IN

Based on the virtual screening data outcomes obtained, possible facile and efficient synthetic routes and chemical reagent availability led us to choose one of the potential ChemBridge ligands containing the hexahydroquinoline scaffold. This compound, *N*-(4-chlorophenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide, and its derivatives were synthesised and the generated derivatives were further subjected to virtual screening.

The predicted binding model at the HIV-1-IN dimer interface shows the interaction of ChemBridge Ligand *N*-(4-chlorophenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (Figure 2.8 and Figure 2.9) interacting with amino acids residues Glu170 and Thr174 *via* oxygen from the carbonyl groups and pi-interactions with His171.

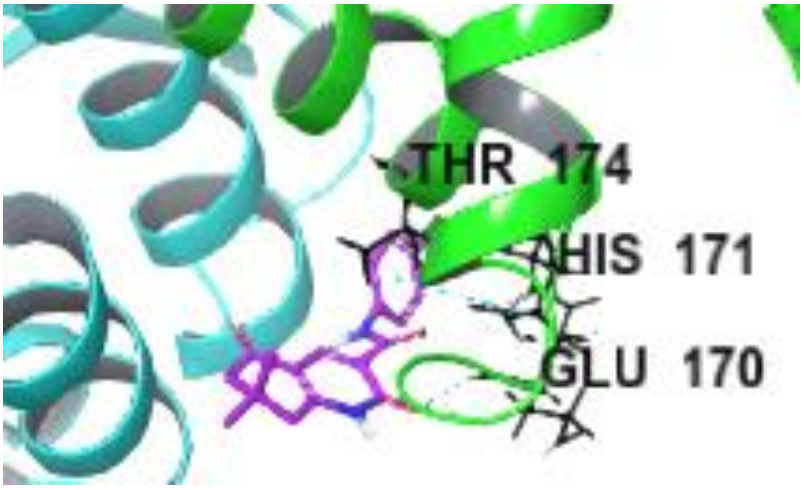



**Figure: 2.8:** 3-Dimensional binding mode of ChemBridge ligand **2.8a** in the LEDGF/p75 binding site of HIV-1 IN

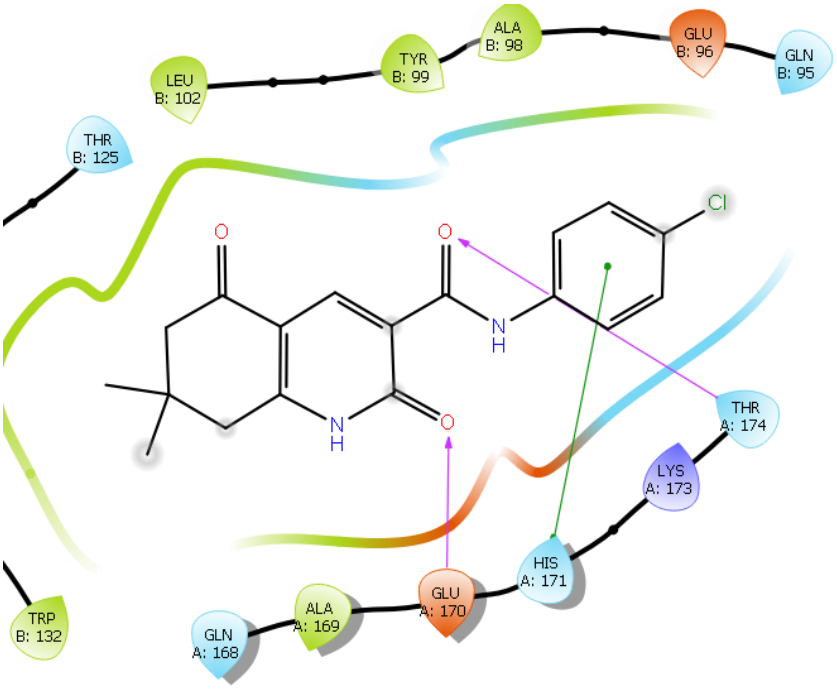



**Figure: 2.9:** 2-Dimensional binding mode of ChemBridge ligand **2.8a** in the LEDGF/p75 binding site of HIV-1 IN

The docking scores of the reference compounds were Mut101 (-6.176) and CX05168 (-5.329). The ChemBridge ligand (-4.333) docking score was poorer than that of both control compounds. The Glide Emodel, Glide docking and MMGBSA were obtained after subjecting the ligands to the "MMGBSA" protocol. The interactions of the ChemBridge ligand, CX05168 and Mut101 with HIV-IN are represented in Table 2.1. The residues Glu170, His171 and Thr174 from HIV-IN were found to interact with the ChemBridge ligand, CX05168 and Mut101.

**Table 2.1**: Molecular docking results of ChemBridge ligand **6.8a**, CX04328 and Mut101.

| | **Docking score (Kcal/mol)** | **Glide score (Kcal/mol)** | **XP G score** | **Glide emodel (Kcal/mol))** | **MMGBSA (Kcal/mol)** | **Hydrogen bond interactions** | **Oxygen Interactions** |
|---|---|---|---|---|---|---|---|
| 2.8a | -4.333 | -4.333 | -4.333 | -58.112 | -52.523 | | Glu170<br>Thr174 |
| CX05168 | -5.329 | -5.339 | -5.339 | -52.558 | -51.171 | Glu170 | His171<br>Thr174 |
| Mut101 | -6.176 | -6.621 | -6.621 | -63.191 | -59.389 | His171<br>Thr174 | Glu170 |

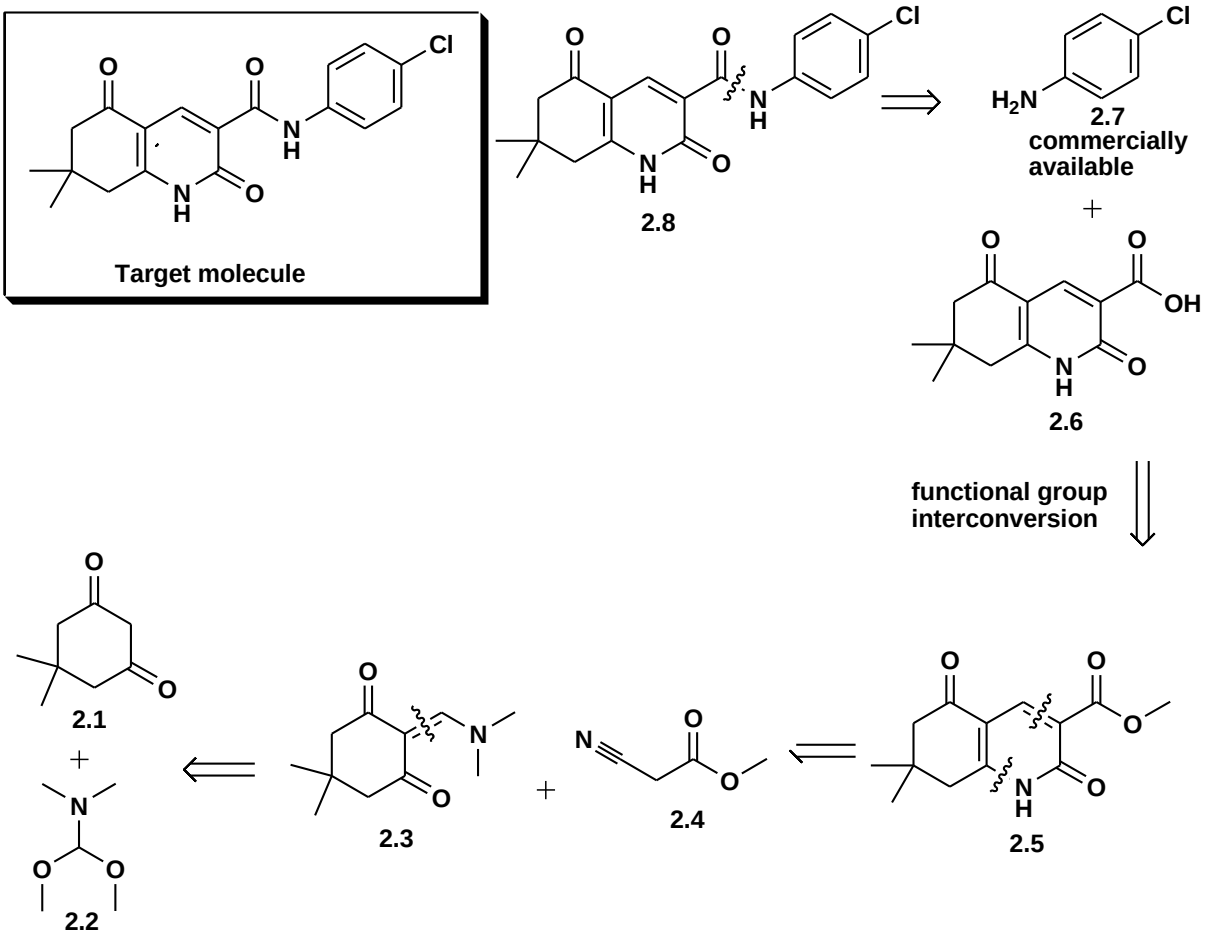
## 2.3 Synthetic route toward *N*-(4-chlorophenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide and its derivatives

In our quest to develop bioactive compounds we present the synthesis of *N*-(4-chlorophenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide and its derivatives, chosen from virtual screening data. The synthesis involves the reaction of aniline derivatives and the prepared hexahydroquinoline-3-carboxylic acid using carbonyldiimidazole (CDI)[160] as a mediator. CDI is a useful coupling reagent that promotes one-pot amide formation. Quinolines are important scaffolds in drug-discovery and show pharmacological activities such as HIV-1 inhibition,[161]antitubercular,[162] antimalarial[163,164] and antioxidant[165] activity, cytotoxicity,[166] cytochrome inhibition,[167] and antimicrobial,[168,169] antiproliferative,[170] and anticancer[171,172,173] activity. This type of scaffold remains one of the most attractive classes of nitrogen-heterocycles in pharmaceutical agents. Fossa and co-workers synthesised and evaluated the cardiotonic effect of a family of functionalised hexahydroquinoline carboxylates on guinea pig myocardial preparations.[174] Dzhavakhishvili and co-workers also reported one pot methods for the synthesis of dihydroquinoline-diones.[175] Similar hexahydroquinoline carboxyl derived scaffolds were reported by Gorobets and co-workers.[176]

### *2.3.1 Retrosynthesis of target compounds N-(4-chlorophenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamides*

The initial step was to design a facile and an efficient synthetic route towards the desired *N*-(4-chlorophenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide target

molecule shown in Scheme 2.1. The *retrosynthesis* concept was first used by Prof E. J. Corey[177] and has since been frequently used to develop synthetic pathways.



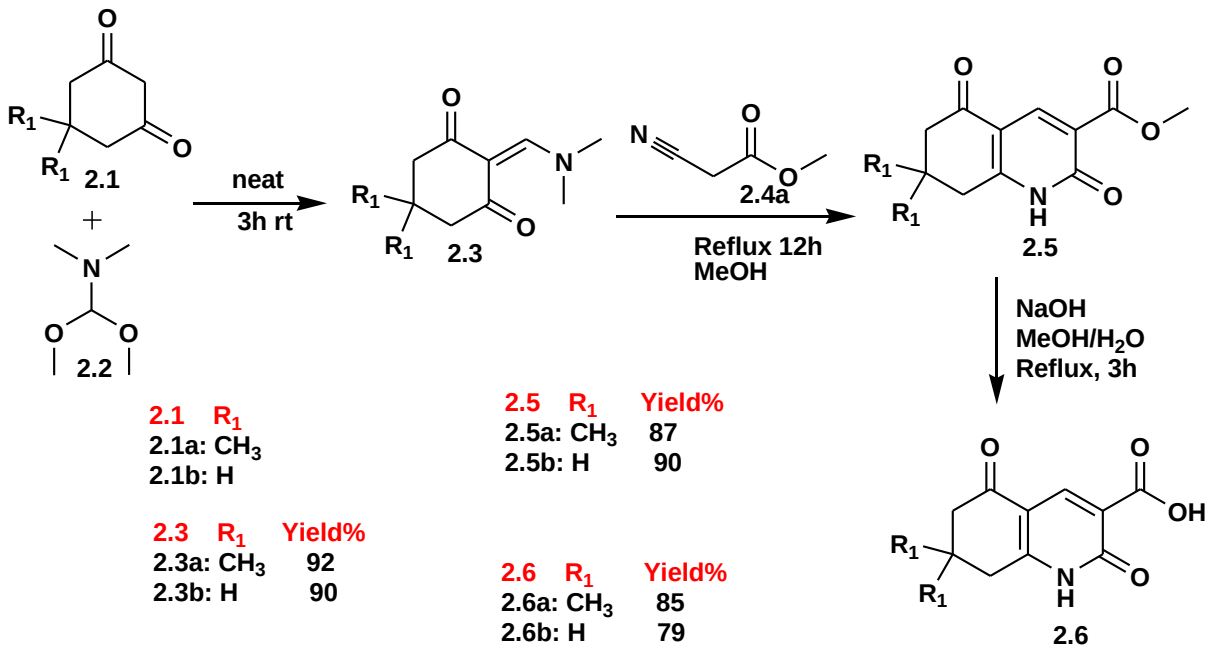

**Scheme 2.1**: Retrosynthesis of target molecule *N*-(4-chlorophenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide **2.8**.

The retrosynthesis of target compound **2.8** shows that it can be directly obtained in four steps as shown in Scheme 2.1. The disconnection of compound **2.8** can lead to commercially available 4-chloroaniline **2.7** and compound **2.6**. Functional group interconversion of **2.6** leads to **2.5**. The disconnection of bonds on compound **2.5** may lead to commercially available methyl cyanoacetate **2.4** and compound **2.3**. Disconnection of the double bond on the chemical structure **2.3** can lead to a commercially available *N*,*N*-dimethylformamide dimethyl acetal **2.2** and 4,4-dimethyl-1,3-cyclohexanedione **2.1**.

The stepwise synthesis reactions: the reaction of *N*,*N*-dimethylformamide dimethyl acetal **2.2** and 4,4-dimethyl-1,3-cyclohexanedione **2.1** will give rise to compound **2.3**. The reaction of compound **2.3** and methyl cyanoacetate **2.4** give rise to compound **2.5**. Hydrolysis of the ester group of compound **2.5** will give carboxylic acid **2.6**. The reaction of 4-chloroaniline **2.7** and compound **2.6** forms an amide bond to afford **2.8**.

## 2.4. Stepwise preparation of 2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid intermediates (2.6a-b)

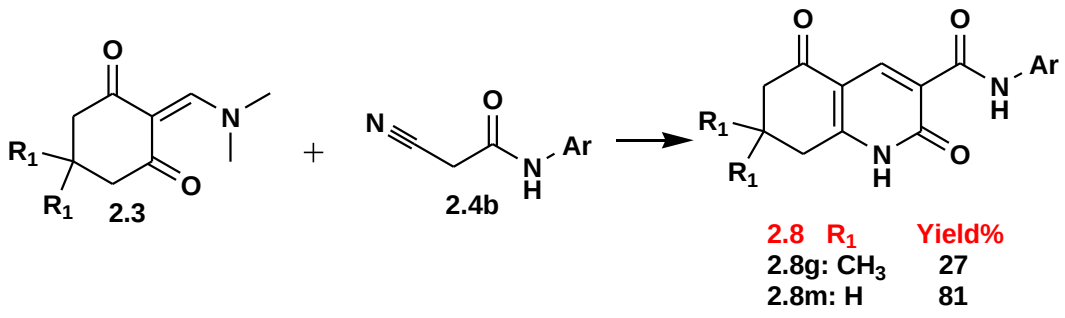
After planning the synthetic pathway, the preparation of 2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid derivatives was pursued as shown in Scheme 2.2, based on accessibility of chemical reagents. The preparation of intermediates 2-((dimethylamino)methylene)-5,5-dimethylcyclohexane-1,3-dione derivatives (**2.3a-b**) has been reported by Schenone and co-workers[178] in good yields at 0 ºC and under reflux using ether. Al-Mousawi and co-workers[179] reported preparation of 2-((dimethylamino)methylene)-5,5-dimethylcyclohexane-1,3-dione derivatives **2.3a-b** under reflux using xylene, which resulted in average yields. We adopted the method of Dzhavakhishvili and co-workers[175] by a neat reaction of 1,3-cyclohexanedione derivatives **2.1a-b** and *N*,*N*-dimethylformamide dimethyl acetal **2.2** that gave rise to 2-((dimethylamino)methylene)-5,5-dimethylcyclohexane-1,3-dione derivatives (**2.3a-b**) after three hours at room temperature. The reaction of methyl cyanoacetate **2.4a** and **2.3a-b** under reflux using methanol as solvent gave rise to cyclised methyl 7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate derivatives **2.5a-b** in good yields after twelve hours as previously reported by Fossa and co-worker.[174] Methyl 7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate derivatives **2.5a-b** were hydrolysed under reflux using methanol and water as co-solvents (1:1) in the presence of sodium hydroxide pellets for three hours. After cooling, 1M hydrochloric acid was introduced dropwise to obtain precipitated 2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid derivatives **2.6a-b** in good yields as shown in Scheme 2.2.




**Scheme 2.2**: Multistep synthesis of 2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acids **2.6a-b**.

## 2.5. Synthesis of 7,7-dimethyl-2,5-dioxo-*N*-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide derivatives 2.8a-m and 2.10a-d

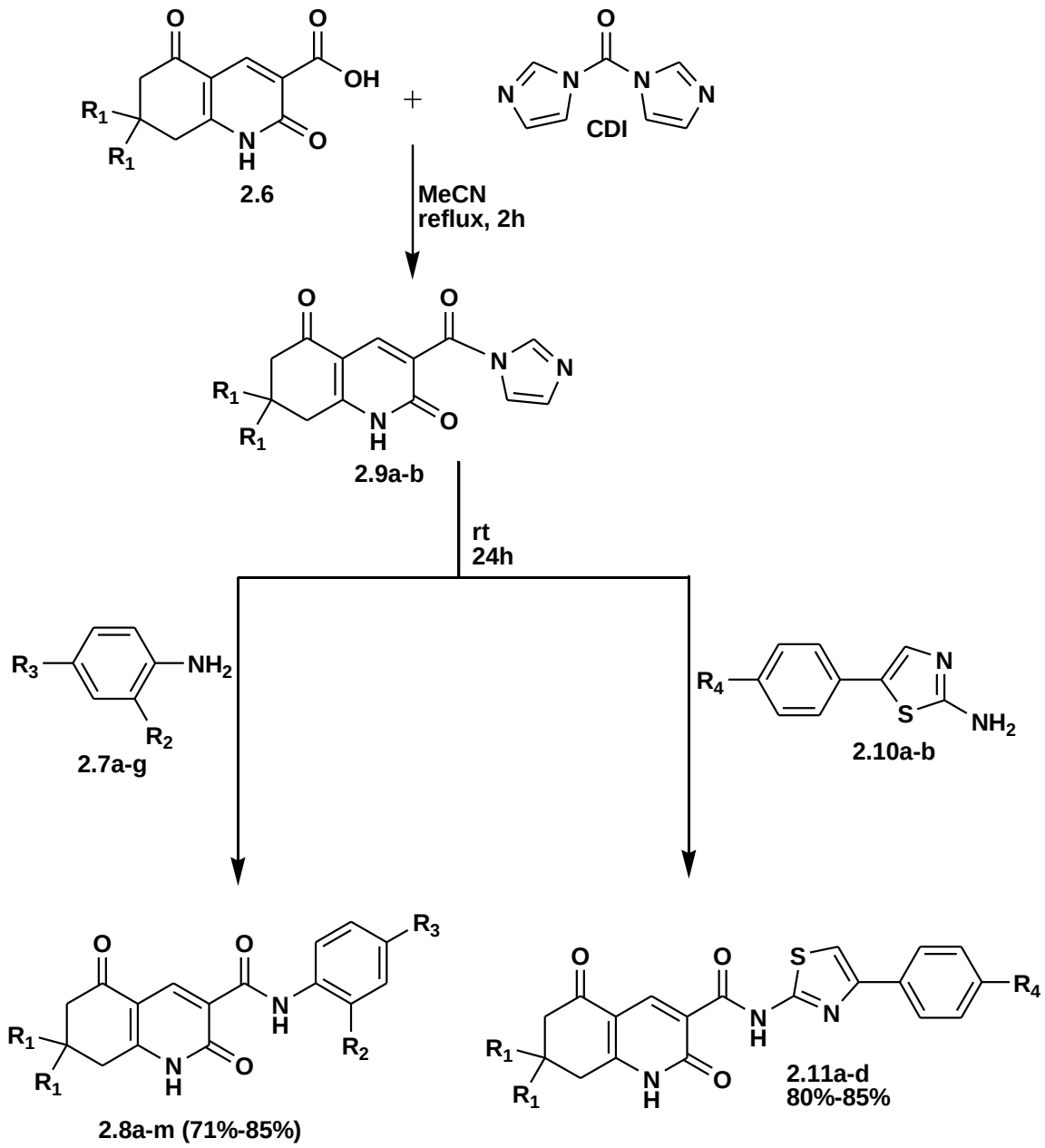
Gorobets and co-workers[176] reported a multistep reaction for the synthesis of **2.8g** and **2.8m** by preparing *N*1-substituted 2-cyanoacetamides **2.4b** in order to react with 2-((dimethylamino) methylene)-cyclohexane-1,3-dione derivatives **2.3** to form the products **2.8g** (27% yield) and **2.8m** (81% yield) (Scheme 2.3).




**Scheme 2.3:** Synthesis of **2.8g** and **2.8m** previously reported by Gorobets.

Our synthesis of the 7,7-dimethyl-2,5-dioxo-N-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide derivatives **2.8a-m** and **2.11a-d** was pursued according to the retrosynthetic

sequence shown in Scheme 2.1. The reactions were carried out by treating 2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid derivatives **2.6a-b** with CDI under reflux for two hours in acetonitrile as solvent. After cooling to room temperature, an appropriate aniline derivative (**2.7**) was introduced to the reaction for 24 hours to give rise to 7,7-dimethyl-2,5-dioxo-*N*-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxamides **2.8a-m**. The success of these reactions led us to the use of 4-phenylthiazol-2-amines derivatives **2.10** which gave rise to **2.11a-d**. The reaction of di-substituted halide anilines such as **2.7h**, **2.7i**, and **2.7j** with **2.6a-b** were not successful and resulted in recovery of starting material. The reactions of anilines **2.7k** and **2.6a-b** were not successful either.




| 2.8: | $R_1$ | $R_2$ | $R_3$ | Yied % |
|---|---|---|---|---|
| 2.8a: | $CH_3$ | H | Cl | 82 |
| 2.8b: | $CH_3$ | H | Br | 72 |
| 2.8c: | $CH_3$ | H | $CH_3$ | 76 |
| 2.8d: | $CH_3$ | $CH_3$ | $CH_3$ | 78 |
| 2.8e: | $CH_3$ | OH | H | 80 |
| 2.8f: | $CH_3$ | $CH_3O$ | $CH_3O$ | 82 |
| 2.8g: | $CH_3$ | H | H | 79 |
| 2.8h: | H | H | Cl | 80 |
| 2.8i: | H | H | Br | 86 |
| 2.8j: | H | H | $CH_3$ | 71 |
| 2.8k: | H | OH | H | 76 |
| 2.8l: | H | $CH_3O$ | $CH_3O$ | 83 |
| 2.8m: | H | H | H | 81 |

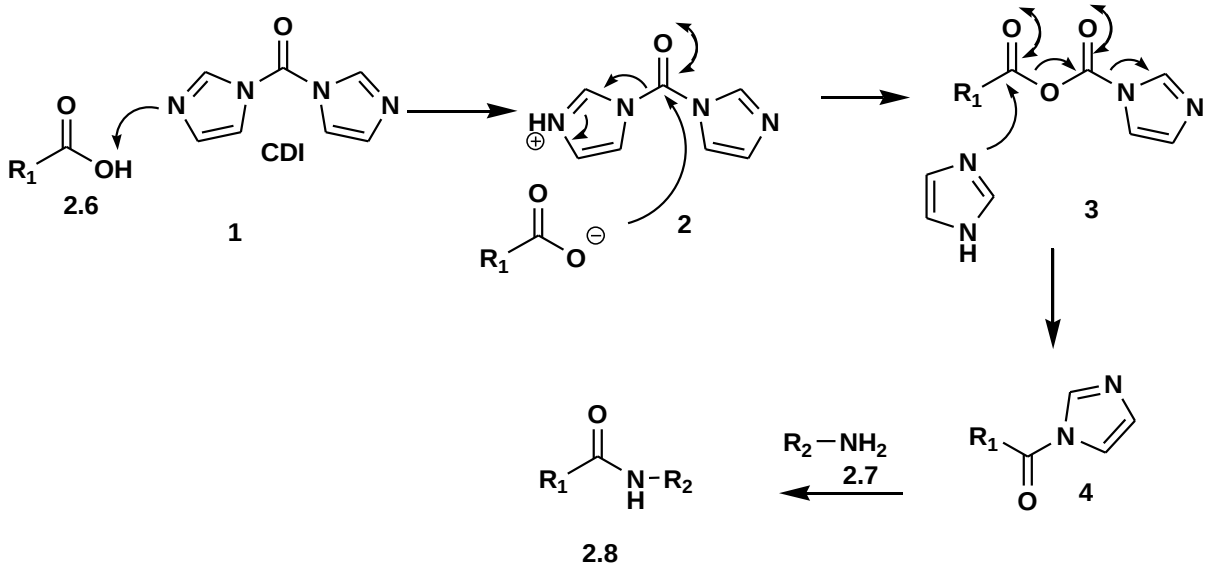
| 2.7: | $R_2$ | $R_3$ |
|---|---|---|
| 2.7a: | H | Cl |
| 2.7b: | H | Br |
| 2.7d: | $CH_3$ | $CH_3$ |
| 2.7e: | OH | H |
| 2.7f: | $OCH_3$ | $OCH_3$ |
| 2.7g: | H | H |
| 2.7h | Br | Br |
| 2.7i | Cl | Cl |
| 2.7j | F | F |
| 2.7k | $NO_2$ | H |

| 2.10: | $R_4$ |
|---|---|
| 2.10a: | F |
| 2.10b: | $OCH_3$ |

| 2.11: | $R_1$ | $R_4$ | Yield |
|---|---|---|---|
| 2.11a: | $CH_3$ | F | 80 |
| 2.11b: | $CH_3$ | $OCH_3$ | 82 |
| 2.11c: | H | F | 83 |
| 2.11d: | H | $OCH_3$ | 85 |

**Scheme 2.4:** Synthesis of **2.8a-m** and **2.11a-d**

The reaction mechanism for the formation of compounds **2.8** and **2.11** proceeds by CDI mediation (see Paul and Anderson[160]) as shown in Scheme 2.5. The initial setup involves the carboxylic acid **2.6** being deprotonated by CDI to form two intermediates at event **2** which react to form imidazole and an acyl carboxy imidazole intermediate at event **3** leading to the activated acylimidazole intermediate at event **4**.The aniline derivative **2.7** is then introduced to the acylimidazole to afford the products **2.8**.




**Scheme 2.5:** The reaction mechanism of amide bond formation using CDI

*2.5.1 The effect of aniline substituents and 4-phenylthiazol-2-amine derivatives on synthesis*

Compounds **2.8** and **2.11** were isolated in fairly good yields, with some variation based on the nature of the substituent groups of the aniline **2.7** and 4-phenylthiazol-2-amine **2.10**derivatives as shown in Scheme 2.4. The highest yields for this set of compounds was obtained from **2.8i** giving 86% with a *para*-bromine substituent on the aniline. The lowest yield was from **2.8j** giving 71% with a *para*-methyl substituent on the aniline. The use of CDI as a mediator resulted in good yields while using anilines **2.7a-g** and 4-phenylthiazol-2-amines derivatives **2.10a-b** as primary amine sources. When di-halo substituted anilines (2,4-dibromoaniline, 2,4-difluoroaniline **2.7h** , 2,4-dichloroaniline **2.7i** and 2-nitro aniline (**2.7j**)) were treated with CDI and compound **2.6** the reactions were not successful. When 4-phenylthiazol-2-amines derivatives **2.10a-b** were treated with CDI compound **2.6** the reactions as described in Scheme 6.3, good yields of **2.11-a-d** were obtained (80-85%). Anilines with the strongly electron-

withdrawing nitro group or two electron-withdrawing halo-groups seem insufficiently nucleophilic to participate in the reaction.

## 2.6. The structural elucidation of compounds 2.8a-m and 2.11a-d using FTIR, NMR and HRMS

The obtained structures **2.8a-m** and **2.11a-d** were analysed by FTIR, NMR and HRMS. By way of example we describe the characterization data for compound **2.8a** shown in Figure 2.10.

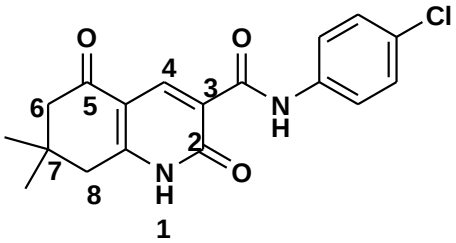



**Figure 2.10.** Structure of compound **2.8a**

The $^{1}$H NMR spectrum showed a broad singlet for two NH groups at δ = 13.15 and δ = 11.70, one singlet from a CH group at 8.71 for the proton at position **4**, two doublets from the aromatic ring at δ = 7.74 (*J* = 8.4 Hz) and δ = 7.42 (*J* = 8 Hz), two singlets for the $CH_2$ groups at positions **6** and **8** at δ = 2.85 and δ = 2.44 and one singlet corresponding to two $CH_3$ groups at δ = 1.04. The $^{13}$C NMR spectrum of **2.8a** showed 14 distinct resonances due to two methyl groups overlapping with each other and one $CH_2$ group overlapping with the DMSO-$d_6$ residual peak (see DEPT 135 in Appendix). The mass spectrum of **2.8a** showed the protonated molecular ion $[M+H]^+$ peak at m/z 345.1012 which is consistent with the mass of the proposed product for which the calculated value is 345.1000. The FTIR spectrum displayed characteristic absorption bands for carbonyl groups at 1679 $cm^{-1}$, 1621 $cm^{-1}$ and 1598 $cm^{-1}$and for the amine group at 2971 $cm^{-1}$ and 2864 $cm^{-1}$.

For the groups of compounds **2.8** and **2.11**, FTIR spectra showed characteristic absorption bands for N-H in the range of 3266 $cm^{-1}$ to 2860 $cm^{-1}$, while the C=O stretching band appeared between 1770 $cm^{-1}$ and 1570 $cm^{-1}$. The $^{1}$H NMR spectra exhibited two singlet signals in the region of δ 13.33- 11.48 ppm corresponding to the NH and one singlet at δ 8.76-8.71 ppm corresponding to CH. $^{13}$C NMR spectra showed characteristic signals in the region of δ 193.4 159.9 ppm corresponding to three C=O groups. ESI-LCMS was used to confirm the masses of

**2.8a-m** and **2.11a-d.** Chromatograms exhibited the protonated parent molecular ion $[M+H]^+$ represented in Table 2.2.

**Table 2.2**: The chemical formulae, calculated and measured molecular ion masses of **2.8a-m** and **2.11a-d**

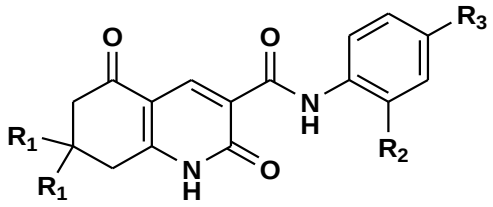
| Compound | Chemical Formula $[M+H]^+$ | Calculated m/z | Measured m/z |
|---|---|---|---|
| **2.8a** | $C_{18}H_{18}ClN_2O_3^+$ | 345.1000 | 345.1012 |
| **2.8b** | $C_{18}H_{18}BrN_2O_3^+$ | 391.0495 | 391.0481 |
| **2.8c** | $C_{19}H_{21}N_2O_3^+$ | 325.1547 | 325.1578 |
| **2.8d** | $C_{20}H_{23}N_2O_3^+$ | 339.1703 | 339.1711 |
| **2.8e** | $C_{18}H_{19}N_2O_4^+$ | 327.1339 | 327.1335 |
| **2.8f** | $C_{20}H_{23}N_2O_5^+$ | 371.1600 | 371.1604 |
| **2.8g** | $C_{18}H_{19}N_2O_3^+$ | 311.1390 | 311.1419 |
| **2.8h** | $C_{16}H_{14}ClN_2O_3^+$ | 317.0687 | 317.0688 |
| **2.8i** | $C_{16}H_{14}BrN_2O_3^+$ | 363.0182 | 363.0164 |
| **2.8j** | $C_{17}H_{17}N_2O_3^+$ | 297.1234 | 297.1240 |
| **2.8k** | $C_{16}H_{15}N_2O_4^+$ | 299.1026 | 299.1050 |
| **2.8l** | $C_{18}H_{19}N_2O_5^+$ | 343.1288 | 343.1275 |
| **2.8m** | $C_{16}H_{15}N_2O_3^+$ | 283.1077 | 283.1113 |
| **2.11a** | $C_{21}H_{19}FN_3O_3S^+$ | 412.1126 | 412.1144 |
| **2.11b** | $C_{22}H_{22}N_3O_4S^+$ | 424.1326 | 424.1345 |
| **2.11c** | $C_{19}H_{15}FN_3O_3S^+$ | 384.0813 | 384.0825 |
| **2.11d** | $C_{20}H_{18}N_3O_4S^+$ | 396.1013 | 396.1023 |

A total of seventeen (17) 7,7-dimethyl-2,5-dioxo-*N*-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide derivatives **2.8a-m** and *N*-(4-(4-fluorophenyl)thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide derivatives **2.11a-d** were synthesised and to the best of our knowledge only **2.8g** and **2.8m** have been previously reported using a different synthetic method.[176]

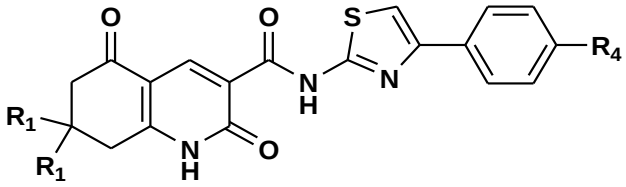
## 2.7. Biological evaluations of library of hexahydroquinoline-3-carboxamide 2.8 and 2.11 derivatives

A set of seventeen synthesised hexahydroquinoline-3-carboxamide compounds **2.8** and **2.11** were tested using the Alphascreen assay for determination of percentage inhibition between the interactions of HIV-1-IN and LEDGF/p75 proteins. In addition multimerization, cytotoxicity and antiviral activities were also measured. The experiments were carried out at a final concentration of 100 µM of test compound and were done in triplicate with average results shown in Table 2.3. CX05168 and Mut101 were used as controls for IN-LEDGF/p75 inhibition and multimerization. Auranofin was used as a control for cytotoxicity studies and Raltegravir was used as a control for antiviral activity.

**Table 2.3**: LEDGF/p75-HIV-1-IN inhibition, HIV-1-IN multimerization, cytotoxicity and antiviral activities of hexahydroquinoline-3-carboxamides **2.8** and **2.11**.




**2.8a-m**




**2.11a-d**

| Compound Codes | % Inhibition His-LEDGF-IN-*Tag* | | | % Multimerization IN-IN | | Cytotoxicity MT4 | %Antiviral activity (infected MT4) | |
|---|---|---|---|---|---|---|---|---|
| # | Sub B *His* | Sub B *Gst* | Sub C *Gst* | Sub B | Sub C | | Sub B | Sub C |
| **2.8a** | 8.86 | -8.03 | -0.22 | 4.12 | -1.63 | 11.6 | Non | Non |
| **2.8b** | 30.46 | -7.32 | -6.30 | 5.09 | 1.35 | 24.98 | 23.94 | 22.36 |
| **2.8c** | 31.05 | 1.15 | 0.20 | 5.70 | 1.02 | Non toxic | 36.02 | 32.06 |
| **2.8d** | 19.20 | 6.70 | -6.20 | 2.17 | 0.68 | 5.60 | 35.98 | 30.26 |

| Compound Codes | % Inhibition His-LEDGF-IN-*Tag* | | | % Multimerization IN-IN | | Cytotoxicity MT4 | %Antiviral activity (infected MT4) | |
|---|---|---|---|---|---|---|---|---|
| **2.8e** | 52.32 | 5.25 | -2.5 | -0.89 | -0.08 | 2.65 | 35.62 | 29.56 |
| **2.8f** | 4.34 | 3.17 | -0.50 | -5.03 | -1.72 | 7.45 | 20.19 | 18.26 |
| **2.8g** | 2.09 | 12.56 | -3.33 | 0.23 | -1.12 | 2.44 | 24.59 | 20.36 |
| **2.8h** | 35.70 | -9.28 | 1.08 | -1.80 | -1.29 | Non toxic | Non | Non |
| **2.8i** | -10.35 | -2.10 | 2.98 | -1.60 | -1.01 | Non toxic | 27.67 | 20.36 |
| **2.8j** | 36.63 | 4.70 | -9.45 | 3.39 | 1.25 | Non toxic | 28.79 | 22.68 |
| **2.8k** | 53.85 | 5.90 | -17.5 | 5.4 | 0.09 | Non toxic | 20.99 | 17.56 |
| **2.8l** | 11.19 | 0.50 | 0.67 | 3.07 | 0.30 | Non toxic | 32.17 | 10.69 |
| **2.8m** | -4.88 | 3.45 | -3.43 | -0.43 | 0.28 | Non toxic | 38.09 | 34.59 |
| **2.11a** | 11.90 | 3.45 | -2.77 | -13.53 | -4.88 | Non toxic | 33.50 | 26.31 |
| **2.11b** | -0.65 | 8.00 | 0.94 | -3.90 | -1.78 | Non toxic | 29.79 | 15.69 |
| **2.11c** | 16.04 | 5.57 | 5.69 | 19.75 | 4.16 | Non toxic | 34.81 | 22.18 |
| **2.11d** | -5.03 | -19.9 | 5.69 | 0.20 | 1.12 | Non toxic | 22.19 | 12.22 |
| **CX05168** | 44.74 | 67.85 | 68.16 | 125.30 | 100.30 | | | |
| **Mut101** | 95.81 | 97.66 | 96.77 | 100.00 | 100.00 | | | |
| **Auranofin** | | | | | | 100.00 | | |
| **Raltegravir** | | | | | | | 100.00 | 100.00 |

From the initial seventeen hexahydroquinoline-3-carboxamide (**2.8** and **2.11)** compounds screened for IN-LEDGF/p75 inhibition, only two compounds showed more than 50% inhibition at a dose concentration of 100 µM (Table 2.3). The best inhibitor for this set of compounds with 53% inhibition was **2.8k**, which contains hydrogen at $R_1$ and a hydroxyl group at $R_2$. The replacement of hydrogen at $R_1$ with a methyl group corresponds to compound **2.8e** with the second best inhibition of 52%. Four compounds showed between 30% and 50% inhibition at a dose concentration of 100 µM. Compound **2.8j** and **2.8h** showed inhibition of 37% and 36%, respectively, and they each have hydrogen at $R_1$. Compound **2.8j** has a methyl group at $R_2$, while **2.8h** has chlorine at $R_2$. Compounds **2.8c** and **2.8b** showed inhibition of 31% and they contain a methyl group at $R_1$. Compound **2.8c** also has a methyl group at $R_2$, while **2.8b** contains bromine at $R_2$. These data suggest that the hydroxyl group from **2.8k** and **2.8e** at the $R_2$ position might play a significant role in the binding ability within the HIV-1-IN dimer. The virtual screening results of **2.8k** and **2.8e** shows the 2-dimensional mode of interaction of the hydroxyl group with Gnl168 and Gnl95, respectively, as shown in Figure 2.10 and Figure 2.11. Interestingly, these compounds are interacting with different residues from **2.8a**, CX05168 and Mut101 which interact with the HIV-1-IN through Glu170, His171 and Thr174.

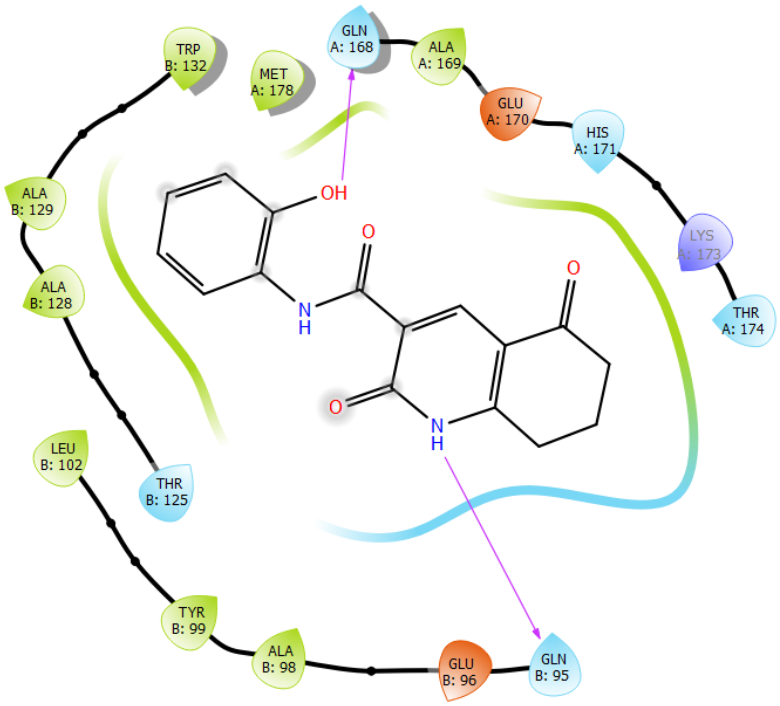



**Figure: 2.10:** 2-Dimensional binding mode of **2.8k** in the LEDGF/p75 binding site of HIV-1 IN

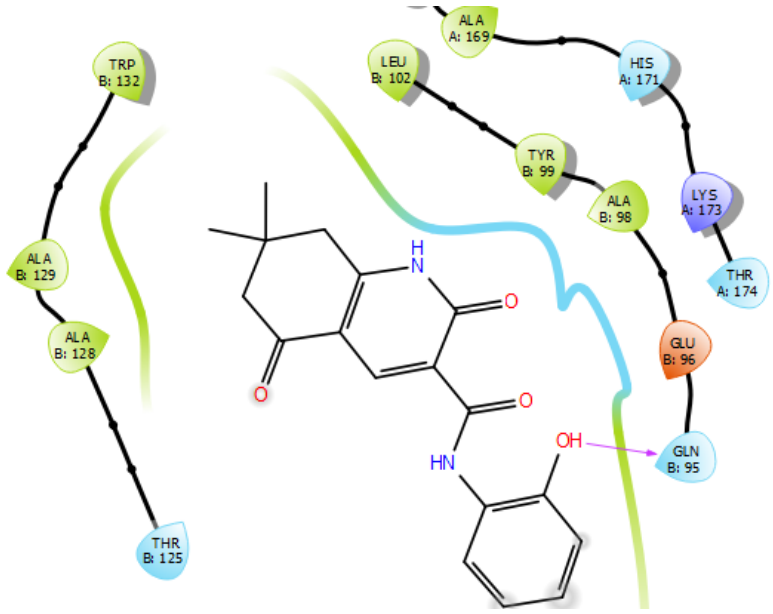



**Figure: 2.11:** 2-Dimensional binding mode of **2.8e** in the LEDGF/p75 binding site of HIV-1 IN

The hexahydroquinoline-3-carboxamide compounds **2.8** and **2.11** were also put through an Alphascreen dimerization assay for HIV-1 IN Subtype B and Subtype C to determine if they are capable of IN multimerization. Briefly, protein multimerization refers to the assembly of the IN quaternary protein structure consisting of multimeric proteins from their composite IN protein subunits. The tests were done in duplicate at 100 µM of test compound and results are recorded in Table 2.4. The evaluation results show that this set of compounds did not significantly induce multimerization in the HIV-1 IN Alpha Screen assay. Compound **2.11c** gave the highest multimerization of only 19%. This might suggest possible protein shearing and breaking of the integrase- integrase interaction. The hexahydroquinoline-3-carboxamide compounds **2.8** and **2.11** were subjected to toxicity evaluation in the MT4 cell line at 100 µM. Compounds **2.8c**, **2.8h**, **2.8i**, **2.8j**, **2.8k**, **2.8l**, **2.8m** and **2.11a-2.11d** were found to be non-toxic as recorded in Table 2.4. Compounds **2.8a**, **2.8b**, **2.8d-2.8g** were found to be somewhat toxic. Finally the hexahydroquinoline-3-carboxamide compounds **2.8** and **2.11** were evaluated for their antiviral activities in infected MT4 cells (Subtype B and Subtype C assays). The tests were carried out in a cell-based assay and each compound was analysed at a single dose of 100 µM. Compounds **2.8a** and **2.8h** were found to possess no antiviral activity. Non-toxic compounds **2.8c**, **2.8i**, **2.8j**, **2.8k**, **2.8l**, **2.8m** and **2.11a-2.11d** showed antiviral activity of between 20 and 38% against subtype B. The activity percentage of **2.8b** and **2.8d-6.8g** were considered to be partially or fully attributed to the toxicity of the compounds, for example **2.8b**

was found to be 24% toxic and showed 23% antiviral activity. The highest antiviral activity of 38% was seen for **2.8h** in subtype B and 34% antiviral activity in subtype C. This compound **2.8h** was found to be non-toxic. This compound has hydrogen at $R_1$, $R_2$ and $R_3$. Compound **2.8c** which contains a methyl group at $R_1$ and $R_3$ shows 36% antiviral activity in subtype B and 32% in subtype B. Compound **2.8e** which showed 52% IN-LEDGF/p75 inhibition was also found to have 35% antiviral activity in subtype B and 29% in subtype C. The antiviral activity of non-toxic compound **2.8k** with 53% IN-LEDGF/p75 inhibition was found to be 20% antiviral activity in subtype B and 17% in subtype C. This set of compounds was also evaluated for antimicrobial activities against two Gram-positive bacterial species (*Staphylococcus aureus* and *Bacillus cereu*s) and Gram negative bacterial species (*Escherichia coli* and *Pseudomonas aeruginosa)* using the Minimum Inhibitory Concentration (MIC) assay determined by the iodonitrotetrazolium (INT) indicator. Unfortunately, none of the compounds showed any significant activity against the bacterial strains.

In order to remove the possibility of false positives from the hexahydroquinoline-3-carboxamide compounds (**2.8** and **2.11**), the synthesised compounds were subjected to false positive remover PAINS (pan assay interference compounds) and they passed the filter.[180]

## 2.8. Conclusion

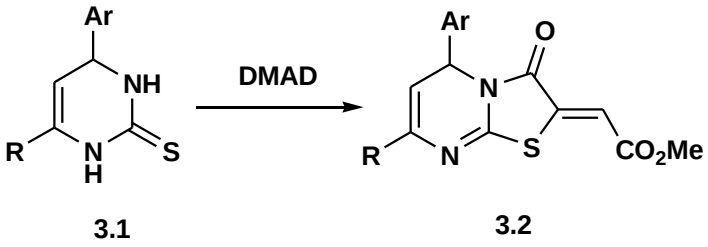
A series of seventeen hexahydroquinoline derivatives (**2.8** and **2.11**) were prepared using CDI as a mediator from **2.6a-b** and aniline derivatives **2.7a-g** in good yields of 71%-85%, a big improvement compared to the method used by Gorobets and co-workers[176] (27% for **2.12g**). The di-substituted halide anilines **6.7h-j** and 2-nitroaniline **6.7k** reactions under similar conditions were not successful. To the best of our knowledge fifteen (15) of the synthesised compounds are novel, while two compounds **2.8g** and **2.8m** have been previously reported using a different method. The initial virtual screening molecular modeling docking revealed ChemBridge compound **2.8a** and the control compounds CX05168 and Mut101 interacting with HIV-1-IN through Glu170, His171 and Thr174. Based on the biological results compounds **2.8e** and **2.8k** gave more than 50% HIV-1-LEDGF/p75 inhibition. The virtual screening of **2.8e** and **2.8k** with HIV-IN dimer shows the hydroxyl group from the compounds interacting with the Gnl168 and Gnl95. Multimerization results showed no significant activity, while most of the compounds were found to be non-toxic.

# CHAPTER 3

# Synthesis of fused pyrimidine derivatives *via* 3-CR using zwitterion adduct as intermediate

## 3.1 Introduction

This chapter is concerned with the reactivity of the zwitterion adduct generated by reaction of an isocyanide and DMAD with substrates containing both an acidic proton and internal nucleophiles for the preparation of a number of different 6-5 fused ring systems. The first such system we aimed to prepare was a series of thiazolo[3,2-*a*]pyrimidines. One of the known approaches to synthesise these compounds proceeds by the reaction of 3,4-dihydropyrimidine-2(1*H*)-thione **3.1** and DMAD to give pyrimidine derivative such as **3.2**. Generally a five-membered ring is constructed onto the existing 6-membered ring (Scheme 3.1).[181,182,183,184]



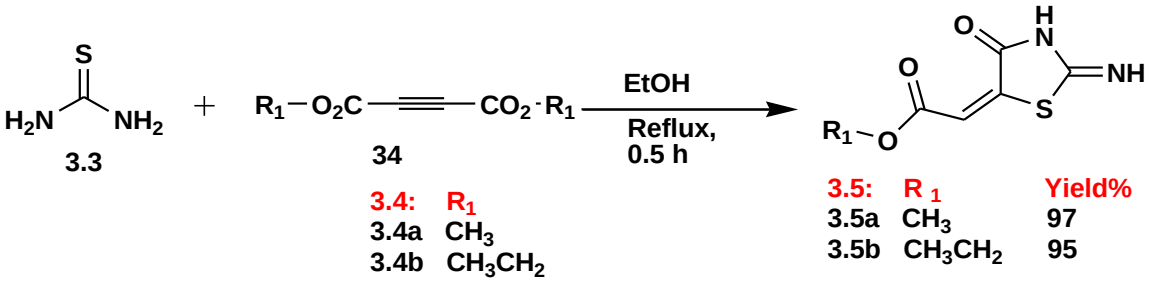

**3.1** **3.2**

**Scheme 3.1**: Synthesis of thiazolo[3,2-*a*]pyrimidines.

Our approach to these compounds involves the preparation of substrates containing both an acidic proton and internal nucleophile in order to construct the 6-membered ring onto the prepared 5-membered ring substrate. Our aim was to use vinylogous ester derivatives prepared from thiourea and other possible prepared substrate to increase the substrate scope of in this synthetic approach.

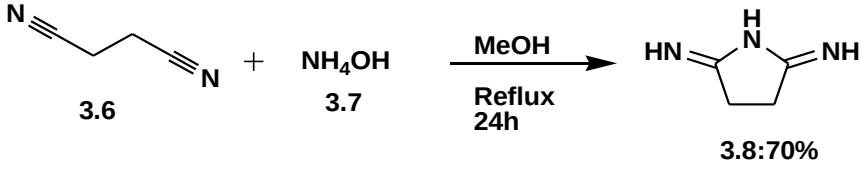
### 3.2. Preparation of 5-membered ring intermediates

Thiourea **3.3** and acetylene dicarboxylate **3.4** were used to prepare compounds **3.5a-b** by means of a modified procedure reported by Hendrickson and co-workers[107] using methanol as solvent (Scheme 3.2). They obtained **3.5a** in 91% yield while Choudhary and Peddinti[108] prepared **3.5a-b** in water to achieve 100% yield. When using a water and ethyl acetate solvent mixture (40%:60%) they got **3.5a** in 95% and **3.5b** in 94% yield. Using ethanol under reflux we obtained **3.5a** in 97% yield and **3.5b** in 95%. The reactions were carried out by mixing ethanol and thiourea **3.3** under reflux conditions for 30 min and dialkyl acetylenedicarboxylate **3.4** was added through the condenser to obtain the products **3.5a-b** in excellent yield.



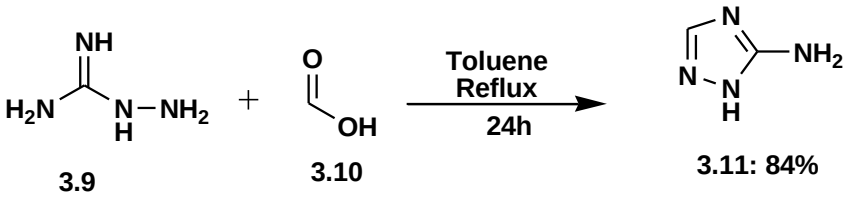

**Scheme 3.2:** Synthesis of thiazole-based substrates **3.5a-b**.

The third substrate was prepared according to the method reported by Elvidge and Linstead,[185] by treating 1,4-dinitrile **3.6** with excess ammonium hydroxide **3.7** in dry methanol under reflux conditions to afford pyrrolidine-2,5-diimine **3.8** as shown in Scheme 3.3. The solid product was obtained with 70% yield, lower than the 85% yield reported by Elvidge and Linstead.[185]



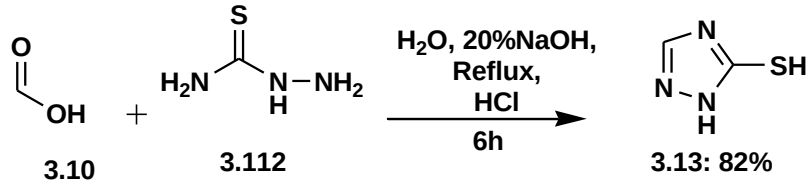

**Scheme 3.3:** Synthesis of pyrrolidine-2,5-diimine **3.8**

The fourth substrate was prepared as shown in Scheme 3.4 by heating a solution of aminoguanidine bicarbonate **3.9** and formic acid **3.10** using toluene as solvent for 24 h to afford 1*H*-1,2,4-triazol-5-amine[186] **3.11** as described by Boechat and co-workers[186] Our yield of 84% was lower than the 93% reported by Boechat and co-workers.[186]



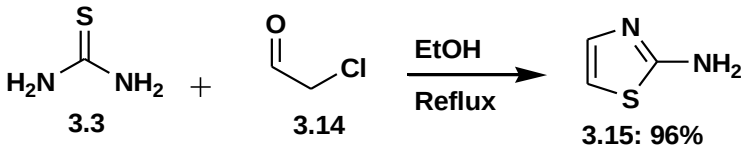

**Scheme 3.4:** Synthesis of 1*H*-1,2,4-triazol-5-amine **3.11**

The fifth substrate was prepared from a solution of formic acid **3.10** and thiosemicarbazide **3.12** with boiling water followed by addition of 20% NaOH solution. The reaction was refluxed for 2 h then neutralised using concentrated HCl to obtain 82% yield of product **3.13** (Scheme 3.5). This compares favourably to the 71% reported by Hu and co-workers.[187]



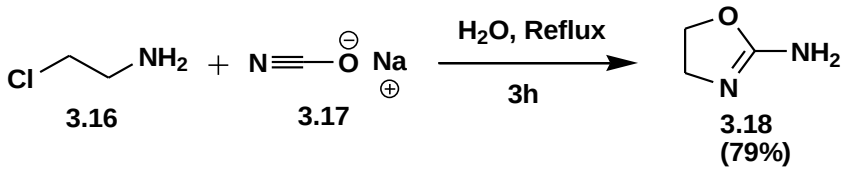

**Scheme 3.5:** Synthesis of 1*H*-1,2,4-triazole-5-thiol **3.13**

The reaction of thiourea **3.3** and α-halo-aldehyde **3.14** in absolute ethanol under reflux conditions gave rise to thiazol-2-amine **3.15** as shown in Scheme 3.6. Compound **3.15** was prepared by Zarnegar and co-workers[188] obtaining yield of 98% from methylcarbonyl activated by HBr or HI, while using ethanol and α-halo-aldehyde we obtained 96% yield.




**Scheme 3.6:** Synthesis of thiazol-2-amine **3.15**

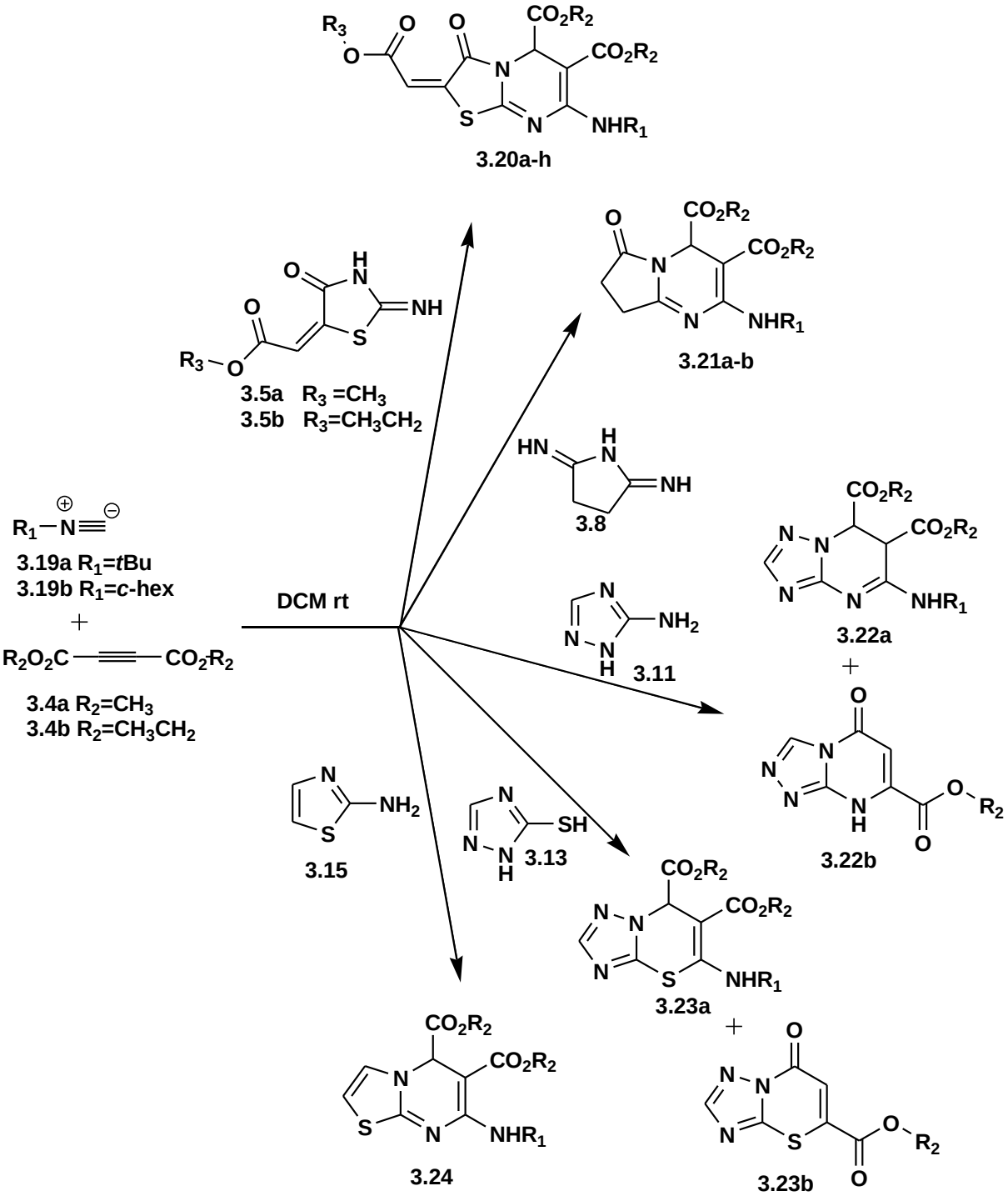
The reaction of 2-chloroethylamine **3.16** and sodium cyanate **3.17** gave rise to 79% yield of 2-imino-1,3-oxazolidine **3.18**[189] (Scheme 3.7).




**Scheme 3.7.** Synthesis of 2-imino-1,3-oxazolidine **3.18**.

### 3.3 Synthesis of pyrimidine derivatives and their analogues using an isocyanide-based 3-CR.

The synthesis of highly functionalised pyrimidines and their analogues were successfully achieved using two different methods: Method A and Method B. Our initial procedure (Method A) involved the addition of DMAD **3.4a** to the 5-membered ring compound **3.5a** in dry $CH_2Cl_2$ at room temperature (rt) followed by slow addition of isocyanide derivative **3.19** in dichloromethane. This protocol resulted in moderate yields (Table 3.1). The reactions were carried by out by treating ((*E*)-methyl 2-(2-amino-4-oxothiazol-5(4*H*)-ylidene)acetate **3.5a** or ((*E*)-ethyl 2-(2-amino-4-oxothiazol-5(4*H*)-ylidene)acetate **3.5b** with DMAD **3.4a** or diethylacetylene dicarboxylate (DEtAD) and *tert*-butyl isocyanide **3.19a** which gave rise to compounds **3.20a-d** (Scheme 3.8). When this reactions were repeated with cyclohexyl isocyanide **3.19b** gave rise to **3.20e-h**, as expected (Table 3.1). In order to improve the moderate yields obtained using Method A, we applied differend order of compounds addition and the reaction temperature. Isocyanide was added to DMAD under inert conditions at 0 ºC and the reaction continued for 0.5 h followed by the addition of 5-membered ring compound **3.5a** to afford the products in improved yield (Method B) as recorded in Table 3.1.




**Scheme 3.8:** Synthesis of highly functionalised pyrimidines *via* one pot 3-CR.

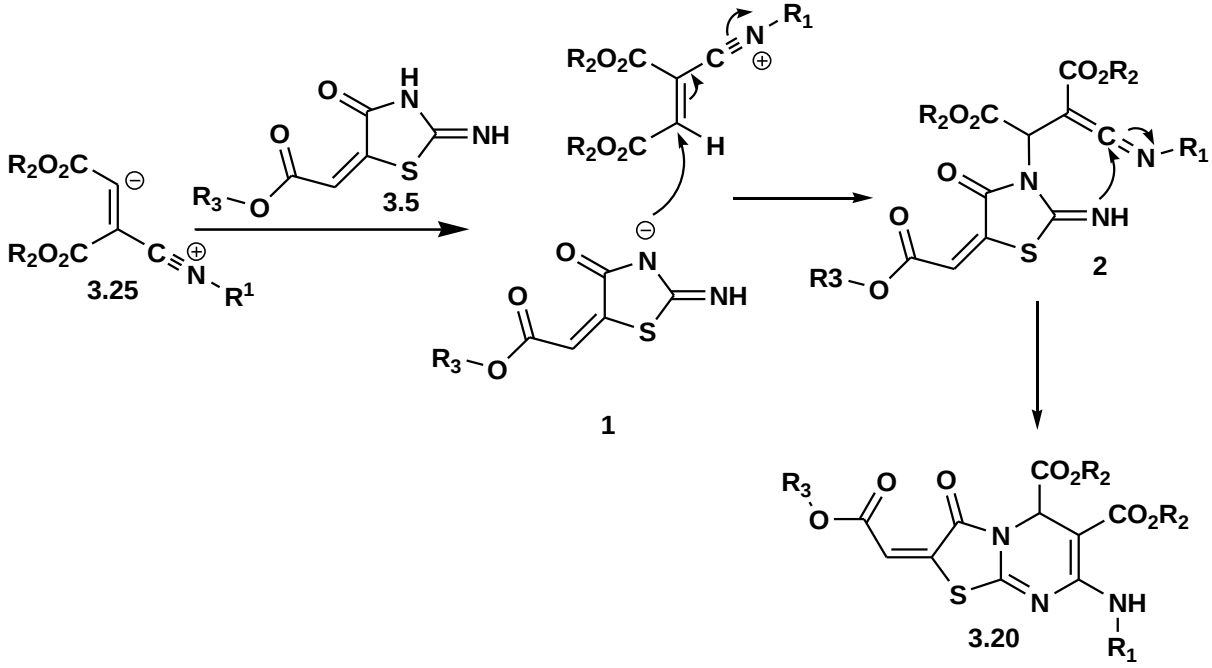
**Table 3.1**: The isolated yields of products **3.20a-h**, **3.21a-b**, **3.22a-b**, **3.23a-b** and **3.24**

| Compound | $R_1$ | $R_2$ | $R_3$ or X | Method A Yield (%) | Method B Yield (%) |
|---|---|---|---|---|---|
| **3.20a** | *t*-Bu | $CH_3$ | $CH_3$ | 68 | 82 |
| **3.20b** | *t*-Bu | $CH_2CH_3$ | $CH_3$ | 66 | 80 |
| **3.20c** | *t*-Bu | $CH_3$ | $CH_2CH_3$ | 69 | 87 |
| **3.20d** | *t*-Bu | $CH_2CH_3$ | $CH_2CH_3$ | 71 | 86 |
| **3.20e** | Cyclohexyl | $CH_3$ | $CH_3$ | 61 | 79 |
| **3.20f** | Cyclohexyl | $CH_2CH_3$ | $CH_3$ | 55 | 79 |
| **3.20g** | Cyclohexyl | $CH_3$ | $CH_2CH_3$ | 67 | 84 |
| **3.20h** | Cyclohexyl | $CH_2CH_3$ | $CH_2CH_3$ | 62 | 75 |
| **3.21a** | *t*-Bu | $CH_3$ | | 65 | 78 |
| **3.21b** | *t*-Bu | $CH_2CH_3$ | | 52 | 75 |
| **3.22a** | *t*-Bu | $CH_3$ | N | | 30 |
| **3.22b** | | $CH_3$ | N | 25 | 20 |
| **3.3a** | *t*-Bu | $CH_3$ | S | | 24 |
| **3.23b** | | $CH_3$ | S | 20 | 18 |
| **3.24** | *t*-Bu | $CH_3$ | | | 27 |

The reaction of 5-membered ring substrates **3.5a-b** towards the zwitterion adduct proceeds smoothly and resulted in good yields. The highest yield was obtained from compound **3.20c** with 87% containing *tert*-butyl at $R_1$ and a methyl group at $R_2$ while **3.20h** gave 75% as the lowest yield using Method B.

Esmaeili and co-workers[95] proposed the reaction mechanism of this similar reactions as shown in Scheme 3.9. Zwitterion intermediate **3.25** deprotonates substrate **3.5** to give an intermediate

nitrilium ion which is attacked by the deprotonated nucleophile at event **1**. The resulting ketenimine intermediate at event **2** cyclises to give pyrimidine **3.20**.




**Scheme 3.9.** Proposed reaction mechanism.

These 5-membered ring substrates **3.5a-b** have low predicted $pK_a$ values of approximately 0.5[190] as they contain an acidic proton which is suitable to initiate the reaction with the zwitterion adduct, as indicated in the mechanism shown in Scheme 3.9. The electron deficient alkynes DMAD **3.4a** or DEtAD **3.4b** never showed any tendency to react directly with 5-membered ring substrates **3.5a-b** to form any extra products during the reaction and isolation.

When pyrrolidine-2,5-diimine **3.8** was used as a substrate using Method A the yields were also moderate and eventually increased when Method B was applied (Table 3.1). It was interesting for us to note that pyrrolidine-2,5-diimine[185] **3.8**, with a predicted $pK_a$ of 10.8,[190] containing an amidine functionality known to be nucleophilic,[191] was not reactive towards DMAD directly. When we examined product **3.21** closely, it was clear that hydrolysis of one imine group to a carbonyl group had occurred (Scheme 3.8). We speculate that this hydrolysis occurred prior to reaction, leading to 5-iminopyrrolidin-2-one (predicted $pK_a$ =2.3)[190] which contains an acidic proton and is suitable to initiate the reaction with the zwitterion adduct to afford **3.21a-b** as recorded in Table 3.1.

The fourth 5-membered ring substrate which was put to the test was 1*H*-1,2,4-triazol-5-amine **3.11**, with a predicted $pK_a$ of 9.2.[190] When Method A was applied using 1*H*-1,2,4-triazol-5-amine **3.11** the reaction gave rise to product **3.22b** from the direct reaction of DMAD and 1*H*-1,2,4-triazol-5-amine **3.11** in a yield of 25%. The 3-CR product of isocyanide **3.19** with DMAD **3.4** and 1*H*-1,2,4-triazol-5-amine **3.11** was not observed during the reaction and isolation. The results obtained indicate that DMAD as a Michael acceptor was found to be more reactive towards 1*H*-1,2,4-triazol-5-amine **3.11** than to the zwitterion adduct using Method A. This might suggest that the nucleophilicity of substrate 1*H*-1,2,4-triazol-5-amine **3.11** makes it suitable to react with DMAD directly, and less suitable as a substrate for the 3-CR. In addition, product **3.22b** was also reported by Reimlinger and co-workers[104] after reacting DMAD (in the absence of isocyanide) with 1*H*-1,2,4-triazol-5-amine **3.11** under ethanol reflux achieving two isomeric products in yields of 10% and 29%. Subsequently, Method B was put to the test and the reaction resulted in a mixture of two products (Scheme 3.8). After isolation, desired product **3.22a** was obtained in a low yield of 30% and co-product **3.22b** in 20% yield.

The fifth 5-membered ring substrate 1*H*-1,2,4-triazole-5-thiol **3.13** was tested using Method A and gave rise to **3.23b** in 20% yield by directly reacting with DMAD (Scheme 3.8). Substrate **3.13** has a predicted $pK_a$ of 11.1,[190] hence its nucleophilicity allows it to be reactive towards DMAD as a Michael acceptor. Product **3.23b**,[105] after reacting DMAD with substrate **3.13** at room temperature using acetonitrile as a solvent to obtain 74% yield. When Method B was applied the reaction gave desired product **3.23a** in 24% yield and a co-product **3.23b** in 18% yield. The isomers shown in Scheme 3.8 were those isolated from the reactions and identified by X-ray crystallography

The sixth 5-membered ring tested was 2-aminothiazole **3.15** with $pK_a$ of 5.3,[192] which gave rise to product **3.24**. There were no traces of the starting material or the product from direct reaction with DMAD, however the isolated yield was only 27%. The overall outcome of these reactions shows that factors and conditions such as order of reagents addition, inert conditions and the temperature, influences the reaction yields.

When green solvents such as acetone, ethanol and isopropanol were tested in this reaction they resulted in inferior yields of **3.20a-h** and **3.21a-b** (Table 3.2), while products **3.22a** and **3.23a** were not obtained. Method B gave these lower yields, while there were no products observed at all using Method A which resulted in starting materials being recovered after the reaction.

Using acetone as a solvent for synthesis of **3.20a** gave 25% yield using Method B, whereas ethanol and isopropanol gave 13% and 15% yield, respectively. Compound **3.21a** gave 27% yield as the highest yield for all the three green solvents put to the test. Thus acetone seem to be better in terms of yields compared to isopropanol which is comparatively better than ethanol. This may suggest poor solubility of the 5-membered ring substrates used in acetone, ethanol and isopropanol.

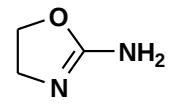
**Table 3.2**: The synthesis of **3.20a-h** and **3.21a-b** pyrimidines in green solvents

| **Compound** | **Acetone Yield (%)** | **Ethanol Yield (%)** | **Isopropanol Yield (%)** |
|---|---|---|---|
| **3.20a** | 25 | 13 | 15 |
| **3.20b** | 18 | 10 | 12 |
| **3.20c** | 22 | 12 | 17 |
| **3.20d** | 19 | 11 | 16 |
| **3.20e** | 21 | 8 | 11 |
| **3.20f** | 23 | 14 | 17 |
| **3.20g** | 18 | 13 | 16 |
| **3.20h** | 15 | 10 | 12 |
| **3.21a** | 27 | 17 | 22 |
| **3.21b** | 24 | 15 | 18 |

In relation to the substrates tested for the reactions, substrates **3.5a-b** and **3.8** are suitable for use in the three-component reaction while **3.11**, **3.13** and **3.15** are not as they gave poor yields of products **3.22a**, **3.23a** and **3.24**, whilst there were no products obtained when using isocyanide **3.19b**. Nair and co-workers[193] have remarked previously that three component reactions of this type are most successful when the third component (substrate) is non-reactive towards DMAD **3.4** and the isocyanide **3.19** to avoid unexpected or co-products. Based on the results obtained it was evident that substrates **3.11** and **3.13** were too reactive towards DMAD

directly, hence they gave rise to co-products **3.22b** and **3.23b**. When Adib and co-workers used 2-oxo-2-(1,3-thiazol-2-ylamino)acetate, an amide derivative of thiazol-2-amine **3.15**, as a third component, to react with a DMAD-isocyanide zwitterion they obtained good yields.[194] The increased acidity of the amide proton allowed the reaction to occur smoothly. Most of the substrates containing nitrogen heterocycles reported to participate in reaction with the zwitterion have a nitrogen atom next to a carbonyl group.[95,195,196]

When 2-imino-1,3-oxazolidine[189] **3.18** was tested as a third component with isocyanide **3.19a** and DMAD the reaction was not successful and resulted in recovery of starting material. This substrate is considered to be basic with a predicted $pK_a$ of 8.7 as shown in Figure 3.1. Presumably this substrate is unable to react with the zwitterion as it is not acidic, and it is also not sufficiently nucleophilic to react with DMAD directly.




**3.18**

**$pK_a$ 8.7**

**Figure 3.1.** Structure of 2-imino-1,3-oxazolidine and its $pK_a$

### 3.4 Structural elucidation of pyrimidine derivatives by FTIR, NMR, HRMS and Single X-ray Crystallography techniques

The synthesised compounds were characterised by NMR spectroscopy, mass spectrometry, and FTIR spectroscopy. Where applicable the compounds **3.20a, 3.20e, 3.21a, 3.22a** and **3.23b** where subjected for single-crystal X-ray analysis. The analysis of product **3.20a** is presented as an example as shown on Figure 3.2

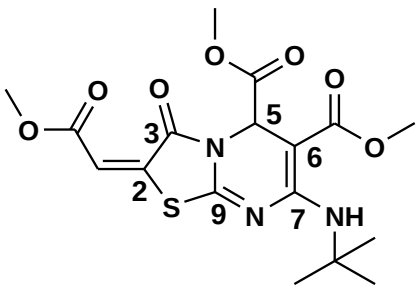



**Figure 3.2.** Structure of compound **3.20a**

The $^{1}H$ NMR spectrum showed a broad singlet for NH at δ= 9.05, a singlet due to the vinyl proton at δ= 7.03, a singlet from the CH of the six-membered ring (position **5**) at δ= 5.78, three singlets due to the methoxy protons at δ= 3.88, 3.74 and 3.70 and one singlet for the nine *t*-butyl protons at δ= 1.44. The $^{13}C$ NMR spectrum of **3.20a** showed 15 distinct resonances in accordance with the proposed structure. The mass spectrum of **3.20a** showed the molecular ion $[M+H]^+$ peak at m/z 412.1207 which is consistent with the mass of the proposed product which is calculated as 412.1173. The FTIR spectrum displayed characteristic absorption bands for carbonyl groups at 1736 $cm^{-1}$, 1688 $cm^{-1}$, 1653 $cm^{-1}$ and 1629 $cm^{-1}$ and for the amino group at 3061 $cm^{-1}$.

FTIR spectra of compounds **3.20b-h**, **3.21a-b**, **3.22a**, **3.23a** and **3.24** showed characteristic absorption bands for N-H in a range of 3061 $cm^{-1}$ to 2852 $cm^{-1}$ , while the C=O stretching band appeared in a range of 1748 $cm^{-1}$ to 1518 $cm^{-1}$. The $^{1}H$ NMR spectra exhibited one singlet signal in the region of δ 9.10- 4.84 ppm corresponding to the NH and one singlet at δ 6.45-4.27 ppm corresponding to CH. $^{13}C$ NMR spectra showed the CH at position **5** with signals in the range δ 61.2-50.4 ppm. ESI-LCMS was used to confirm the exact masses of the compounds. Chromatograms exhibited protonated parent molecular ion $[M+1]^+$ represented in experimental section.

After recrystallization from ethyl acetate and hexane, compounds **3.20a, 3.20e** and **3.21a** were subjected to X-ray crystallographic analysis[197,198,199] (Figure 3.3). The single-crystal X-ray structure of **3.21a** unambiguously established the presence of a third carbonyl group which confirms hydrolysis of the imine group found in the starting material **3.8** to the carbonyl group, found in product **3.21a**. This type of hydrolysis is facile and can occur upon exposure to oxygen or moisture either during reaction, separation or characterization.[185]

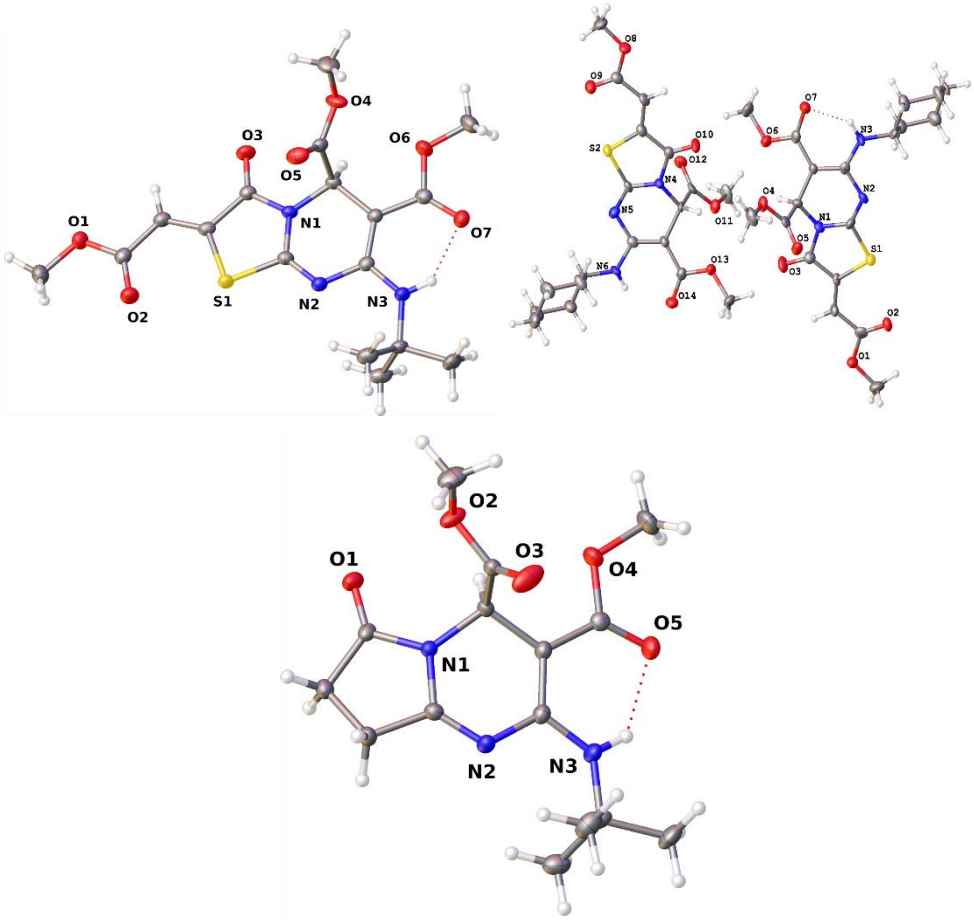



**Figure 3.3:** Single-crystal X-ray structure (ORTEP) of **3.20a** (top left, CCDC 1981633), **3.20e** (top right, (CCDC 1981634) and **3.21a** (bottom right, CCDC 1981635).

In consideration of possible regioisomers that can be obtained when using substrate 1*H*-1,2,4-triazol-5-amine **3.11** the identity of compound **3.22a** was unambiguously confirmed by single-crystal X-ray analysis as shown in Figure 3.4. Interestingly, the single crystal X-ray analysis of compound **3.22a** revealed an unexpected tautomeric form, consisting of two adjacent stereogenic centres allowing the two ester groups to adopt a *trans* arrangement as shown in Figure 3.5.

The NMR data of structure **3.22b** was found to be in agreement with that previously reported.[104] Final product identities for substrate 1*H*-1,2,4-triazole-5-thiol **3.13** were confirmed by single-crystal X-ray analysis of **3.23b** as shown in Figure 3.4 and the structure of **3.23a** was based on NMR data comparison from related reported compounds.[200] Even though the compounds obtained **3.22a-b** and **3.23a-b** were isolated from the reactions, the possibility of the other regioisomers being present in the reaction mixtures cannot be ruled out.

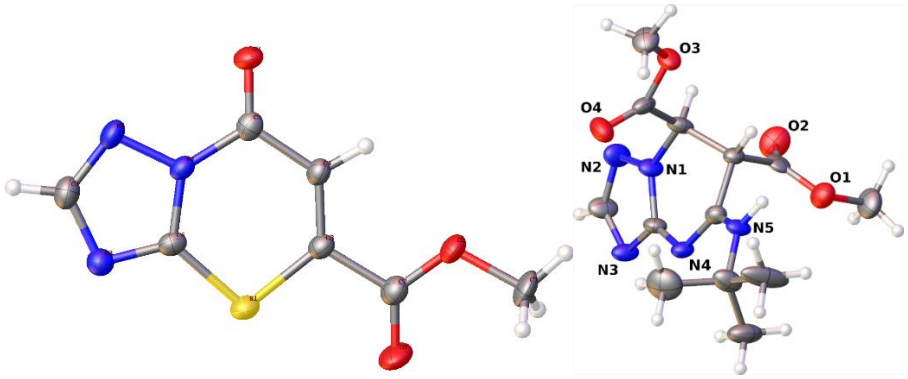



**Figure 3.4:** Structure of compound **3.23b** (left) and **3.22a** (right, CCDC 1986041).

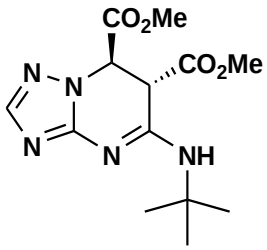


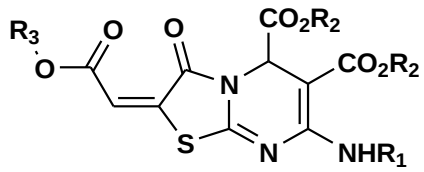
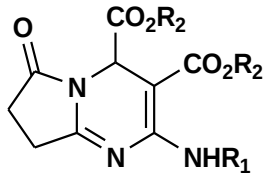
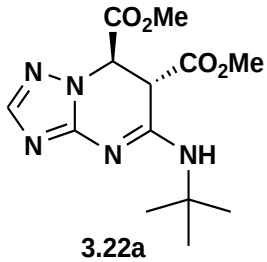
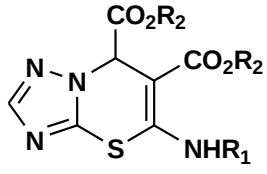
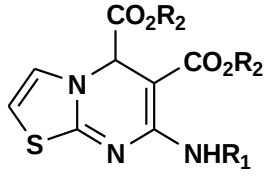

**Figure 3.5:** Structure of compound **3.22a**.

A total of thirteen highly functionalised pyrimidines were synthesised *via* a 3-CR method and to the best of our knowledge these compounds have not been previously described.

### 3.5 Biological evaluation of library of 5-membered ring fused pyrimidine derivatives

The cytotoxicity and antimicrobial MIC evaluations of the synthesised 5-membered ring fused pyrimidine derivatives were conducted at Mintek, Research and Development, Advanced Material Division, Biomed group) under the supervision and guidance of Dr Qasim Fish and Ms Mapula Makgoba. The toxicities were evaluated in an MT-4 cell line at 100 µM. Auranofin was used as a cytotoxic control compound starting at 100 µM serial dilution series and DMSO was used as a background control. At this concentration compounds **3.20a-f**, **3.21a-b**, **3.22a-b** and **3.23a** were found to cause cell reduction at $CC_{50}$ values above 200 µM as recorded in Table 3.3. Only three compounds were found to be relatively toxic with $CC_{50}$ of 54 µM (**3.20g**), 15 µM (**3.20h**) and 27 µM (**3.23b**). Compound **3.20g** contains a cyclohexyl group at $R_1$, methyl group at $R_2$ and ethyl group at $R_3$, while **3.20h** contains a cyclohexyl group at $R_1$, ethyl group at $R_2$ and ethyl group at $R_3$.

**Table 3.3**: $CC_{50}$ values of synthesised pyrimidine derivatives

| Structures | Number | $CC_{50}$ | % Antiviral inhibition (infected MT4) |
|---|---|---|---|
|  3.20a-h | **3.20a** | > 200 µM | -2.13 |
| | **3.20b** | > 200 µM | -6.89 |
| | **3.20c** | > 200 µM | 2.99 |
| | **3.20d** | > 200 µM | -3.44 |
| | **3.20e** | > 200 µM | -7.79 |
| | **3.20f** | > 200 µM | 6.21 |
| | **3.20g** | 54 µM | |
| | **3.20h** | 15 µM | |
|  3.21a-b | **3.21a** | > 200 µM | -6.79 |
| | **3.21b** | > 200 µM | 2.14 |
|  3.22a | **3.22a** | > 200 µM | 7.04 |
| | **3.22b** | > 200 µM | 6.03 |
|  3.23a | **3.23a** | > 200 µM | 1.21 |
| | **3.23b** | 27 µM | |
|  3.24 | **3.24** | > 200 µM | -3.71 |

**$CC_{50}$** - concentration of compounds that causes 50% reduction of cell growth

Out of fifteen compounds subjected to cytotoxicity testing, thirteen compounds were found to have high $CC_{50}$ values of above 200 µM as one example represented in Figure 3.6. This low toxicity means that these compounds are good candidates for further biological evaluations such as antiviral testing.

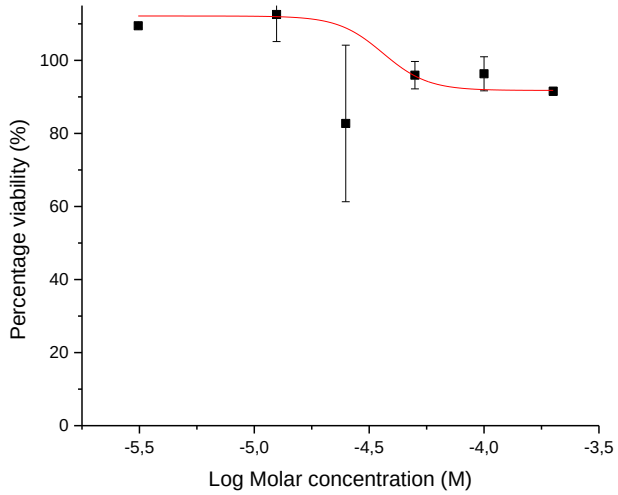



**Figure 3.6**: Over >100 µM cytotoxicity curve of compoun**3.20d**

Compounds **3.20g** and **3.20h** were able to reduce 50% of viable cells at the concentrations of 54 µM and 15 µM, respectively, as presented in Figure 3.7. These compounds were found to reduce viable cell at low concentration (normally below 100 µM), which makes them poor candidates for further biological evaluation.

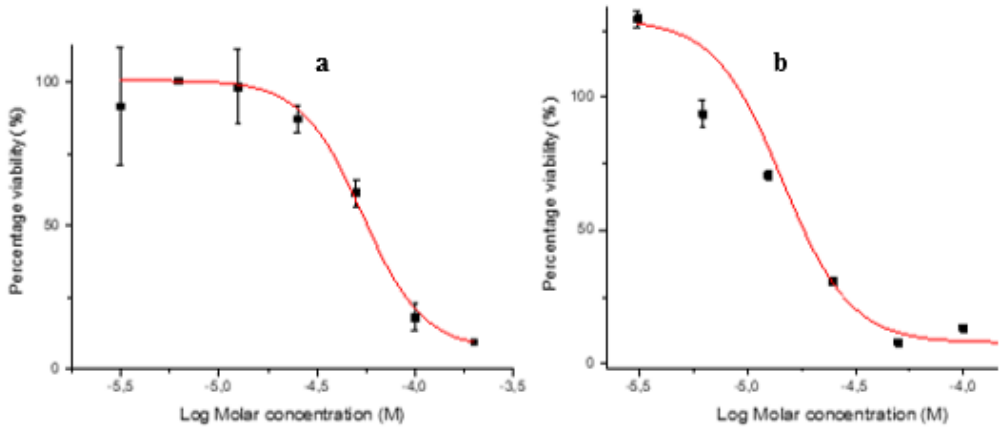



**Figure 3.7**: $CC_{50}$ of compounds a) **3.20h** and b) **3.20g**.

Eventually compounds **3.20a-f**, **3.21a-b**, **3.22a-b**, **3.23a** and **3.24** which were found relatively non-toxic were then evaluated for antiviral activities in infected MT4 cells. The tests were carried out in a cell-based assay and each compound was analysed at a single dose of 100 μM using Dolutegravir and Raltegravir as control compounds. Based on the outcome of this assay, none of the compounds showed significant activity.

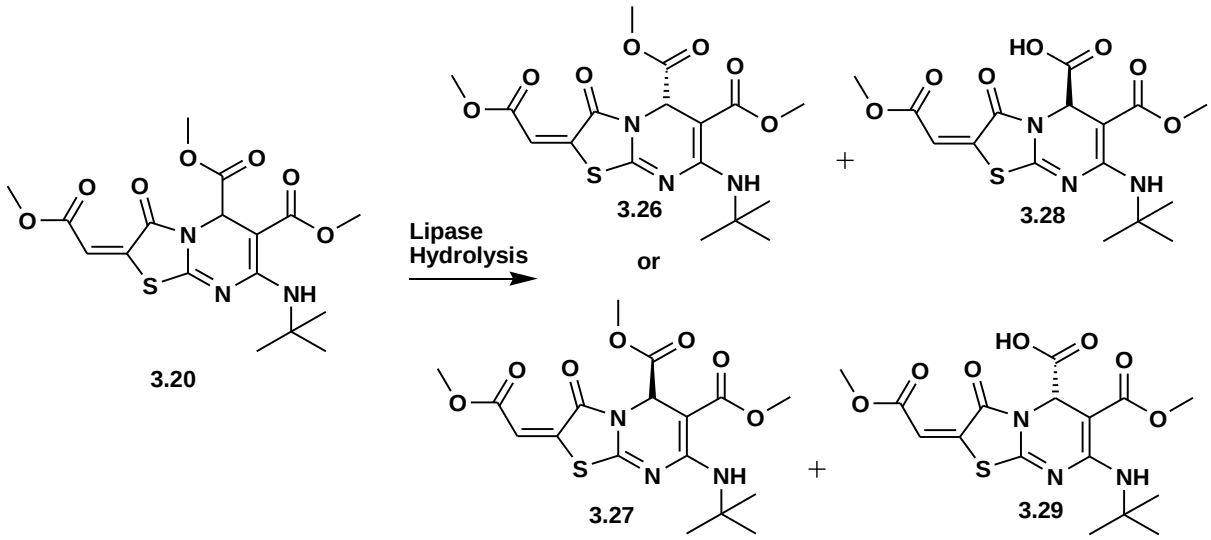
This set of compounds were also evaluated for their antimicrobial activities against a set of Gram-positive bacterial and Gram negative bacterial species. Ampicillin was used as a control for *E.coli* and *S. aureus*, while Gentamycin was used as a control for *B. subtilis* and *P. aeruginosa*.[201] Unfortunately, none of the compounds showed any significant activity against the bacterial strains.

### 3.6. Conclusion

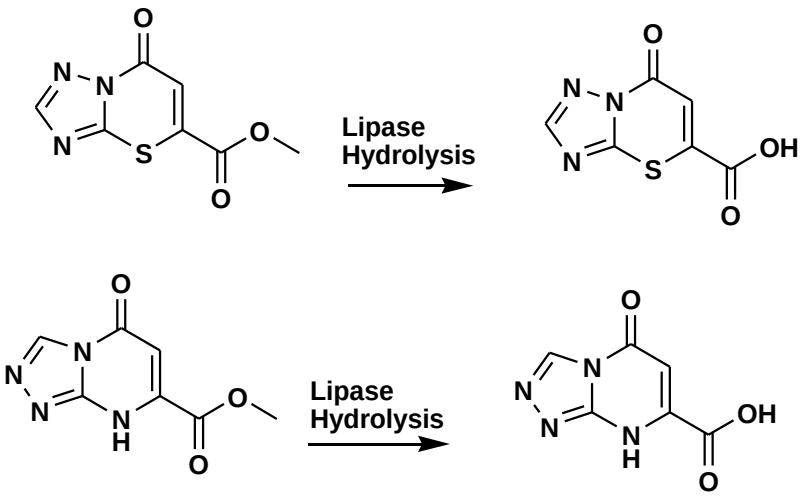
The synthesis of novel pyrimidine derivatives was accomplished using a one-pot, three component reaction. The facile and convenient reaction of isocyanides and DMAD or DEtAD under inert conditions at 0 ºC formed a zwitterion intermediate that further reacted with compounds **3.5a-b** and **3.8**. The reaction of pyrrolidine-2,5-diimine **3.8**, isocyanide and DMAD or DEtAD gave a product with an extra carbonyl group instead of the initial C=NH due to exposure to moisture or air. Unlike substrates **3.5a-b** and **3.8**, the reaction of substrates **3.11**, **3.13** and **3.15** resulted in low yields using Method B and no desired product using Method A. We believe their poor reactivity may in part be a result of their basic nature, and reactivity towards DMAD as Michael acceptor. Attempts to synthesize the pyrimidines in green solvents such as acetone, ethanol and isopropanol resulted in inferior yields. The cytotoxicity studies of this set of compounds showed thirteen compounds to be non-toxic and only two compounds were found to be relatively toxic. There was no significant antimicrobial activity observed for this set of compounds.

### 3.7. Future work

We will use lipase enzymes for enzymatic kinetic resolution to obtain single enantiomers.




**Scheme 3.10:** Possible structural modifications on highly functionalised pyrimidines.




**Scheme 3.11:** Possible structural modifications on the ester group.

# CHAPTER 4

# A facile and efficient one pot 3-CR method for the synthesis of novel thiazine-based heterocyclic compounds using zwitterion adduct intermediates

## 4.1 Introduction

In accordance with the aim of the study to develop biologically active heterocyclic scaffolds using 3-CR, we present here an efficient method for the synthesis of a library of thiazines. The synthesis involves the reaction of the prepared 2-amino-4*H*-1,3-thiazin-4-one derivatives with alkyl isocyanides and DMAD/DEtAD. This chapter is concerned about the ability of zwitterion adducts formed from the reaction of an alkyl isocyanide and DMAD (shown in Figure 4.1), to react with six-membered thiazine. The aim is to construct a new six-membered ring onto a six-membered thiazine in order to form fused [6-6] heterocyclic compounds comparable to the [5-6] pyrimidines prepared in Chapter two from initial five-membered ring substrates.

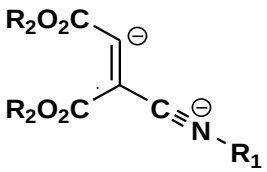



**Figure 4.1.** Structure of zwitterion adduct from the reaction of an isocyanide and DMAD.

## 4.2 Preparation of 2-amino-4*H*-1,3-thiazin-4-one derivatives using alkyl propiolate derivatives and thiourea

Thiazines can be structured in many different ways, as shown in Figure 4.2. Our interest was in using 1,3-thiazine derivatives (Figure 4.2b) as building blocks for the synthesis of heterocyclic scaffolds.

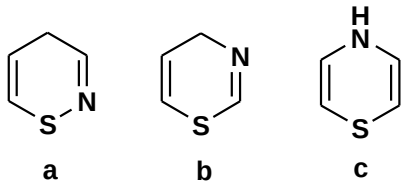
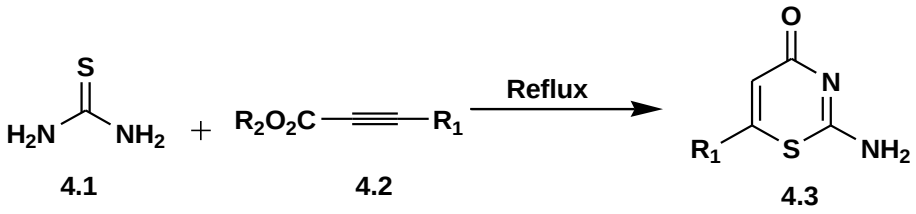



**Figure 4.2.** Structures of 1,2- (**a**), 1,3- (**b**) and 1,4- (**c**) thiazine.

*4.2.1 Preparation of 2-amino-4H-1,3-thiazin-4-one derivatives (**4.3**) using different solvents under reflux.*

In order to achieve the synthesis of 2-amino-4*H*-1,3-thiazin-4-one derivatives, reactions were set up at different temperatures using different solvents, as shown in Schemes 4.1-4.2 and Tables 4.1-4.3. Different solvents were selected to investigate the solvent effect on product conversions and yields. Kishida and Tereda[202] reported the reaction of thiourea **4.1** and alkyl propiolate derivatives **4.2** under ethanol reflux to obtain 2-amino-4*H*-1,3-thiazin-4-one derivatives (**4.3f1-2, 4.3b** and **4.3e, Scheme 4.1**). Choudhary and Peddinti[108] reported the synthesis of 2-amino-4*H*-1,3-thiazin-4-one **(4.3a**) from the reaction of thiourea **4.1** and alkyl propiolate **4.2a** at 0-5 ºC in methanol and ethyl acetate. To the best of our knowledge compounds **4.3c** and **4.3d** have not been previously reported. The reactions of thiourea and alkyl propiolate and thiazines are well documented in literature.[203,204,205]

The 2-amino-4*H*-1,3-thiazin-4-one derivatives were prepared firstly under reflux conditions in different solvents (i.e. methanol, ethanol, isopropanol, ethyl acetate and water). The reactions were set up for at least 2 h after introducing an appropriate alkyl propiolate **4.2** to a solution of thiourea **4.1** as shown in Scheme 4.1. After cooling, the resulting precipitates were collected by filtration and washed with water to afford products **4.3b-e** in yields ranging from marginal to good yield as shown in Table 4.1.



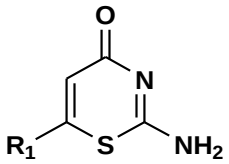

| 4.2 | $R_1$ | $R_2$ |
|---|---|---|
| 4.2a: | H | $CH_3$ |
| 4.2b: | $CH_3$ | $CH_2CH_3$ |
| 4.2c: | $CH_2CH_3$ | $CH_2CH_3$ |
| 4.2d: | $CH_2CH_2CH_3$ | $CH_3$ |
| 4.2e: | Ph | $CH_2CH_3$ |

| 4.3 | $R_1$ | $R_2$ |
|---|---|---|
| 4.3b: | $CH_3$ | $CH_2CH_3$ |
| 4.3c: | $CH_2CH_3$ | $CH_2CH_3$ |
| 4.3d: | $CH_2CH_2CH_3$ | $CH_3$ |
| 4.3e: | Ph | $CH_2CH_3$ |

**Scheme 4.1**: Synthesis of 2-amino-4*H*-1,3-thiazin-4-one derivatives **4.3b-e**

The highest yield obtained from alcohol solutions was 87% for **4.3c** and **4.3d** using isopropanol. The solvent choice was shown to have a strong influence on the formation of the products. For example, products **4.3b** and **4.3c** were obtained in yields of 11% and 17% after 2 h using methanol, while changing the solvent to isopropanol afforded 83% and 87% yields and reaction times were shortened 2-fold.

**Table 4.1**: Yields of derivatives **4.3b-f** under solvent refluxing conditions

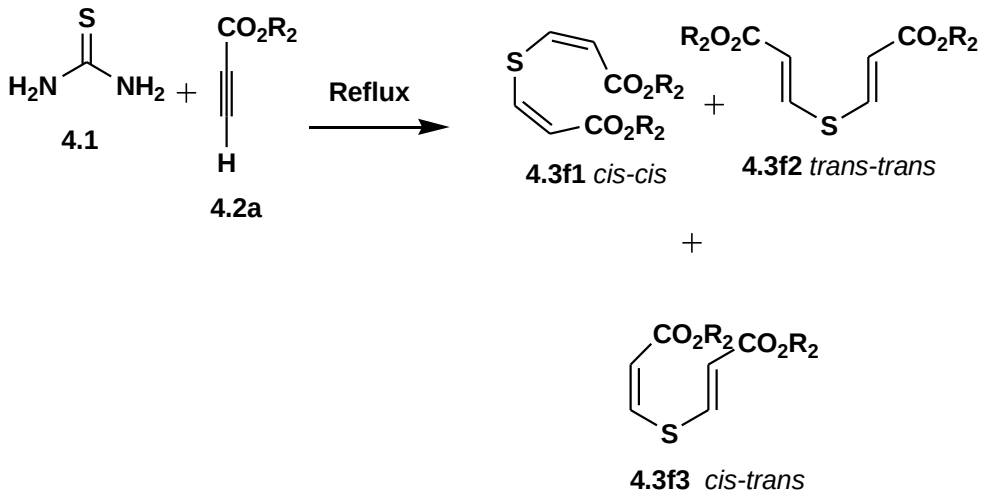



4.3

| Compound | $R_1$ | Water Yield % (time) | EtOAc Yield % Yield % (time) | MeOH Yield % (time) | EtOH Yield % (time) | iPrOH Yield % (time) |
|---|---|---|---|---|---|---|
| **4.3a** | H | - | - | - | - | - |
| **4.3b** | $CH_3$ | - | - | 11 (2 h) | 61 (2 h) | 83 (1 h) |
| **4.3c** | $CH_2CH_3$ | - | - | 17 (2 h) | 66 (2 h) | 87(1 h) |
| **4.3d** | $CH_2CH_2CH_3$ | - | - | 21 (2 h) | 70 (2 h) | 87 (2 h) |
| **4.3e** | Ph | - | - | 25 (2 h) | 72 (2 h) | 84 (2 h) |
| **4.3f 1-3** | H | 87 (0.5 h) | 81 (0.5 h) | 70 (2 h) | 55 (2 h) | 52 (2 h) |

No reaction (-)

It was observed that the longer the alcohol chain the better the isolated yield of the products. Unfortunately, no products were isolated when thiourea **4.1** and alkyl propiolates **4.2b-e** were reacted using water or ethyl acetate as solvents and only starting materials were recovered (Table 4.1). When using methanol as a solvent under reflux the product conversion was marginal, giving from 11% - 25% yield and resulting in recovery of starting materials. When thiourea **4.1** and alkyl propiolate **4.2a** were reacted under reflux using methanol, ethanol, isopropanol, ethyl acetate or water, this resulted in formation of **4.3f1-4** instead of the anticipated **4.3a** in average to good yield of a combination isomers[108]: **4.3f1** *cis-cis***, 4.3f2** *trans-trans* and **4.3af3** *cis-trans* as shown in Scheme 4.2.



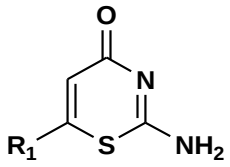

**Scheme 4.2**: Synthesis of **4.3f1-3**

*4.2.2 Preparation of **4.4a-e** using different solvents at room temperature*

The attention was then focused on the formation of **4.3a-e** at room temperature to determine the influence of heating on the formation of the products including **4.3a** which was never achieved under reflux, using the same solvents as used in Section 4.2.1 above. When thiourea **4.1** and alkyl propiolates **4.2a-e** were reacted at room temperature using water or ethyl acetate there was no conversion and the starting materials were recovered as shown in Table 4.2. The formation of product **4.3b** using ethanol and isopropanol gave 31% and 36% yield at room temperature, respectively, while with heating under reflux using ethanol and isopropanol the product **4.3b** was isolated in 61% and 83% yield, respectively. It can be concluded that reaction at room temperature was not favourable for the formation of **4.3e-b**.The reaction of thiourea **4.1** and alkyl propiolate **4.2a** resulted in formation of products **4.3f** in good to excellent yield

instead of the anticipated **4.3a**. The room temperature conditions seem to favour the formation of these products **4.3f** in comparison to reflux conditions.

**Table 4.2**: Achieved yields of derivatives **4.3a-e** at room temperature




**4.3**

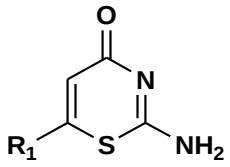
| Compound | $R_1$ | Water Yield % (time) | EtOAc Yield % (time) | MeOH Yield % (time) | EtOH Yield % (time) | iPrOH Yield % (time) |
|---|---|---|---|---|---|---|
| **4.3a** | H | - | - | - | 11 (2 h) | 17 (2 h) |
| **4.3b** | $CH_3$ | - | - | 10 (2 h) | 31(2 h) | 36 (1.5 h) |
| **4.3c** | $CH_2CH_3$ | - | - | 13 (2 h) | 34 (2 h) | 27 (1 h) |
| **4.3d** | $CH_2CH_2CH_3$ | - | - | 21 (2 h) | 22 (2 h) | 34 (2 h) |
| **4.3e** | Ph | - | - | 17 (2 h) | 19 (2 h) | 28 (2 h) |
| **4.3f 1-3** | H | 95 (0.5 h) | 84 (0.5 h) | 90 (2 h) | 73 (2 h) | 65 (2 h) |

No reaction (-)

*4.2.3 Preparation of 2-amino-4H-1,3-thiazin-4-one derivatives at 0-5ºC*

We further investigated the effect of temperature on the formation of the 2-amino-4*H*-1,3-thiazin-4-ones by conducting the reactions at set temperatures between 0-5ºC as shown in Table 4.3. It was interesting to observe that lower temperatures seemed to favour the formation of **4.3a** which was difficult to obtain at room temperature and with heating under reflux. In addition, variation in solvent i.e. ethyl acetate, methanol, ethanol and isopropanol did not influence the reaction outcome for the formation of **4.3a**. Apart from formation of **4.3a** in average to good yield ranging from 50% - 75% after 2h; the only other product observed was **4.3f**, which was isolated in trace amounts when thiourea **4.1** was reacted with alkyl propiolate **4.2a** using ethyl acetate as a solvent at 0-5ºC after 2h. It can be established that there is temperature effect towards products formation.

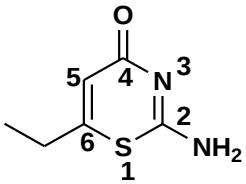
**Table 4.3**: Achieved yields of **4.3** derivatives at 0-5ºC (**4.3a-e**)




**4.3**

| Compound | $R_1$ | Water Yield % (time) | EtOAc Yield % (time) | MeOH Yield % (time) | EtOH Yield % (time) | iPrOH Yield % (time) |
|---|---|---|---|---|---|---|
| **4.3a** | H | 50 (0.5 h) | 70 (2 h) | 72 (2 h) | 72 (2 h) | 75(2h) |
| **4.3b** | $CH_3$ | - | - | - | - | - |
| **4.3c** | $CH_2CH_3$ | - | - | - | - | - |
| **4.3d** | $CH_2CH_2CH_3$ | - | - | - | - | - |
| **4.3e** | Ph | - | - | - | - | - |
| **4.3f 1-3** | H | - | trace (2h) | - | - | - |

*4.2.4 The analysis of derivatives **4.3a-e** by FTIR and NMR techniques*




**4.3c**

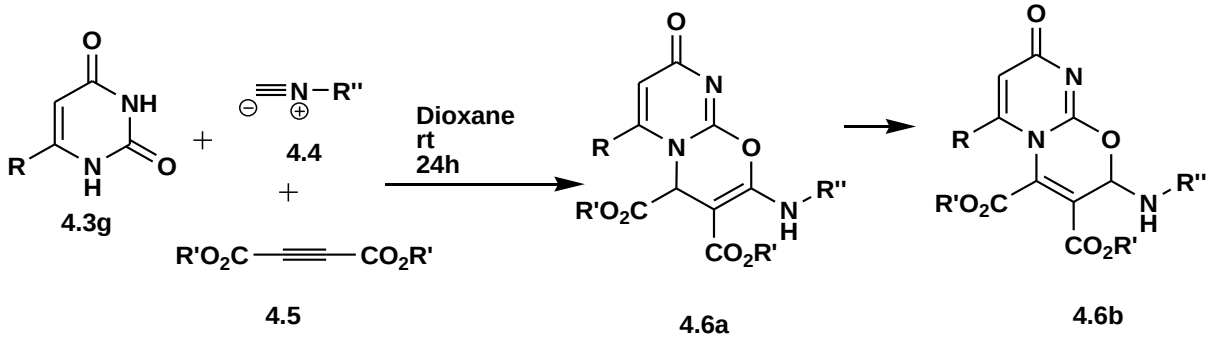
**Figure 4.3**: Structure of **4.3c**

By way of example spectroscopic characteristics of compound **4.3c** are discussed as shown in Figure 4.3. The $^1$H NMR spectrum shows a broad singlet for $NH_2$ at δ = 8.24; a singlet at δ = 6.21 corresponding to a proton at position **5**, a quartet at δ = 2.47 with a *J* value of 7.2 Hz corresponding to the $CH_2$ attached to carbon at position **6**, and a triplet at δ = 1.15 with a *J* value of 7.2 Hz corresponding to the adjacent methyl group. The $^{13}$C NMR spectrum of **4.3c** showed 6 distinct resonances in accordance with the proposed structure. FTIR spectra of **4.3c**

showed the following absorption bands: 3248 $cm^{-1}$, 3045 $cm^{-1}$, 2979 $cm^{-1}$ and 1617 $cm^{-1}$. The rest of the compounds **4.3a-b** and **4.3d-e** followed similar trend patterns.

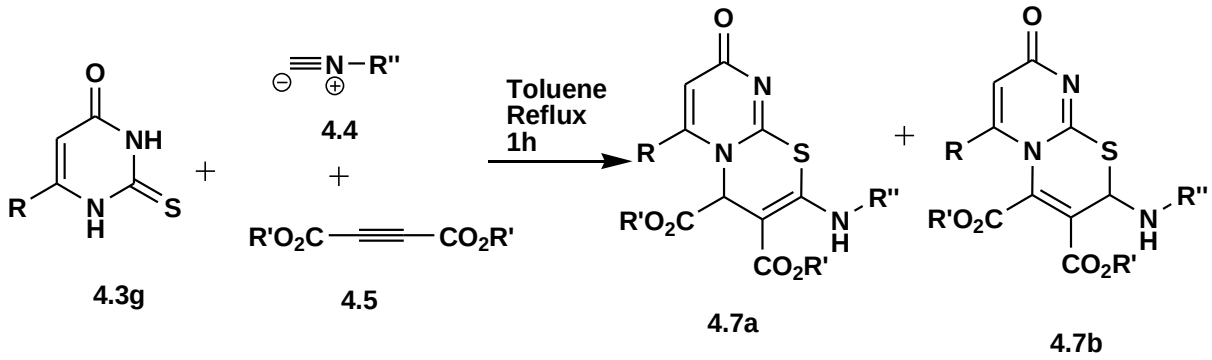
### 4.3. Synthesis of dimethyl 8-(tert-butylamino)-4-oxo-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (4.7) derivatives using zwitterionic adducts

The successful use of zwitterionic adducts for synthesis of highly functionalised pyrimidines from five membered-ring substrates in Chapter 3 led us to the use of 2-amino-4*H*-1,3-thiazin-4-one derivatives **4.3** to react with the zwitterion adduct. 5-Substituted uracil derivatives **4.3g**, which are analogues of 2-amino-4*H*-1,3-thiazin-4-one derivatives **4.3a-e**, have been reported by Baharfar and Baghbanian[206] to undergo reaction with isocyanides **4.4** and dialkyl acetylenedicarboxylates **4.5** to achieve 7-alkyl (or halo)-8-oxo-2*H*,8*H*-pyrimido[2,1-*b*][1,3]oxazine-3,4-dicarboxylate derivatives **4.6a** or **4.6b** using 1,4-dioxane at room temperature as shown in Scheme 4.3.



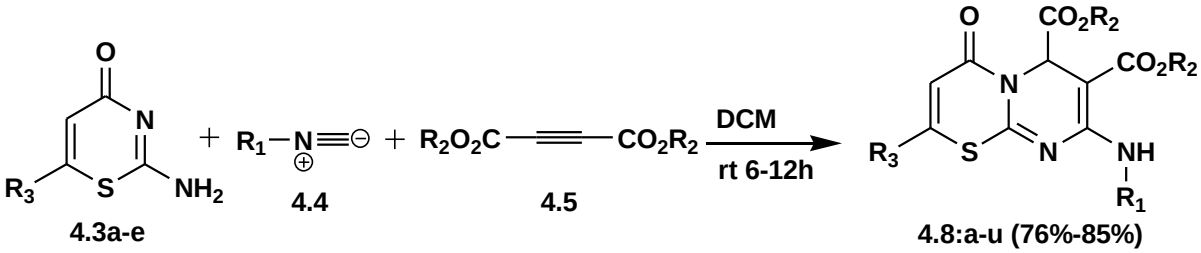

**Scheme 4.3**: Synthesis of **4.6a-b**

Yadollahzadeh and co-workers[207] demonstrated the reaction of 2-thiouracils **4.3h** with isocyanides **4.4** in the presence of dialkyl acetylenedicarboxylates **4.5** under reflux using toluene as a solvent to obtain **4.7a-b** as shown in Scheme 4.4.




**Scheme 4.4**: Synthesis of **4.7a-b**

In our case we present the reaction of 2-amino-4*H*-1,3-thiazin-4-one derivatives **4.3a-e** and isocyanides **4.4** in the presence of dialkyl acetylenedicarboxylates **4.5** in dichloromethane at room temperature to afford novel compounds **4.8a-u** in good yields. The reactions were carried out by the addition of the isocyanide **4.4** to DMAD **4.5** at 0 ºC under inert conditions as shown in Scheme 4.5. After stirring for 0.5 h in dry $CH_2Cl_2$, a solution of compound **4.3a-e** in dry $CH_2Cl_2$ was slowly added and the reaction mixture was allowed to stir for 6-12 h at rt as shown in Scheme 4.5. After purification, novel products **4.8a-u** were obtained in good yields as shown in Scheme 4.5.




**4.4 $R_1$**
**4.4a: *t*-Bu**
**4.4b: tetramethylbutyl**
**4.4c: cyclohexyl**

**4.5 $R_2$**
**4.5a: $CH_3$**
**4.5b: $CH_2CH_3$**

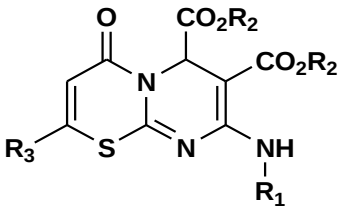
| 4.8: | $R_1$ | $R_2$ | $R_3$ | Yield% |
|---|---|---|---|---|
| 4.8a | *t*-Bu | $CH_3$ | H | 81 |
| 4.8b | *t*-Bu | $CH_2CH_3$ | H | 85 |
| 4.8c | *t*-Bu | $CH_3$ | $CH_3$ | 82 |
| 4.8d | *t*-Bu | $CH_2CH_3$ | $CH_3$ | 83 |
| 4.8e | tetramethylbutyl | $CH_3$ | $CH_3$ | 76 |
| 4.8f | tetramethylbutyl | $CH_2CH_3$ | $CH_3$ | 77 |
| 4.8g | cyclohexyl | $CH_3$ | $CH_3$ | 84 |
| 4.8h | cyclohexyl | $CH_2CH_3$ | $CH_3$ | 85 |
| 4.8i | *t*-Bu | $CH_3$ | $CH_2CH_3$ | 84 |
| 4.8j | *t*-Bu | $CH_2CH_3$ | $CH_2CH_3$ | 79 |
| 4.8k | tetramethylbutyl | $CH_3$ | $CH_2CH_3$ | 81 |
| 4.8l | tetramethylbutyl | $CH_2CH_3$ | $CH_2CH_3$ | 81 |
| 4.8m | *t*-Bu | $CH_3$ | $CH_2CH_2CH_3$ | 84 |
| 4.8n | *t*-Bu | $CH_2CH_3$ | $CH_2CH_2CH_3$ | 85 |
| 4.8o | tetramethylbutyl | $CH_3$ | $CH_2CH_2CH_3$ | 79 |
| 4.8p | tetramethylbutyl | $CH_2CH_3$ | $CH_2CH_2CH_3$ | 79 |
| 4.8q | *t*-Bu | $CH_3$ | $C_6H_6$ | 77 |
| 4.8r | *t*-Bu | $CH_2CH_3$ | $C_6H_6$ | 80 |
| 4.8s | tetramethylbutyl | $CH_3$ | $C_6H_6$ | 76 |
| 4.8t | tetramethylbutyl | $CH_2CH_3$ | $C_6H_6$ | 83 |
| 4.8u | cyclohexyl | $CH_3$ | $C_6H_6$ | 79 |

**Scheme 4.5**: Synthesis of dimethyl 8-(*tert*-butylamino)-4-oxo-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate derivatives (**4.8a-u**).

The yields of **4.8a-u** were high due to the acidic nature of the 2-amino-4*H*-1,3-thiazin-4-one derivatives **4.3a-e** which possess an acidic proton that initiates the reaction with the zwitterion adduct. The highest yields obtained were 85% for **4.8b**, **4.8h** and **4.8n** and the lowest yield was 76% from **4.8h**. The reactions were achieved smoothly in dichloromethane. The study was limited due to availability of a narrow substrate scope of alkyl isocyanides **4.4**, di-*tert*-butyl acetylenedicarboxylate **4.5** and alkyl propiolate derivatives **4.2**.

It is worth mentioning that performing the reactions using green solvents such as acetone, ethanol and isopropanol resulted in average to low yields of thiazine compounds **4.8a-u** (Table 4.4), while for some substrates the reaction was not tested in ethanol or isopropanol solvents due to limited reagent availability. Using acetone as a solvent for synthesis of thiazine compounds **4.8a-u** was tested for all thiazine starting materials **4.3a-e** and resulted in average yields. The highest yield obtained was 58% for **4.8o** which contains tetramethylbutyl at $R_1$, a methyl group at $R_2$ and propyl at $R_3$. The lowest yield was 41% from **4.8g** containing a cyclohexyl at $R_3$, and methyl groups at $R_1$ and $R_2$. Despite only average yields being obtained, acetone can be considered as an option for the synthesis of thiazine compound **4.8a-u** using 3-CR. When using ethanol and isopropanol the yields comparatively dropped. Unlike pyrimidines discussed in Chapter 3 the thiazines **4.8** seem to give reasonable average yields.

**Table 4.4** The synthesis of **4.8a-u** thiazine in green solvents



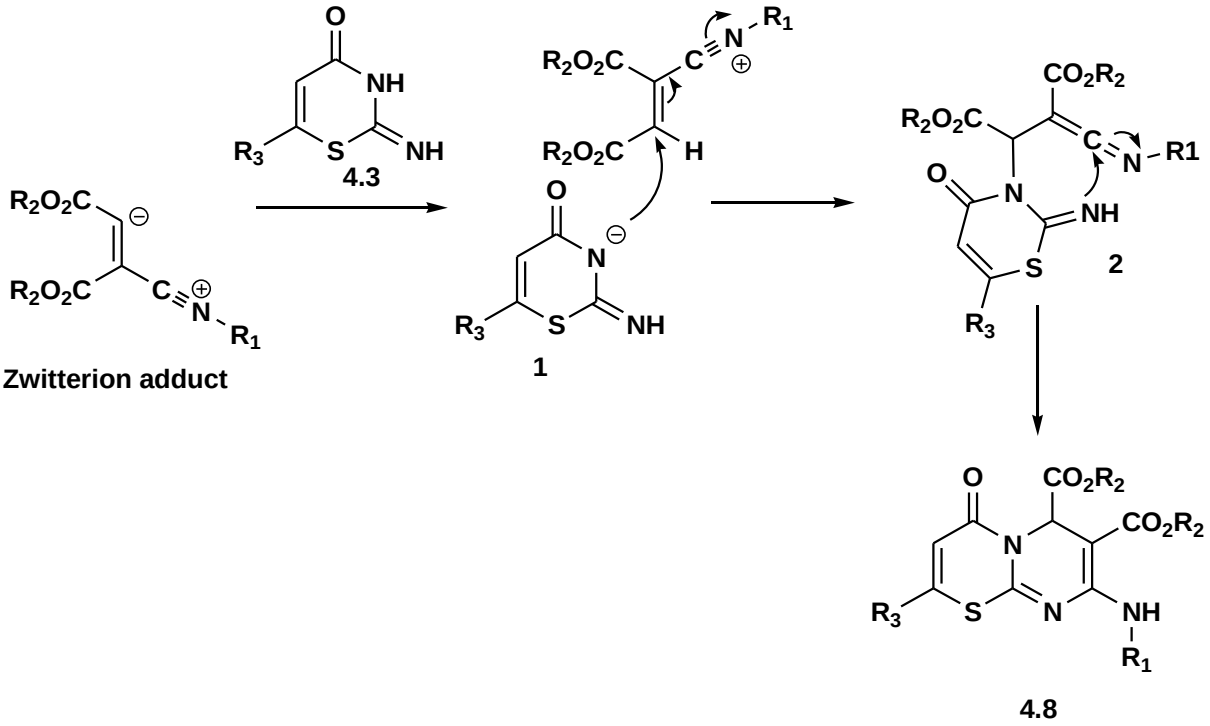

| Compound | Acetone Yield (%) | Ethanol Yield (%) | Isopropanol Yield (%) |
|---|---|---|---|
| **4.8a** | 44 | 36 | 42 |
| **4.8b** | 55 | 43 | 47 |
| **4.8c** | 45 | 35 | 41 |
| **4.8d** | 57 | 41 | 38 |
| **4.8e** | 53 | 46 | 42 |
| **4.8f** | 48 | 39 | 43 |
| **4.8g** | 41 | 33 | 43 |

| Compound | Acetone Yield (%) | Ethanol Yield (%) | Isopropanol Yield (%) |
|---|---|---|---|
| **4.8h** | 48 | 37 | 35 |
| **4.8i** | 55 | 37 | 42 |
| **4.8j** | 53 | 37 | 46 |
| **4.8k** | 43 | - | - |
| **4.8l** | 48 | - | - |
| **4.8m** | 51 | - | - |
| **4.8n** | 47 | - | - |
| **4.8o** | 58 | - | - |
| **4.8p** | 55 | - | - |
| **4.8q** | 53 | - | - |
| **4.8r** | 47 | - | - |
| **4.8s** | 56 | - | - |
| **4.8t** | 43 | - | - |
| **4.8u** | 40 | | |

- the reaction was never tested

The derivatives **4.3** are strong NH/$NH_2$-acids with predicted $pK_a$ values of -1.80 for **4.3b,** -1.83 for **4.3a** and -2.21 for **4.3e**.[190] The novel substrates **4.3c** and **4.3d** are also expected to fall into strongly acidic $pK_a$ regions. The more acidic substrates seem to favour 3-CR using zwitterion adducts from alkyl isocyanides **4.4** and dialkyl acetylenedicarboxylates **4.5** as previously discussed in Chapter 3. Based on the isolated yields it is clear that the 2-amino-4*H*-1,3-thiazin-4-one derivatives **4.3** were completely inert towards direct reaction with either dialkyl acetylenedicarboxylates or alkyl isocyanides, while they demonstrated very high reactivity

towards the zwitterion adduct. The more basic the substrates, the more likely they are to be reactive towards DMAD and undergo Michael addition reaction leading to an alternative product, as discussed in Chapter 3. Although we have not definitively established the mechanism for the formation of **4.8**, it is likely to be similar to that previously described (see Chapter 3, Mohlala and co-workers[208]) and is shown in Scheme 4.6. The zwitterion adduct deprotonates **4.3** to form an intermediate that then attacks the nitrilium ion at event **1**. The resulting ketenimine intermediate cyclises at event **2** to give compound **4.8** as the final product.




**Scheme 4.6:** Proposed reaction mechanism for the formation of **4.8.**

### 4.4 The structural elucidation of derivatives 4.8a-u by FTIR, NMR and HRMS techniques

The obtained structures **4.8a-u** were analysed by FTIR, NMR spectroscopy and HRMS. By way of example for this group of compounds, spectroscopic characteristics for compound **4.8a** (Figure 4.4) are discussed. The $^{1}$H NMR spectrum shows a broad singlet for NH at δ = 8.73; two doublets, one for the CH at position **2** at 7.36-7.33 ppm with a *J* value of 10.8 Hz and one for the CH at position **3** at 6.51-6.48 ppm with a *J* value of 10.4 Hz; a singlet for the CH at δ =6.35 from position **6**; two singlets due to methoxy protons at δ = 3.75 and δ = 3.71 and one

singlet for the nine *t*-Bu protons at δ = 1.42. The $^{13}$C NMR spectrum of **4.8a** showed 13 distinct resonances in accordance with the proposed structure.

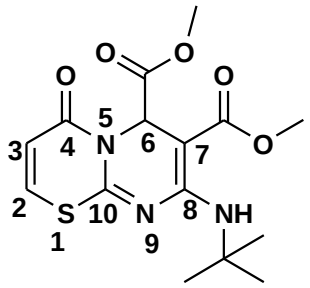



**Figure 4.4:** Structure of dimethyl 8-(*tert*-butylamino)-4-oxo-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate **4.8a**

The mass spectrum of **4.8a** showed the molecular ion $[M+H]^+$ peak at m/z 354.1113 which is consistent with the mass of the proposed product which is calculated as 354.1118 as shown in Table 4.3. The FTIR spectrum displayed characteristic absorption bands for carbonyl groups at 1728 $cm^{-1}$, 1690 $cm^{-1}$ and1648 $cm^{-1}$ and for the amine group at 2982 $cm^{-1}$.

Generally the rest of the series **4.8 b-u** showed similar patterns: the FTIR spectra of this set of compounds showed characteristic absorption bands of N-H in the range of 3285 $cm^{-1}$ to 2851 $cm^{-1}$, while the C=O stretching band appeared in the range of 1780 $cm^{-1}$ to 1602 $cm^{-1}$. The $^1$H NMR spectra of compounds **4.8b-u** exhibited one singlet signal in the region of δ 8.77-8.00 ppm corresponding to the NH, and one singlet at δ 6.39-6.24 ppm corresponding to CH. $^{13}$C NMR spectra showed characteristic signals in the region of δ 171.2-160.7 corresponding to three C=O groups and a CH at position **6** with signals at δ 53.0-51.1 ppm. ESI-LCMS was used to confirm synthesis of compounds **4.8b-u** within an acceptable tolerance of ± 0.005. Chromatograms exhibited protonated parent molecular ions $[M+1]^+$ represented in Table 4.5.

**Table 4.5**: The chemical formulae, calculated and measured molecular ion values of **4.8a-u**

| Compound | Chemical Formula [M+1] | Calculated m/z | Measured m/z |
|---|---|---|---|
| **4.8a** | $C_{15}H_{20}N_3O_5S^+$ | 354.1118 | 354.1113 |
| **4.8b** | $C_{17}H_{24}N_3O_5S^+$ | 382.1431 | 382.1439 |
| **4.8c** | $C_{16}H_{22}N_3O_5S^+$ | 368.1275 | 368.1279 |
| **4.8d** | $C_{18}H_{26}N_3O_5S^+$ | 396.1588 | 396.1597 |
| **4.8e** | $C_{20}H_{30}N_3O_5S^+$ | 424.1901 | 424.1906 |
| **4.8f** | $C_{22}H_{34}N_3O_5S^+$ | 452.2214 | 452.2223 |
| **4.8g** | $C_{18}H_{24}N_3O_5S^+$ | 394.1431 | 394.1433 |
| **4.8h** | $C_{20}H_{28}N_3O_5S^+$ | 422.1744 | 422.1712 |
| **4.8i** | $C_{17}H_{24}N_3O_5S^+$ | 382.1430 | 382.1427 |
| **4.8j** | $C_{19}H_{28}N_3O_5S^+$ | 410.1744 | 410.1749 |
| **4.8k** | $C_{21}H_{32}N_3O_5S$ | 438.2057 | 438.2021 |
| **4.8l** | $C_{23}H_{36}N_3O_5S^+$ | 466.2370 | 466.2333 |
| **4.8m** | $C_{18}H_{26}N_3O_5S$ | 396.1588 | 396.1545 |
| **4.8n** | $C_{20}H_{30}N_3O_5S^+$ | 424.1901 | 424.1873 |
| **4.8o** | $C_{22}H_{34}N_3O_5S^+$ | 452.2214 | 452.2190 |
| **4.8p** | $C_{24}H_{38}N_3O_5S^+$ | 480.2527 | 480.2526 |
| **4.8q** | $C_{21}H_{24}N_3O_5S^+$ | 430.1431 | 430.1445 |
| **4.8r** | $C_{23}H_{28}N_3O_5S^+$ | 458.1744 | 458.1744 |
| **4.8s** | $C_{25}H_{32}N_3O_5S^+$ | 486.2057 | 486.2044 |
| **4.8t** | $C_{27}H_{36}N_3O_5S^+$ | 514.2370 | 514.2350 |
| **4.8u** | $C_{23}H_{26}N_3O_5S^+$ | 456.1588 | 456.1592 |

During $^1H$ NMR characterisation it was noticed that the $CH_2$ in the middle of the tetramethylbutyl group for compound **4.8f**, **4.8l** and **4.8t** had an equal gap between the lines, giving a signal that appeared like a quartet as shown in Figure 4.5. This set of compounds seem to have a large geminal coupling constant ranging from 14.4-14.8 Hz. Thus it appears that the

two protons are not equivalent and are appearing as two doublets that are too close to each other to be properly resolved because there are not three adjacent protons that could give rise to a quartet splitting pattern with such large coupling constants. This pattern was only present when 1,1,3,3-tetramethylbutyl isocyanide **4.4b** and DEtAD **4.5b** were used as part of the 3-CR with thiazines **4.3b-c** and **4.3e**.

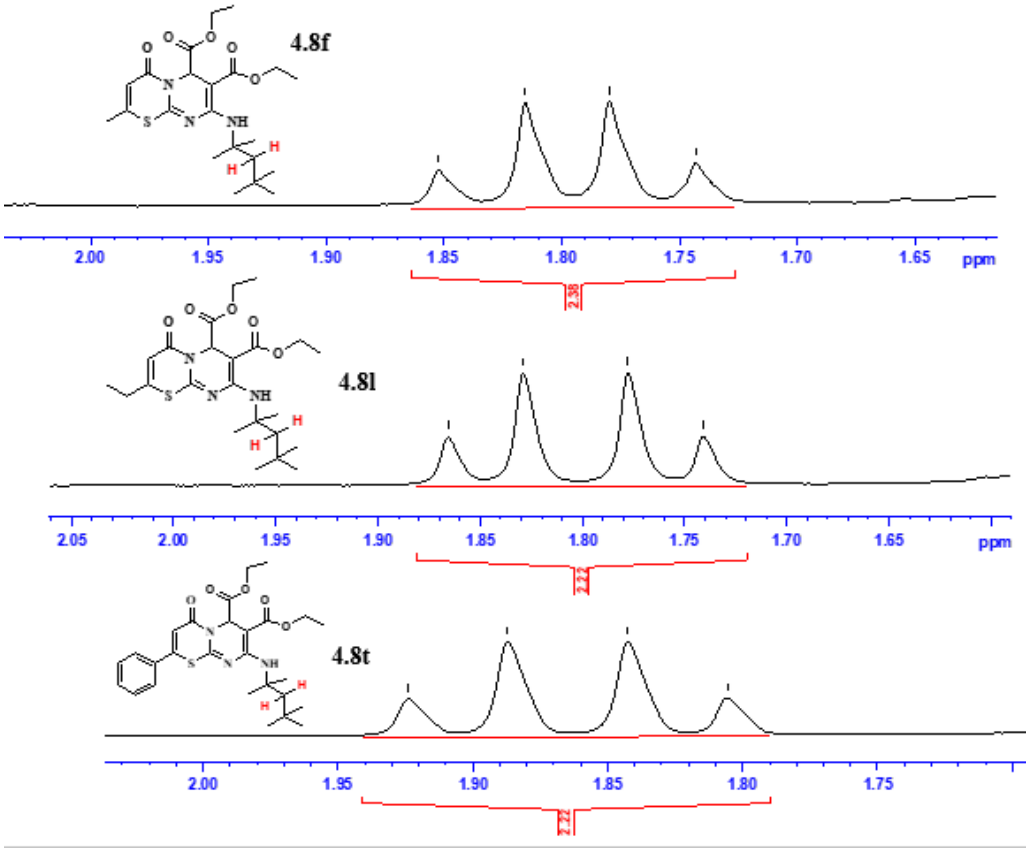



**Figure 4.5:** Splitting patterns of $CH_2$ in the middle of the tetramethylbutyl

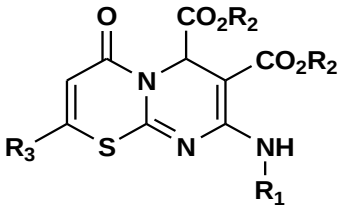
A total of twenty one dimethyl 8-(*tert*-butylamino)-4-oxo-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate derivatives **4.8a-u** were synthesised and to the best of our knowledge none of these compounds have ever been reported before. Compounds **4.3c** and **4.3d** are also novel.

### 4.5 Biological evaluations of thiazine derivative

The synthesised thiazines **4.8a-u** were subjected to toxicity evaluations in the MT4 cell line at 100 µM. Auranofin was used as a cytotoxic control compound starting at 100 µM serial dilution series and DMSO was used as a background control. At this concentration nineteen compounds

**4.8a-b**, **4.8d-l** and **4.8n-u** were found to cause cell reduction at $CC_{50}$ values below 80μM, as recorded in Table 4.6. The fact that these compounds were found to be able to kill viable cells at a concentration below 100 μM makes them relatively toxic. Only two compounds **4.8c** and **4.8m** were found to have $CC_{50}$ values above 100 μM which makes them the least toxic of this set of compounds. Compound **4.8c** contains *tert*-butyl group at $R_1$ and methyl groups at $R_2$ and $R_3$, while compound **4.8m** contains *tert*-butyl at $R_1$, a methyl group at $R_2$ and an ethyl group at $R_3$. Most of this set of compounds were found to be toxic, which is in contrast to the pyrimidines synthesised in Chapter 3.

**Table 4.6**: $CC_{50}$ values of synthesised thiazine derivatives **4.8a-u**




| Compound | $R_1$ | $R_2$ | $R_3$ | $CC_{50}$ |
|---|---|---|---|---|
| **4.8a** | *t*-Bu | $CH_3$ | H | 7.3 μM |
| **4.8b** | t-Bu | $CH_3CH_2$ | H | 12 μM |
| **4.8c** | t-Bu | $CH_3$ | $CH_3$ | 102 μM |
| **4.8d** | t-Bu | $CH_2CH_3$ | $CH_3$ | 51 μM |
| **4.8e** | tetramethylbutyl | $CH_3$ | $CH_3$ | 16 μM |
| **4.8f** | tetramethylbutyl | $CH_2CH_3$ | $CH_3$ | 33 μM |
| **4.8g** | cyclohexyl | $CH_3$ | $CH_3$ | 33 μM |
| **4.8h** | cyclohexyl | $CH_2CH_3$ | $CH_3$ | 51 μM |
| **4.8i** | *t*-Bu | $CH_3$ | $CH_2CH_3$ | 49 μM |
| **4.8j** | *t*-Bu | $CH_2CH_3$ | $CH_2CH_3$ | 80 μM |
| **4.8k** | tetramethylbutyl | $CH_3$ | $CH_2CH_3$ | 25uM |
| **4.8l** | tetramethylbutyl | $CH_2CH_3$ | $CH_2CH_3$ | 20 μM |
| **4.8m** | *t*-Bu | $CH_3$ | $CH_2CH_2CH_3$ | 120 μM |
| **4.8n** | *t*-Bu | $CH_2CH_3$ | $CH_2CH_2CH_3$ | 17 μM |
| **4.8o** | tetramethylbutyl | $CH_3$ | $CH_2CH_2CH_3$ | 21 μM |
| **4.8p** | tetramethylbutyl | $CH_2CH_3$ | $CH_2CH_2CH_3$ | 16 μM |

| Compound | $R_1$ | $R_2$ | $R_3$ | $CC_{50}$ |
|---|---|---|---|---|
| **4.8q** | *t*-Bu | $CH_3$ | $C_6H_6$ | 39 µM |
| **4.8r** | *t*-Bu | $CH_2CH_3$ | $C_6H_6$ | 26 µM |
| **4.8s** | tetramethylbutyl | $CH_3$ | $C_6H_6$ | 57 µM |
| **4.8t** | tetramethylbutyl | $CH_2CH_3$ | $C_6H_6$ | 33 µM |
| **4.8u** | cyclohexyl | $CH_3$ | $C_6H_6$ | 27 µM |

$CC_{50}$ - concentration of compounds that causes 50% reduction of cell growth

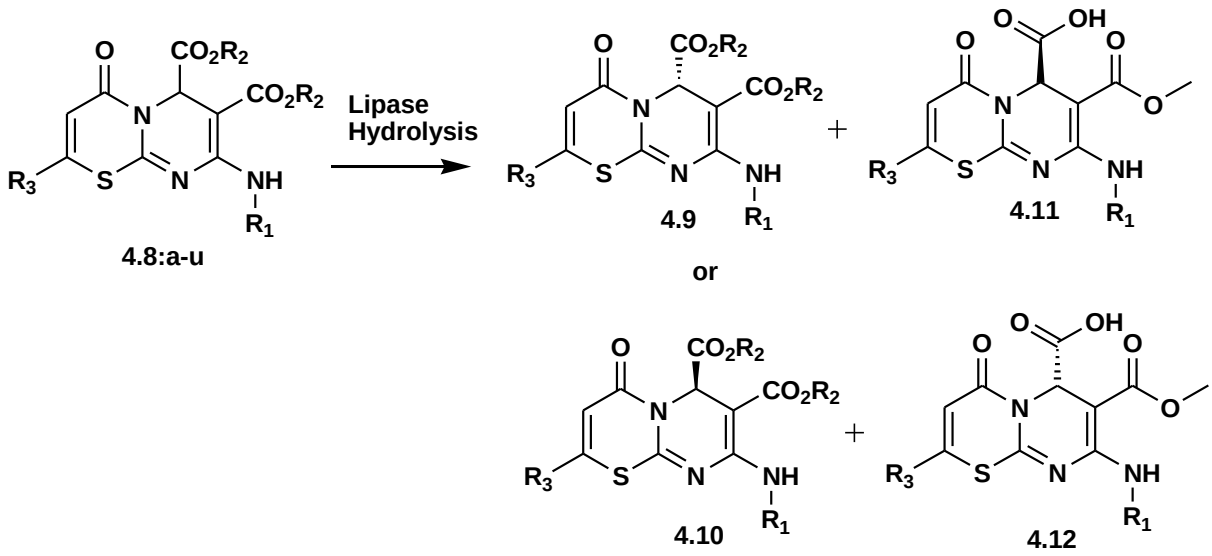
Based on the cytotoxicity studies this set of compounds were found to be relatively toxic and antiviral activities studies were not conducted. The antimicrobial activity studies of this set of compounds showed no significant activity against the bacterial strains.

### 4.6. Conclusions

A series of dimethyl 8-(*tert*-butylamino)-4-oxo-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate derivatives were achieved *via* 3-CR in order to develop drug-like compounds. The novel compounds **4.8a-u** were prepared by reaction of alkyl isocyanides **4.4** with dialkyl acetylenedicarboxylates **4.5** in the presence of 2-amino-4*H*-1,3-thiazin-4-one derivatives **4.3** in good yields. The same reactions were repeated to investigate solvent effects using acetone, ethanol and isopropanol as green solvents which resulted in moderate yields. The acidic $pK_a$ values of **4.3a-e** likely contributed to the selectivity and ease of the reaction to form the products. 2-Amino-6-propyl-4*H*-1,3-thiazin-4-one **4.3d** and 2-amino-6-ethyl-4*H*-1,3-thiazin-4-one **4.3c** were found to be novel compounds. The attempt to replace dichloromethane with green solvents such as acetone, ethanol and isopropanol gave average to low yields, however acetone was found to give better average yields compared to ethanol and isopropanol. None of the compounds showed significant antimicrobial activity.

### 4.7. Future work

The plan for future work is to apply enzymatic kinetic resolution of the ester group on compounds **4.8a-u** using a collection of lipase enzymes, as shown in Scheme 4.7. This will give rise to the enantiopure products of **4.9-4.12** using green chemistry principles.




**Scheme 4.7:** Possible structural modifications of dimethyl 8-(*tert*-butylamino)-4-oxo-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate derivatives.

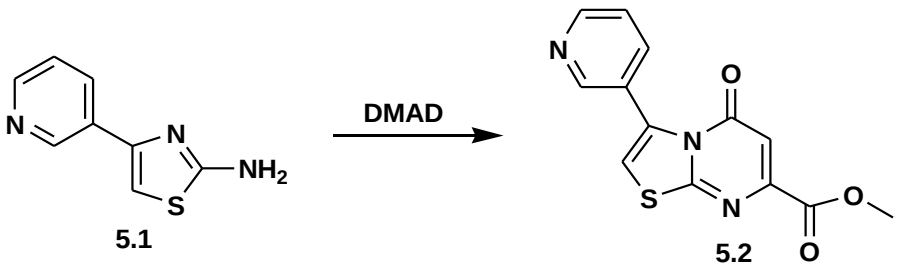
# CHAPTER 5

# Synthesis of fused thiazole-pyrimidine derivatives *via* 2-CR and 3-CR using electron deficient alkynes as Michael acceptors.

## 5.1. Introduction

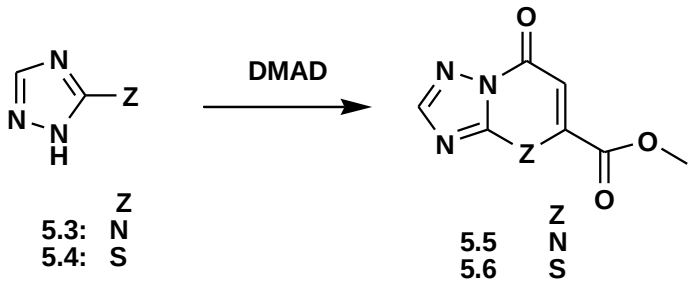
One of the study objectives was to investigate the reactivity of electron deficient alkynes such as DMAD\DEtAD towards the prepared five-membered ring and six-membered ring substrates discussed in Chapters 3 and 4. In this chapter we present 2-CR and 3-CR methods for the preparation of a library of thiazolo-pyrimidinesand their analogues. The synthesis of these scaffolds was also triggered by the co-products obtained in Chapter 3 where DMAD was found to be directly reacting with some of the prepared five-membered rings *via* 2-CR Michael additions in the presence of a third component, an isocyanide. Thus, in these reactions there is competition between reactions of the substrate with DMAD itself and reaction with the DMAD-isocyanide zwitterion adduct. As discussed in Chapter 3 and Chapter 4 the acidity or basicity of the starting heterocyclic substrates influences the product formation.

Using a 2-CR, thiazolo-pyrimidines can be obtained from suitable 2-aminothiazole derivatives **5.1** and the appropriate Michael acceptor[106] such as DMAD, diethylmalonate, ethyl cyanoacetate or cyanoacetamide, ketonitriles, trichloromethyl,[209] ethyl acetoacetate[210] or bis(2,4,6-trichlorophenyl) malonate[211] as the second component under various conditions to give the desired products. Scheme 5.1 illustrates the reaction with DMAD to give product **5.2**.




**Scheme 5.1**. Existing route to thiazolo-pyrimidine carboxylates *via* 2-CR.

On the other hand the reactions of triazolo-based compounds such as 1*H*-1,2,4-triazol-5-amine[186] **5.3** and 1*H*-1,2,4-triazole-5-thiol[187] **5.4** with DMAD afford methyl 5-oxo-1,5-dihydro-[1,2,4]triazolo[4,3-a]pyrimidine-7-carboxylate[104] **5.5** and methyl 5-oxo-5*H*-[1,2,4]triazolo[3,4-*b*][1,3]thiazine-7-carboxylate[105] **5.6** as shown in Scheme 5.2.




**Scheme 5.2.** Existing route to compounds **5.5** and **5.6** *via* 2-CR.

The investigation of substrate reactivity with dialkyl acetylenedicarboxylate (DMAD/DEtAD/DTAD) *via* a 2-CR proceeded by firstly subjecting eleven (11) weakly acidic to basic substrates **5.3**, **5.4**, **5.7a-h** and **5.8**, to the reaction as shown in Figure 4.1. The substrate scope was increased by using eight (8) more acidic substrates **5.9a-b**, **5.10** and **5.11a-e**. The use of substrates **5.8**, **5.9**, **5.10** and **5.11** to react with dialkyl acetylenedicarboxylates has not been reported previously and so we investigated their suitability as substrates in this reaction.

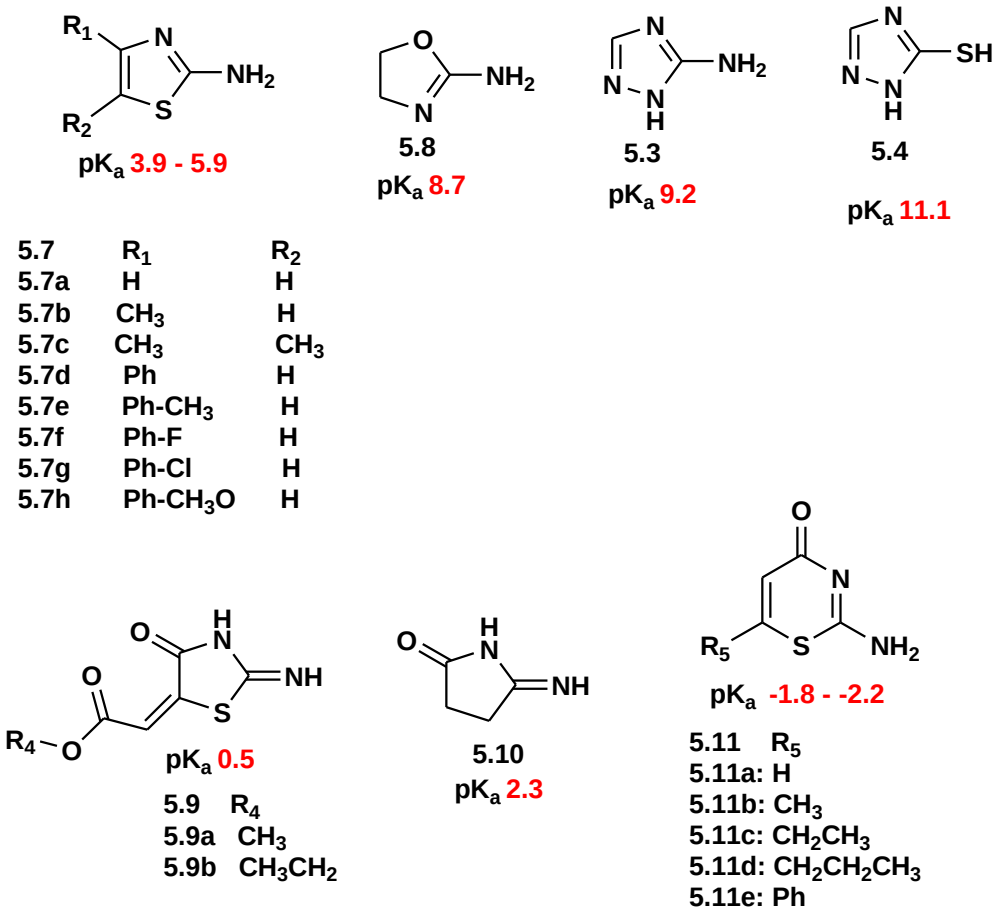
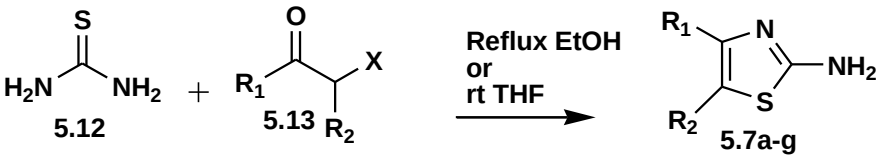



**Figure 5.1**: Acidic and basic substrates **5.3**, **5.4**, **5.7a-h**, **5.8**, **5.9a-b**, **5.10** and **5.11a-e**.

### 5.2 Preparation of substrates 5.3, 5.4, 5.7a-h, 5.8, 5.9a-b, 5.10 and 5.11a-e.

In order to obtain thiazole-pyrimidine derivatives and their analogues using 2-CR, the following substrates **5.3, 5.4, 5.7a-h, 5.8, 5.9a-b, 5.10** and **5.11a-e** were prepared as intermediates to react with DMAD/DEtAD. Substrates **5.3, 5.4**, **5.7a**, **5.8** and **5.9** were prepared as reported in Chapter 3, whereas substrates **5.11** were prepared and discussed in Chapter 4. The reaction of thiourea **5.12** and α-chloroketones **5.13** in absolute ethanol under reflux conditions gave intermediates **5.7b-c** as shown in Scheme 5.3. Compounds **5.7d-h** were achieved under mild conditions at room temperature using THF as solvent.

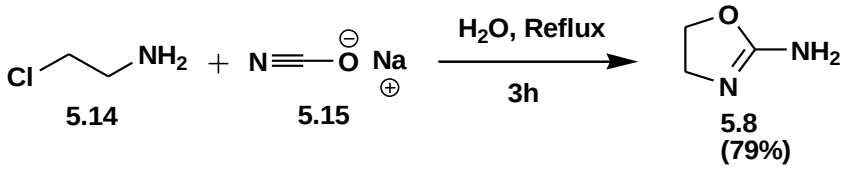



| 5.7 | $R_1$ | $R_2$ | X | Yield% |
|---|---|---|---|---|
| 5.7a | H | H | Cl | 96 |
| 5.7b | $CH_3$ | H | Cl | 91 |
| 5.7c | $CH_3$ | $CH_3$ | Cl | 81 |
| 5.7d | Ph | H | Br | 92 |
| 5.7e | 4-Ph-$CH_3$ | H | Br | 85 |
| 5.7f | 4-Ph-F | H | Cl | 79 |
| 5.7g | 4-Ph-Cl | H | Br | 87 |
| 5.7h | 4-Ph-$OCH_3$ | H | Br | 86 |

**Scheme 5.3.** Synthesis of 2-aminothiazole derivatives.

Preparation of substrates **5.7b** (86%), **5.7d** (89%), **5.7f** (75%) and **5.7h** (94%) has been reported by Keshari and co-workers[212] using carbon tetrabromide at room temperature in the presence of triethylamine as a base and acetonitrile as solvent. Preparation of substrate **5.7c** was reported by Veretennikov and Pavlov[210] using 2-propanol as a solvent obtaining a yield of 72%, which was improved to 81% by using ethanol and increasing the reaction time. Substrate **5.7d** was prepared by Zarnegar and co-workers[188] who obtained a yield of 93% from methylcarbonyl activated by HBr or HI, whereas we obtained a comparable yield of 92% using ethanol and α-haloketones. Compounds **5.7e** and **5.7g** were prepared by Das and co-workers[213] using ammonium-12-molybdophosphate as a catalyst and methanol as solvent at room temperature in percentage yields of 97% and 98%, respectively. Lin and co-workers[214] also reported the preparation of **5.7d** (94%), **5.7f** (87%) and **5.7h** (90%) using poly(ethylene glycol-400) and sodium carbonate. Morales-Bonilla and co-workers[215]reported the synthesis of **5.7d** (69%), **5.7e** (66%), **5.7h** (36%) and **5.7g** (86%) using iodine and ammonium hydroxide.

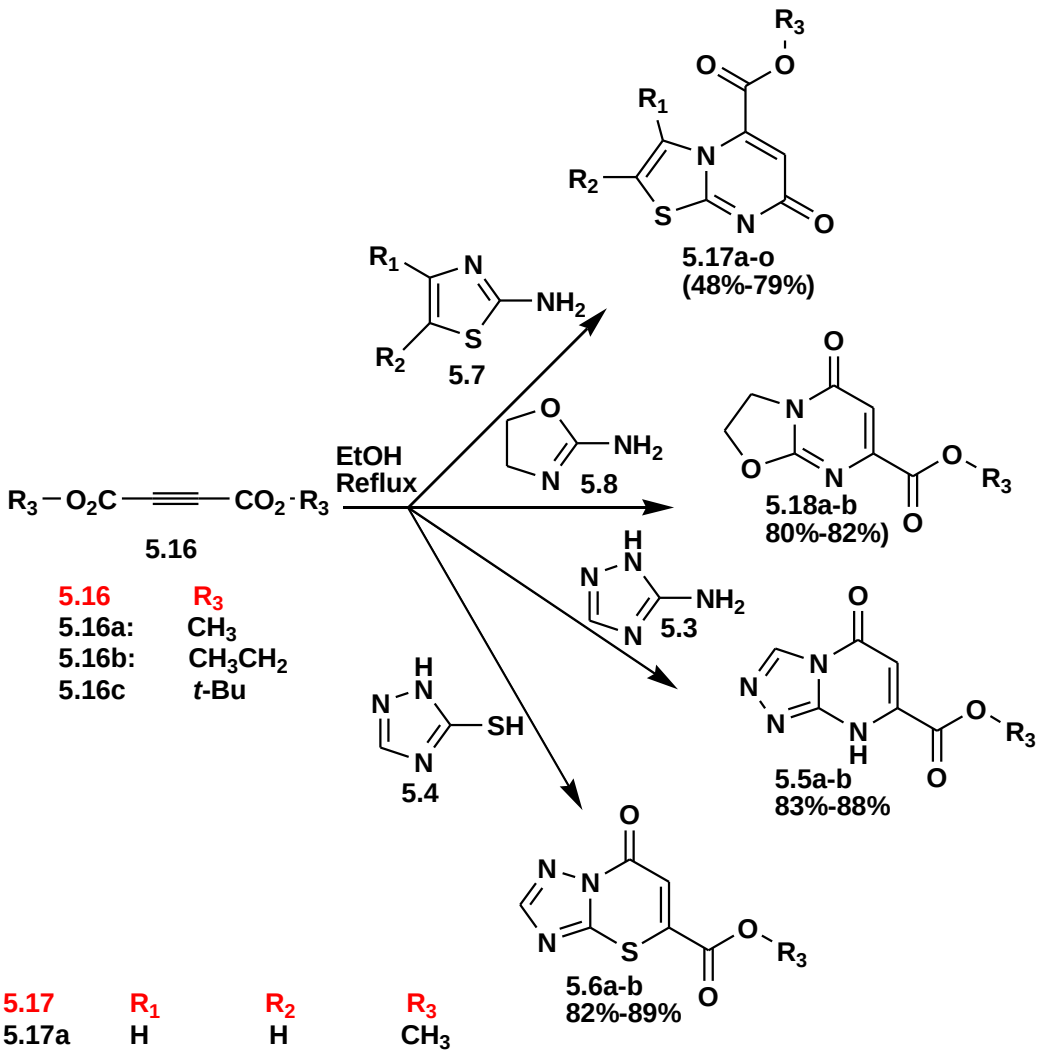
2-Imino-1,3-oxazolidine **5.8** was prepared as discussed in Chapter 3 according to Latli and co-workers resulting in 79% yield[189] (Scheme 5.4).




**Scheme 5.4.** Synthesis of 2-imino-1,3-oxazolidine **5.8**.

## 5.3. Synthesis of thiazole-pyrimidine derivatives and their analogues *via* 2-CR using electron deficient dialkyl acetylenedicarboxylates as Michael acceptors.

After the successful preparation of the required substrates; DMAD **5.16a** was firstly treated with substrate **5.7a-h** under ethanol reflux conditions to obtain product **5.17a-g**. Repetition of these reactions using DEtAD **5.16b** gave rise to product **5.17h-m** as expected whilst DTAD **5.16c** and substrate **5.7a** gave rise to product **5.17o** (Scheme 5.5). The success of this reaction led us to the use of substrate **5.3, 5.4** and **5.8**. These substrates gave rise to products **5.5a-b**, **5.6a-b** and **5.18a-b** respectively as shown in Scheme 5.5 in good yields. Substrate **5.3** is capable of forming two different isomeric products of **5.5** as reported by Reimlinger and co-workers.[104] This results from either the ring-nitrogen reacting first with DMAD to form the first isomer or by initial reaction of the side chain nitrogen with DMAD to form the second isomer. Surprisingly, the reactions of substrate 4-(4-methoxyphenyl)thiazol-2-amine **5.7h** with **5.16a-b** were not successful under the above mentioned conditions resulting in the recovery of starting materials. This might suggest the possibility of steric hindrance or electronic effect due to the methoxy group.




| 5.16 | $R_3$ |
|---|---|
| 5.16a: | $CH_3$ |
| 5.16b: | $CH_3CH_2$ |
| 5.16c | *t*-Bu |

| 5.17 | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 5.17a | H | H | $CH_3$ |
| 5.17b | $CH_3$ | H | $CH_3$ |
| 5.17c | $CH_3$ | $CH_3$ | $CH_3$ |
| 5.17d | Ph | H | $CH_3$ |
| 5.17e | 4-Ph-$CH_3$ | H | $CH_3$ |
| 5.17f | 4-Ph-F | H | $CH_3$ |
| 5.17g | 4-Ph-Cl | H | $CH_3$ |
| 5.17h | H | H | $CH_3CH_2$ |
| 5.17i | $CH_3$ | H | $CH_3CH_2$ |
| 5.17j | $CH_3$ | $CH_3$ | $CH_3CH_2$ |
| 5.17k | Ph | H | $CH_3CH_2$ |
| 5.17l | 4-Ph-$CH_3$ | H | $CH_3CH_2$ |
| 5.17m | 4-Ph-F | H | $CH_3CH_2$ |
| 5.17n | 4-Ph-Cl | H | $CH_3CH_2$ |
| 5.17o | H | H | *t*-Bu |

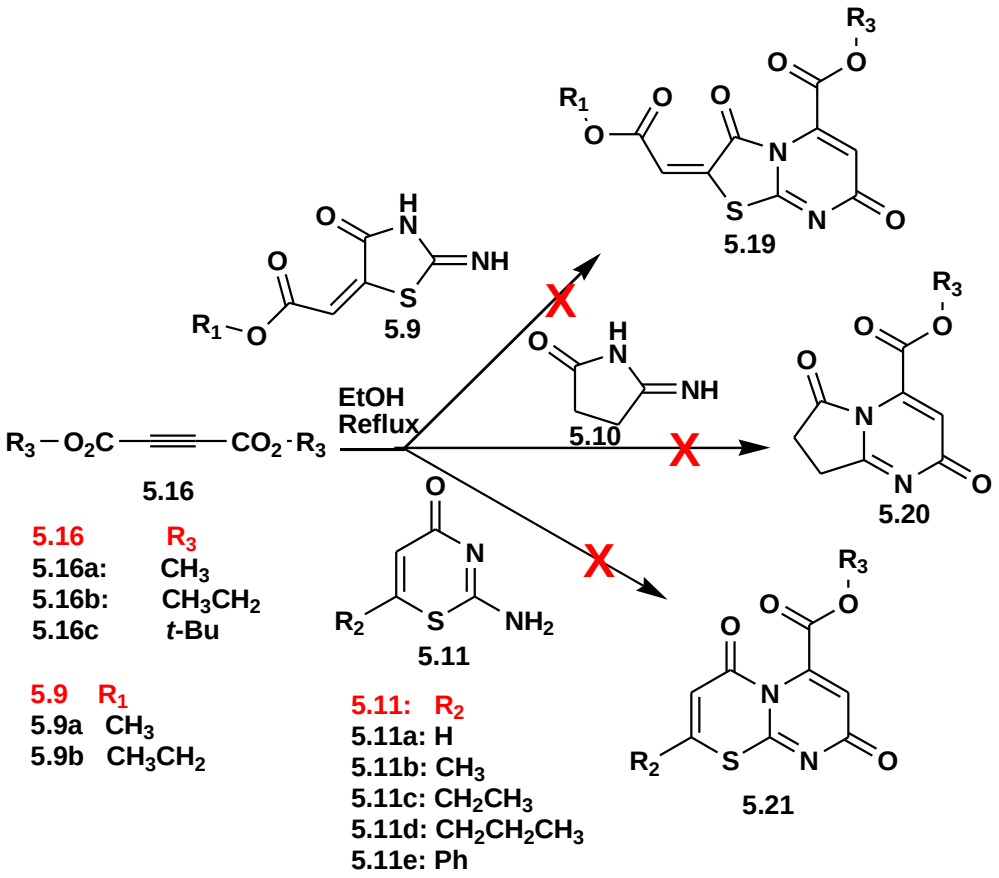
| 4.5 | $R_3$ |
|---|---|
| 5.5a: | $CH_3$ |
| 5.5b: | $CH_3CH_2$ |

| 5.5 | $R_3$ |
|---|---|
| 5.6.b: | $CH_3$ |
| 5.6.d: | $CH_3CH_2$ |

| 5.18 | $R_3$ |
|---|---|
| 5.18a: | $CH_3$ |
| 5.18b: | $CH_3CH_2$ |

**Scheme 5.5:** Synthesis of **5.5**, **5.6**, **5.17** and **5.17** *via* 2-CR.

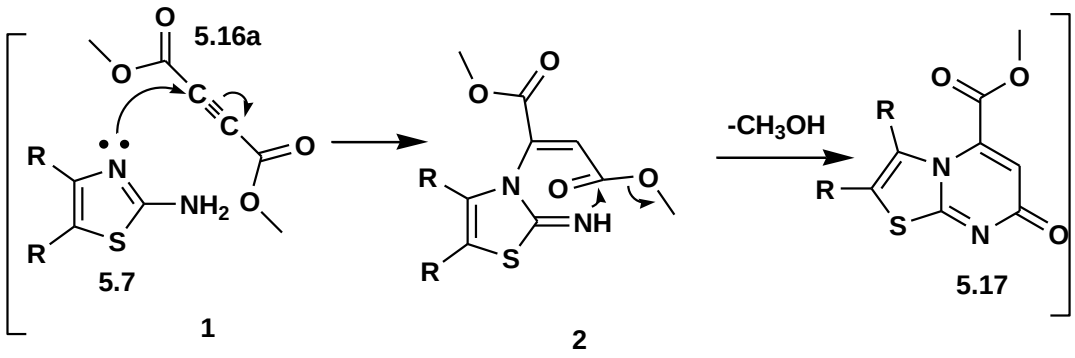
Attempts to use the acidic substrates **5.9a-b**, **5.10** and **5.11a-e** to react with DMAD **5.16** as shown in Scheme 5.6 under the same conditions (Scheme 5.5) to give product **5.19-5.21** were not successful. The starting materials were recovered instead of desired products.




**Scheme 5.6:** Attempted synthesis of **5.19**, **5.20** and **5.21**.

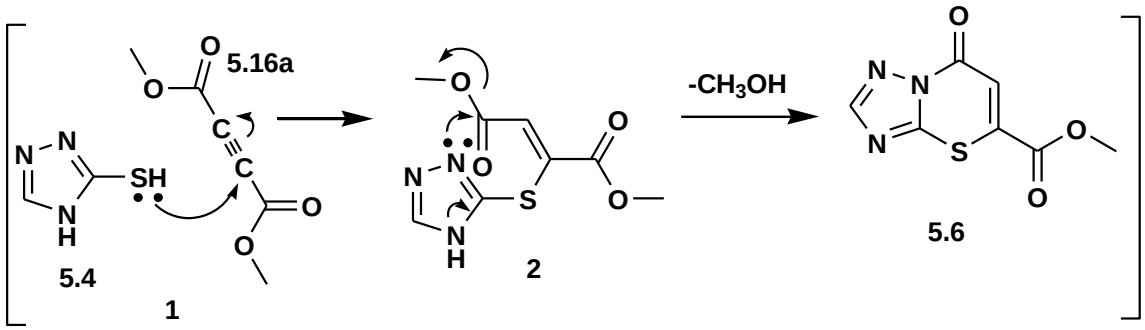
Based on literature records Reimlinger[216] reported preparation of product **5.17a** and later on Acheson and Wallis[217] also reported the reaction of substrate **5.7a** and DMAD **5.16a** using methanol under reflux to obtain product **5.17a** with a melting point of 268-273ºC. When using the 2-CR of substrate **5.7a** and **5.16a** method we obtained product **5.17a** in a yield of 71%. Crank and Khan[218] reported synthesis of product **5.17b** from substrate 4-methylthiazol-2-amine **5.7b** and **5.16a** at room temperature using acetonitrile, obtaining 30% yield. In our case using 2-CR we obtained product **5.17b** in a much better yield of 68%. Herrling[219] reported the synthesis of products **5.17d**, **5.17k** and **5.17l**. Shah and co-workers[105] reported synthesis of product **5.6a** using acetonitrile at room temperature in 74% yield and Reimlinger and co-workers[104] reported preparation of product **5.5a** using ethanol under reflux in 10% yield and 29% yield of an isomeric product. When using the 2-CR of substrate **5.3** and **5.4** with DMAD **5.16a** we obtained products **5.5a** and **5.6a** in good yields of 89% and 83%, respectively. To the best of our knowledge fourteen (14) compounds (**5.17c**, **5.17e-j**, **5.17m-o**, **5.18a-b**, **5.5b** and

**5.6b**) have not been reported previously. Scheme 5.7 represents a proposed mechanism from compound **5.7** to the cyclised product **5.17** (see Acheson and Wallis[217]). The reaction proceeds by initial nucleophilic attack of the ring-nitrogen of **5.7** on the α,β-unsaturated ester of **5.16a** at event **1** forming intermediate which undergoes internal cyclisation at event **2** by nucleophilic attack of the side-chain nitrogen on the ester carbonyl carbon, followed by the loss of methanol, to afford product **5.17**.The ring-nitrogen react first with one of the acetylenic carbon atoms followed by the side chain nitrogen attacking the carbonyl carbon leading to the structural arrangement **5.17** at Scheme 5.7. This arrangement concurs with the reported structures from Acheson and Wallis[217] and Herrling.[219]




**Scheme 5.7:** Proposed reaction mechanism for the formation of product **5.17**.

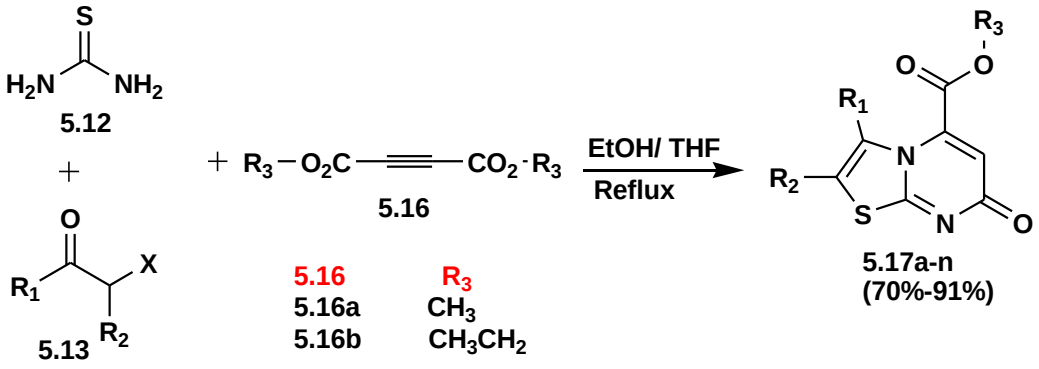
The proposed mechanism for the synthesis of product **5.6** is represented in Scheme 5.8 (see Shah and co-workers[105]). The initial attack from the sulphur lone pairs on one of the acetylenic carbon atoms from **5.16a** at event **1** is followed by proton transfer to give rise to the intermediate at event **2** which undergoes cyclisation to give product **5.6**. The structural formation of product **5.6** results from the side chain sulphur reacting first with one of the acetylenic carbon atoms then the ring-nitrogen attacks the carbonyl carbon leading to the product (Scheme 5.8).




**Scheme 5.8.** Proposed mechanism for synthesis of product **5.6** *via* 2-CR

## 5.4 Synthesis of thiazole-pyrimidine derivatives via 3-CR of thiourea, α-haloketone and DMAD.

In order to improve the average yields obtained using the 2-CR for thiazole-pyrimidines **5.17a-n** we applied a second approach *via* 3-CR of thiourea **5.12**, α-haloketone **5.13** and DMAD **5.16** to give thiazole-pyrimidines **5.17**. The procedure was done by reacting thiourea **5.12** with chloroacetaldehyde **5.13a** in the presence of DMAD **5.16a** using a 1:1 solution of ethanol and THF under reflux for at least 12 h, which gave rise to product **5.17a** in a yield of 87%, a significant improvement from the 72% yield obtained *via* 2-CR. After the success of this reaction we extended the scope of the 3-CR approach by reacting thiourea **5.12** with α-haloketones **5.13b-g** in the presence of DMAD **5.16a** to obtain products **5.17b-5.17g** in a better yield compared the 2-CR approach. The reactions were repeated using DEtAD **5.16b** and gave rise to products **5.17h-n**, as expected (Scheme 5.9). The reactions of substrate **5.13h** and **5.13i** were not successful and resulted in recovery of starting materials after the reaction.



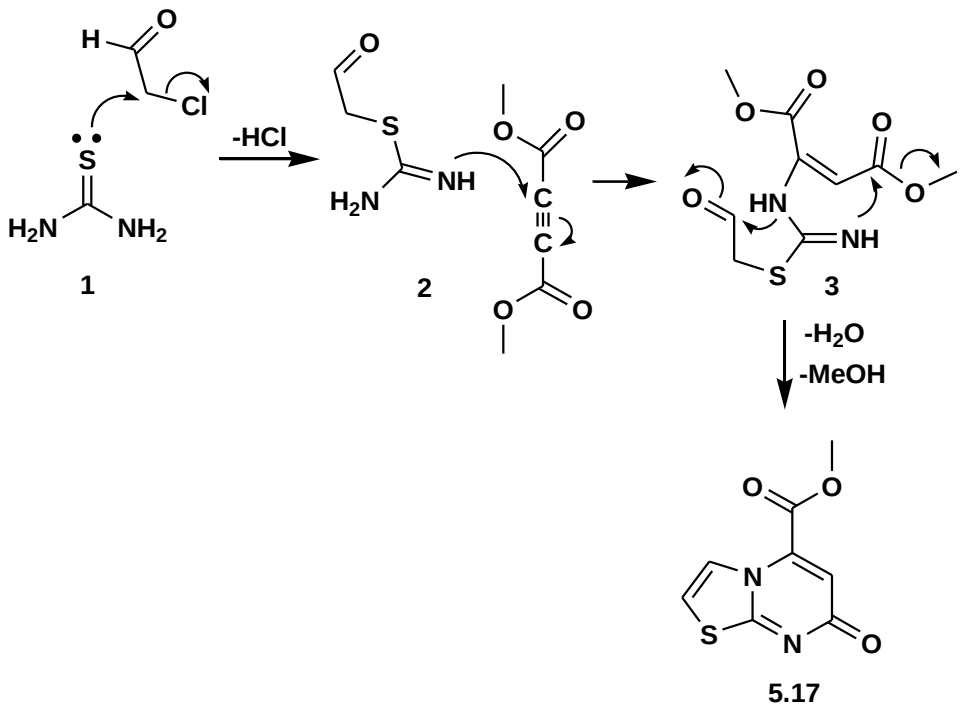

| 5.16 | $R_3$ |
|---|---|
| 5.16a | $CH_3$ |
| 5.16b | $CH_3CH_2$ |

| 5.13 | $R_1$ | $R_2$ | X |
|---|---|---|---|
| 5.13a | H | H | Cl |
| 5.13b | $CH_3$ | H | Cl |
| 5.13c | $CH_3$ | $CH_3$ | Cl |
| 5.13d | Ph | H | Br |
| 5.13e | 4-Ph-$CH_3$ | H | Br |
| 5.13f | 4-Ph-F | H | Cl |
| 5.13g | 4-Ph-Cl | H | Br |
| 5.13h | 4-Ph-$OCH_3$ | H | Br |
| 5.13i | 4-Ph-$NO_2$ | H | Br |

| 5.17 | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 5.17a | H | H | $CH_3$ |
| 5.17b | $CH_3$ | H | $CH_3$ |
| 5.17c | $CH_3$ | $CH_3$ | $CH_3$ |
| 5.17d | Ph | H | $CH_3$ |
| 5.17e | 4-Ph-$CH_3$ | H | $CH_3$ |
| 5.17f | 4-Ph-F | H | $CH_3$ |
| 5.17g | 4-Ph-Cl | H | $CH_3$ |
| 5.17h | H | H | $CH_3CH_2$ |
| 5.17i | $CH_3$ | H | $CH_3CH_2$ |
| 5.17j | $CH_3$ | $CH_3$ | $CH_3CH_2$ |
| 5.17k | 4-Ph | H | $CH_3CH_2$ |
| 5.17l | 4-Ph-$CH_3$ | H | $CH_3CH_2$ |
| 5.17m | 4-Ph-F | H | $CH_3CH_2$ |
| 5.17n | 4-Ph-Cl | H | $CH_3CH_2$ |

**Scheme 5.9.** Synthesis of **5.17a-n** *via* 3-CR.

Attempts to synthesise products **5.17a-n** at room temperature using 3-CR were not successful and resulted in recovery of starting materials. The best conditions for the synthesis of **5.17a-n** were found to be using ethanol/THF from room temperature to reflux conditions, *via* the 3-CR with a highest yield of 91% from **5.15i** as represented in Table 5.1. Based on literature reports on the mechanism of thiazole formation[220,221] we propose that the reaction is likely to proceed by initial attack of the sulphur lone pairs on the α-halo carbon at event **1** to form an imine intermediate. The imine nitrogen attacks one of the acetylenic carbon atoms from DMAD at event **2**, resulting in an intermediate which then undergoes internal cyclisation at event **3** to afford **5.17**, as shown in Scheme 5.10. The reactivity of the intermediate at event **2** attacking the DMAD directly suggests a possible reason for the difference in yields between the 3-CR and 2-CR reactions (Scheme 5.7). However, this is speculation only and there is no evidence available as to the exact sequence of events.




**Scheme 5.10.** Proposed possible reaction mechanism for the formation of compounds methyl 7-oxo-7*H*-thiazolo[3,2-*a*]pyrimidine-5-carboxylates **5.17** *via* 3-CR.

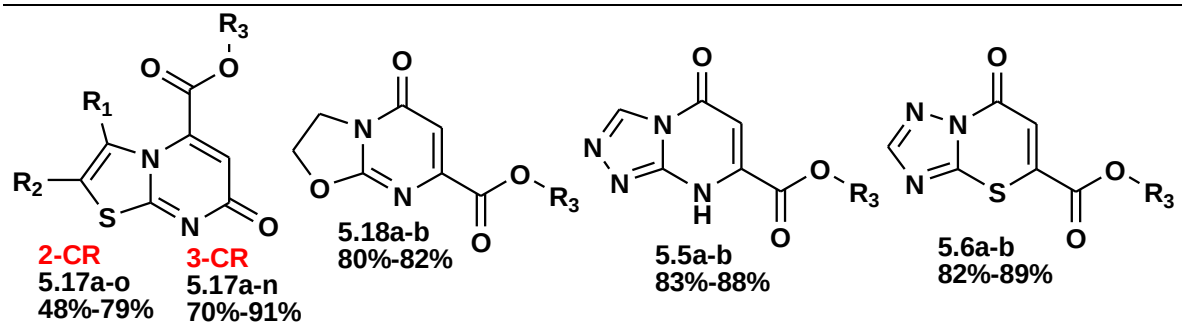
## 5.5 Comparison between 2-CR and 3-CR approach

### *5.5.1 Isolated yields of thiazole-pyrimidine derivatives synthesised via 2-CR and 3-CR*

Table 5.1 presents isolated yields of thiazole-pyrimidine derivatives and their analogues obtained from 2-CR and 3-CR. Initially the 2-CR was applied by treating 2-aminothiazole derivatives **5.7** with DMAD\DEtAD and the highest yield obtained was 79% for **5.17j**, 72% for **5.17a** and 71% for **5.17i**. Most of the products resulted in average yields, whilst **5.17o** gave 70% from the reaction of DTAD and substrate **5.7a**. When using 2-CR substrates 1*H*-1,2,4-triazol-5-amine **5.3**, 1*H*-1,2,4-triazole-5-thiol **5.4** and 2-imino-1,3-oxazolidine **5.8**, they reacted smoothly with DMAD\DEtAD and resulted in good yields of between 80%-89% of products **5.5**, **5.6** and **5.18**. DMAD and DEtAD seem to be most reactive towards substrates **5.3**, **5.4** and **5.8**. No improvement of the 2-CR method was needed for these reactants. The 3-CR method was applied by reacting thiourea **5.12**, α-haloketone **5.13** and DMAD\DEtAD which gave rise to thiazole-pyrimidine derivatives **5.17** in a much improved yield compared to 2-CR. The highest yield obtained was 91% for **5.17i** while the lowest yield was 71% for **5.17e** from this set of compounds. There is a large difference in the yields observed between the 2-CR and 3-CR especially where there are electron withdrawing groups on the phenyl ring such as fluorine

and chlorine (**5.17f**, **5.17g**, **5.17m** and **5.17n)**. The components of the 3-CR seem to be more reactive over a wider substrate range in comparison with the 2-CR. This might suggest that the intermediates generated in the 3-CR (Scheme 5.10) smoothly react with DMAD\DEtAD and subsequently undergo internal cyclisation to afford the desired products in good yields.

Table 5.1: Thiazole-pyrimidine derivatives and their analogues obtained from 2-CR and 3-CR methods




| Compounds | $R_1$ | $R_2$ | $R_3$ | Yield (% ) 2-CR | Yield (% ) 3-CR | Time h (2-CR) 3-CI |
|---|---|---|---|---|---|---|
| **5.17a** | H | H | $CH_3$ | 72 | 87 | (12) 12 |
| **5.17b** | $CH_3$ | H | $CH_3$ | 68 | 78 | (12) 12 |
| **5.17c** | $CH_3$ | $CH_3$ | $CH_3$ | 67 | 82 | (12) 12 |
| **5.17d** | Ph | H | $CH_3$ | 57 | 74 | (6) 6 |
| **5.17e** | 4-Ph-$CH_3$ | H | $CH_3$ | 58 | 71 | (6) 6 |
| **5.17f** | 4-Ph -F | H | $CH_3$ | 48 | 70 | (6) 6 |
| **5.17g** | 4-Ph-Cl | H | $CH_3$ | 50 | 71 | (6) 12 |
| **5.17h** | H | H | $CH_3CH_2$ | 69 | 83 | (12) 12 |
| **5.17i** | $CH_3$ | H | $CH_3CH_2$ | 71 | 91 | (12) 12 |
| **5.17j** | $CH_3$ | $CH_3$ | $CH_3CH_2$ | 79 | 86 | (12) 6 |
| **5.17k** | Ph | H | $CH_3CH_2$ | 61 | 81 | (6) 6 |
| **5.17l** | 4-Ph-$CH_3$ | H | $CH_3CH_2$ | 59 | 79 | (6) 6 |
| **5.17m** | 4-Ph -4F | H | $CH_3CH_2$ | 51 | 86 | (6) 6 |
| **5.17n** | 4-Ph-Cl | H | $CH_3CH_2$ | 52 | 84 | 6 |
| **5.17o** | H | H | *t*-Bu | 70 | | 12 |
| **5.18a** | | | $CH_3$ | 82 | | 12 |

| Compounds | $R_1$ | $R_2$ | $R_3$ | Yield (% ) 2-CR | Yield (% ) 3-CR | Time h (2-CR) 3-CI |
|---|---|---|---|---|---|---|
| **5.18b** | | | $CH_3CH_2$ | 80 | | 12 |
| **5.5a** | | | $CH_3$ | 89 | | 12 |
| **5.5b** | | | $CH_3CH_2$ | 82 | | 12 |
| **5.6a** | | | $CH_3$ | 83 | | 12 |
| **5.6b** | | | $CH_3CH_2$ | 88 | | 12 |

*5.5.2. Substituent effects on yields when using 2-CR and 3-CR*

The 2-CR method gave good yields when substrates **5.3**, **5.4** and **5.8** were used for the synthesis of products **5.5a-b** (82%-89%), **5.6a-b** (83%-88%) and **5.18a-b** (80%-82%), as shown in Table 5.1. Using substrates **5.7a-c** gave quite reasonable yields of **5.17a-c** (67%-72%) and **5.17h-j** (69%-79%). However, when using substrates **5.7d-g** with phenyl groups at $\mathbf{R_1}$ the percentage yields dropped slightly. This was seen for all compounds with the phenyl substituent but the yields dropped further where the phenyl group had electron-withdrawing substituents such as fluorine and chlorine. Thus compounds with either fluorine or chlorine at the *para*-position (**5.17g**, **5.17h**, **5.17m** and **5.17n**) showed slightly reduced yields compared to **5.17e** and **5.17l** with a methyl substituent at the *para*-position. The reaction of DTAD and **5.7a** gave an average yield of novel **5.17o** using 2-CR.

The 3-CR was found to be more effective for the synthesis of products **5.17a-n** when compared to the 2-CR, as shown in Table 5.1. Compounds **5.17** were isolated in different yields based on the nature of the functional groups possessed by DMAD\DEtAD **5.16** and 2-aminothizole **5.7**. The highest yields for this set of compounds using DMAD were 87% and 82% for **5.17a** and **5.17c**, respectively, followed by **5.17b** (78%), with methyl group substituents at $\mathbf{R_1}$ and $\mathbf{R_2}$ as shown in Table 5.1. The percentage yields of compounds with phenyl and phenyl-halide substituents at $\mathbf{R_1}$ for products **5.17d-g** were found to be lower than those of **5.17a-c** with hydrogen and methyl substituents on $\mathbf{R_1}$. The presence of fluorine and chlorine at the *para*-position of 2-amino thiazole **5.7f** and **5.7g** resulted in a slightly decreased percentage yield when compared to *para*-methyl and hydrogen substituents while using DMAD. The drop in yield was not nearly as significant as that seen for these substrates in the 2-CR. When using DEtAD the yield of the 3-CR increased compared to DMAD. The highest yield obtained was 91% from **5.17i**.

## 5.6. Structural elucidation of synthesised compounds using FTIR, NMR, HRMS and single X ray Crystallography.

*5.6.1. The structural analysis of thiazole-pyrimidines **5.17a-o**.*

Compounds **5.17a-o** were analysed by FTIR, NMR, HRMS and where suitable by single X-ray crystallography (**5.17b**, **5.17c**, **5.17e** and **5.17g**).

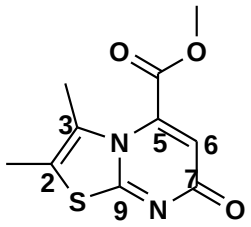



**Figure 5.2.** Structure of product **5.17c**.

By way of example we describe the characterisation data for compound **5.17c (**Figure 5.2). The $^{1}$H NMR spectrum shows a singlet at δ= 6.50 corresponding to the 6-CH, a singlet integrating for 3H due to methoxy group at δ= 3.95 and two singlets at δ= 2.28 and 2.09 integrating for 3H each from the methyl groups at positions 2 and 3. The $^{13}$C NMR spectrum showed ten distinct resonances in accordance with the proposed structure. The mass spectrum of **5.17c** shows the protonated molecular ion peak at m/z= 239.0486 which is consistent with the proposed molecular structure with a calculated value of 239.0485. The FTIR spectrum displayed characteristic absorption bands for the carbonyl groups at 1734 cm$^{-1}$ and 1638 cm$^{-1}$, and at 2993 cm$^{-1}$ for the CH stretch bands.

For the group of compounds **5.17a-o**, the FTIR spectra showed characteristic absorption bands for C=O in the range of 1742 cm$^{-1}$ to 1621 cm$^{-1}$. The $^{1}$H NMR spectra of compounds **5.17a-o** exhibited one singlet signal in the region of δ 6.76-6.47 ppm corresponding to the CH at position 6. $^{13}$C NMR spectra show characteristic signals in the region of δ 167.2-164.7 ppm corresponding to two C=O groups. ESI-LCMS was used to confirm the molecular formulae of synthesised compounds**.** Chromatograms exhibited the protonated parent molecular ion $[M+1]^{+}$.

Single crystal X-ray analysis of **5.17b, 5.17c, 5.17e** and **5.17g** shown in Figure 5.3 unambiguously confirmed the structure of these compounds.

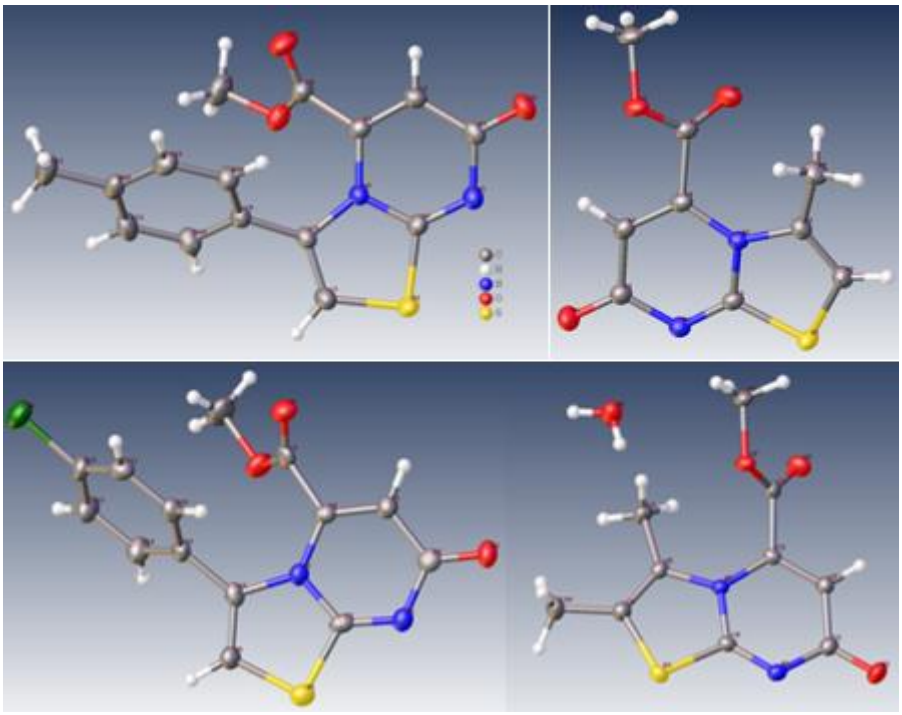


**Figure: 5.3**: Single-crystal X-ray structure (ORTEP) of **5.17b** (top right), **5.17c** (bottom right), **5.17e** (top left) and **5.17g** (bottom left).

*5.6.2. The structural analysis of oxazolo-pyrimidines **5.18a-b** (NMR, FTIR and HRMS)*

The characterisation of compound **5.18a** ($R_3$=Me, Figure 5.4) commenced with $^1$H NMR spectroscopic analysis, which shows a singlet at δ= 6.37 corresponding to the CH at position 6, two triplets at δ 4.68 and δ 4.52 with *J* values of 8.4Hz corresponding to the two $CH_2$ groups and a singlet due to methoxy protons at δ 3.87. $^{13}$C NMR spectroscopy showed eight distinct resonances for **5.18a** ($R_3$=Me) and nine distinct resonances for **5.18b** ($R_3$=Et) in accordance with the proposed structures. The mass spectra of **5.18a** and **5.18b** showed the protonated molecular ion peak at m/z= 197.0549 and 211.0708, respectively, which are consistent with the proposed molecular structures for which the calculated values are 197.0557 and 211.0713. The FTIR spectrum displayed characteristic absorption bands for the carbonyl groups at 1732 $cm^{-1}$ and 1624 $cm^{-1}$.

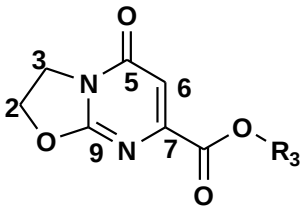



**Figure 5.4.** Structure of **5.16a-b**

Due to poorly resolved peaks in the $^{1}$H NMR spectrum of compound **5.18b**, further NMR studies were carried out at different temperatures in DMSO-$d_6$ (Figure 5.5). The spectrum of **5.18b** at 30 ºC clearly shows poorly resolved peaks, and that the peak resolution could be improved by increasing the temperature to 80 ºC (Figure 5.5). Once resolved, three triplets were seen at 1.29 ppm, 3.63 ppm and 4.05 ppm, with *J* values of 7.2 Hz, 5.6 Hz, 6.0 Hz, respectively. A quartet with a *J* value of 7.2 Hz was observed at 4.30 ppm while a singlet was found at 5.64 ppm. A further increase in temperature to 105 ºC resulted in broader peaks once again.

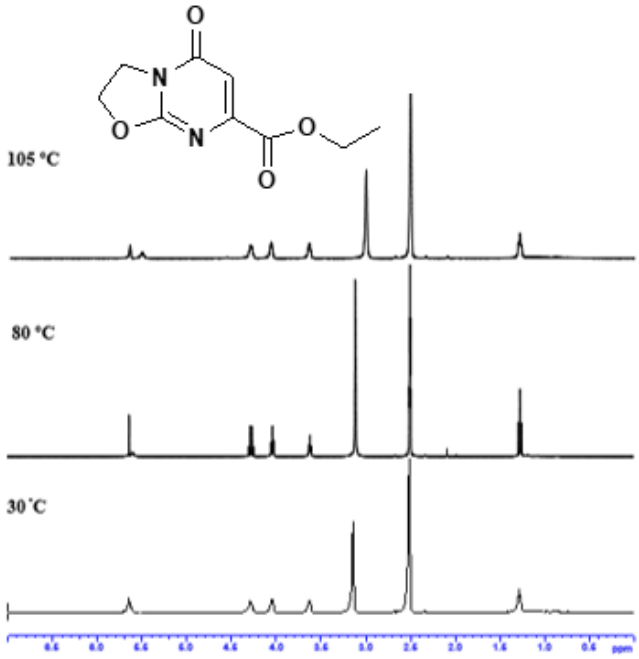



**Figure 5.5**: The effect of temperature on the $^{1}$ H-NMR spectroscopic signals of compound **5.18b** in DMSO-$d_6$

*5.6.3. The structural elucidation of triazolo-thiazine **5.6a-b** (NMR, FTIR, HRMS and single X-ray Crystallography)*

$^{1}$H NMR spectroscopic analysis of compound **5.6a** ($R_3$=Me, Figure 5.6) shows two singlets at δ= 8.69 and δ= 7.54 corresponding to the two CH groups at positions 4 and 6 and a singlet due to the methoxy protons at δ 3.98. $^{13}$C NMR spectra showed seven distinct resonances for **5.6a** and eight distinct resonances for **5.6b** ($R_3$=Et) in accordance with the proposed structures. The mass spectra of **5.6a** and **5.6b** showed the molecular ion peak at m/z= 212.0134 and 226.0288, respectively, which are consistent with the proposed molecular structures for which the calculated values are 212.0124 and 226.0281. The FTIR spectrum for **5.6a** displayed characteristic absorption bands for the carbonyl groups at 1706 cm$^{-1}$ and 1582 cm$^{-1}$.

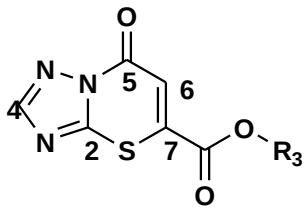



**Figure 5.6.** Structure of triazolo-thiazines **5.6a-b**

The single crystal X-ray analysis of **5.6a** and **5.6b** unambiguously confirmed the structures as shown in Figure 5.6.

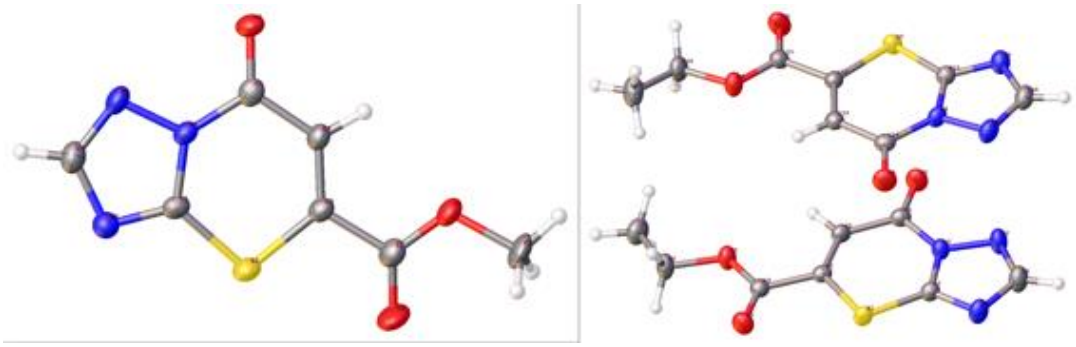


**Figure: 5.6**: Single-crystal X-ray structure (ORTEP) of **5.6a (**left**)** and **5.6b (**right**)**

*5.6.4. The structural analysis of triazolo-pyrimidines **5.5a-b** (NMR, FTIR and HRMS)*

The $^{1}$H NMR spectrum for compound **5.5a** (Figure 5.7) shows two singlets for the two CH groups at positions 5 and 7 at δ= 9.04 and δ= 6.67and a singlet due to the methoxy group at δ 3.95. $^{13}$C NMR spectroscopy showed seven distinct resonances for **5.5a** and eight distinct

resonances for **5.5b** in accordance with the proposed structure. The mass spectra of **5.5a** and **5.5b** showed the molecular ion peaks at m/z= 195.0526.and 209.0677, respectively which is consistent with the proposed molecular structures, for which the calculated values are 195.0513 209.0669. The FTIR spectrum for **5.5a** displayed characteristic absorption bands for the carbonyl groups at 1732 $cm^{-1}$ and 1628 $cm^{-1}$.

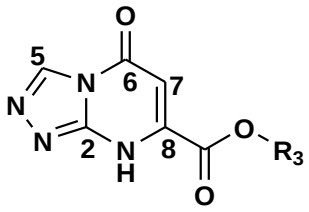



**Figure 5.7.** Structure of triazolo-pyrimidines **5.5a-b.**

The presence of small extra signals in the $^{1}H$ NMR spectrum of compound **5.5b** alerted us to the possible presence of a second tautomeric form (Figure 5.8). The $^{1}H$ NMR spectrum at 30°C inDMSO-$d_6$ shows poorly resolved peaks. An increase in temperature to 80ºC shows a slight change with the disappearance of two broad peaks around 13.00 ppm and 3.10 ppm. At 115ºC the peaks move closer to each other and are almost fully coalesced. At the maximum (instrumental) temperature of 130ºC the resolution improved to observe a triplet at 1.42 ppm, a quartet at 4.50 ppm, and two singlets at 6.66 ppm and 8.09 ppm (Figure 5.8).

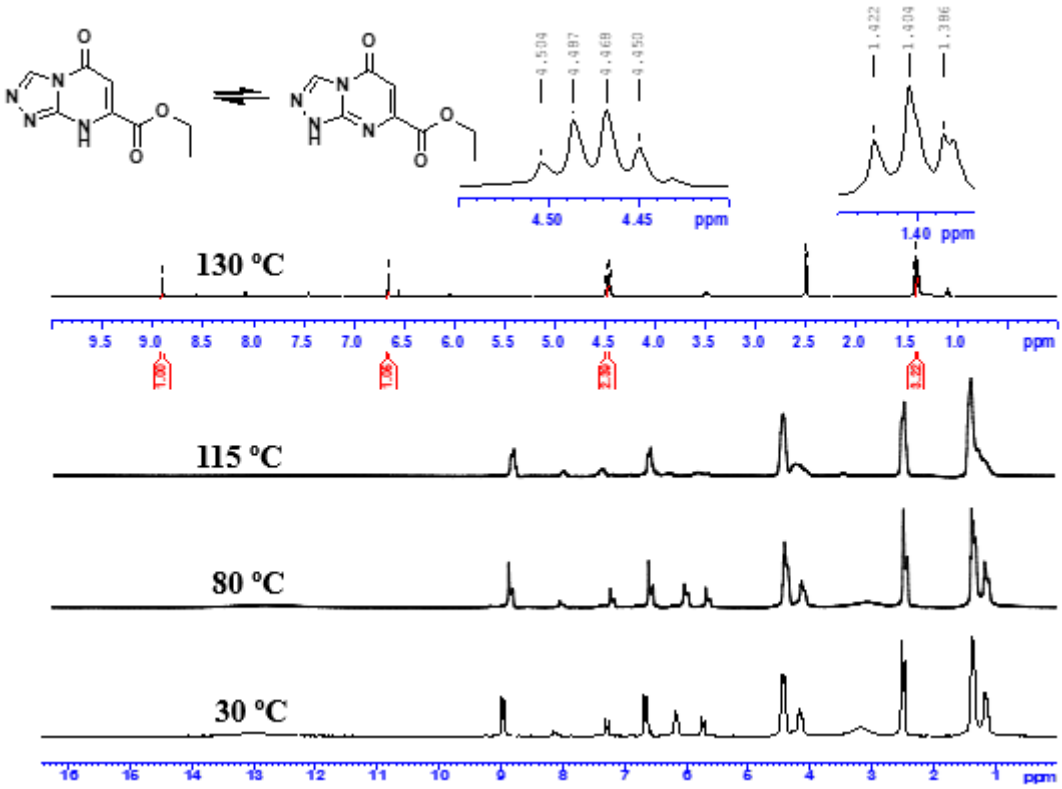



**Figure 5.8.** The effect of temperature on the[1] H-NMR signals in DMSO-$d_6$ of compound **5.5b**

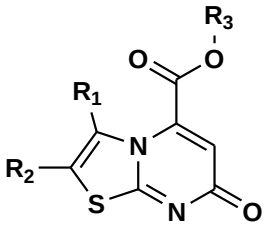
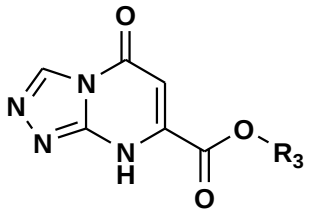
A total of twenty-one pyrimidine derivatives and their analogues were synthesised and to the best of our knowledge fourteen pyrimidine derivatives and their analogues are novel. The 3-CR approach has never been used before for the synthesis of compounds **5.17a-n**. The substrate 2-imino-1,3-oxazolidine **5.8** has never tested previously been used in reaction with DMAD\DEtAD. Seven of the compounds have been reported before: **5.5a**, **5.6a**, **5.17a-b**, **5.17d**, **5.17k** and **5.17l**.

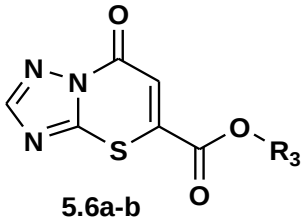
### 5.7. Biological evaluations of pyrimidines and their analogues

The synthesised pyrimidines and their analogues **5.5**, **5.6**, **5.17** and **5.18** were evaluated for toxicity in the MT-4 cell line at 100 µM. Auranofin was used as a cytotoxic control compound starting at a concentration of 100 µM. DMSO was used as a background control. Five of the thiazole-pyrimidine derivatives **5.17b-c**, **5.17h**, **5.17j** and **5.17o** were found to have $CC_{50}$ values above 200 µM as recorded in Table 5.2. Compounds **5.17a** and **5.17g** were found to have a $CC_{50}$ of 101 µM and 162 µM respectively. All of these compounds can be considered relatively non-toxic. The following eight compounds, **5.17d-f**, **5.17i** and **5.17k-n** were considered to be relatively toxic after showing $CC_{50}$ values below 59 µM. The pyrimidines

**5.5a-b**, **5.18a-b** and thiazine **5.6a** had $CC_{50}$ values above 200 µM and are therefore considered non-toxic. Thiazine **5.6b** had a $CC_{50}$ value of 27 µM and is therefore considered to be relatively toxic.

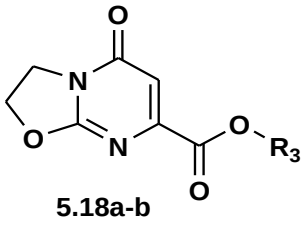
**Table 5.2**: $CC_{50}$ values of synthesised pyrimidine derivatives

| Structures | Number | $CC_{50}$ | % Antiviral inhibition (infected MT4) |
|---|---|---|---|
|  5.17a-o | **5.17a** | 162 µM | -6.89 |
| | **5.17b** | > 200 µM | -4.18 |
| | **5.17c** | > 200 µM | -7.18 |
| | **5.17d** | 22 µM | |
| | **5.17e** | 59 µM | |
| | **5.17f** | 17 µM | |
| | **5.17g** | 101 µM | |
| | **5.17h** | > 200 µM | 0.98 |
| | **5.17i** | 36 µM | |
| | **5.17j** | > 200 µM | -2.63 |
| | **5.17k** | < 3 µM | |
| | **5.17l** | 16 µM | |
| | **5.17m** | 14 µM | |
| | **5.17n** | 17 µM | |
| | **5.17o** | > 200 µM | -9.18 |
|  5.5a-b | **5.5a** | > 200 µM | -2.06 |
| | **5.5b** | > 200 µM | 0.55 |
| | | > 200 µM | |



5.6a-b

| | | |
|---|---|---|
| **5.6a** | | 1.59 |
| **5.6b** | 27 μM | |



5.18a-b

| | | |
|---|---|---|
| **5.18a** | > 200 μM | -4.83 |
| **5.18b** | > 200 μM | 1.19 |

**$CC_{50}$** - concentration of compounds that causes 50% reduction of cell growth

The following compounds, **5.5a-b**, **5.6a-b**, **5.17a-c**, **5.17h**, **5.17j**, **5.17o** and **5.18a-b** are considered relatively non-toxic and were then evaluated for antiviral activities in infected MT4 cells using Dolutegravir and Raltegravir as control compounds. The tests were carried out in a cell-based assay and each compound was analysed at a single dose of 100 μM. Based on the outcome none of these compounds showed significant activity.

The antimicrobial activities of the pyrimidines and their analogues showed no significant activity against the bacterial strains.

## 5.8. Conclusion

A series of methyl 7-oxo-7*H*-thiazolo[3,2-*a*]pyrimidine-5-carboxylate derivatives **5.17** were prepared *via* 2-CR and 3-CR reactions as discussed in Scheme 5.5. These derivatives were obtained from a 3-CR of thiourea **5.12**, α-haloketone **5.13** and DMAD **5.16** using ethanol and THF as solvents from room temperature to reflux in good yields. The presence of deactivating groups such as fluorine and chlorine at the *para*-position of the phenyl rings of **5.17m** and **5.17n**, respectively, didn't show any negative effect towards product formation using the 3-CR method. When **5.13h** and **5.13i** were used as α-haloketones the 3-CR method was not successful in giving the desired products. The 2-CR method was used to produce derivatives **5.17** from the prepared 2-aminothiazole **5.7** and DMAD\DEtAD **5.16** using ethanol under reflux which resulted in moderate yields. When 4-(4-methoxyphenyl)thiazol-2-amine **5.7h** was

subjected to the same 2-CR reaction conditions the reaction was not successful. When using 2-aminothiazole intermediates that contain phenyl groups (**5.7e-g**) at $\mathbf{R_1}$ the percentage yield dropped due to the deactivating groups on the phenyl group for both DMAD and DEtAD reactions. The presence of either fluorine or chlorine at the *para*-position of **5.17f**, **5.17g**, **5.17m** and **5.17n** showed a negative effect on product yield. The reaction of prepared substrates 1*H*-1,2,4-triazol-5-amine **5.3** 1*H*-1,2,4-triazole-5-thiol **5.4** and 2-imino-1,3-oxazolidine **5.8** with DMAD/DEtAD in the presence of ethanol under reflux using the 2-CR method resulted in products **5.5a-b**, **5.6a-b** and **5.18a-b** in good yield. The 3-CR method gave better yields compared to 2-CR. Out of twenty-one synthesised compounds twelve compounds were found to be non-toxic, with $CC_{50}$ values of greater than 100 μM. None of these compounds showed any significant antimicrobial activity.

# CHAPTER 6

# Catalyst-free and solvent-free reactions for the synthesis of diazepines and quinoxalines *via* one pot three-component reaction using benzimidazole, isocyanides and ketones

## 6.1 Introduction

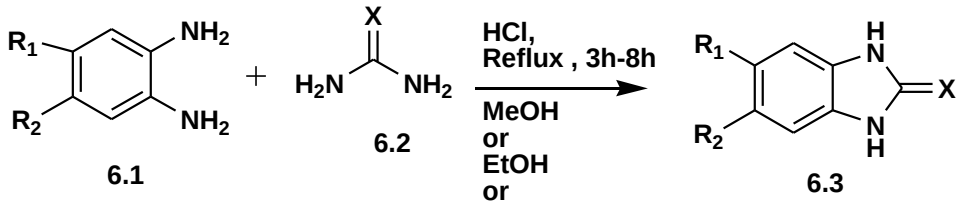
In line with our aim to use the 3-CR method to achieve biologically active scaffolds, this chapter reports on the synthesis of diazepine- and quinoxaline-based derivatives from benzimidazoles, alkyl isocyanide and ketones. In addition, dialkyl acetylenedicarboxylates were used as Michael acceptors to react with 1,2-phenylenediamines or benzimidazole, forming cyclised diazepine-based compounds. Radatz and co-workers[222] reported the synthesis of diazepines from the condensation of *o*-phenylenediamine with ketones and aldehydes under catalyst-free conditions using glycerol as a solvent. Maleki[223] synthesised diazepines from 1,2-diamines with terminal alkynes and isocyanide in the presence of silica-supported superparamagnetic iron oxide nanoparticles. Wang and co-workers[224] reported the synthesis of 1,3-benzodiazepine from propargylamines and *ortho*-bromophenylisocyanates using palladium-catalysed intramolecular alkyne hydroarylation.

Our initial idea was to explore the use of a zwitterion adduct (as described in Chapters 3 and 4) to react with benzimidazole derivatives, and further to use dialkyl acetylenedicarboxylates to construct a new six-membered ring (as described in Chapter 5). However, when we reacted benzimidazole derivatives with DMAD and an isocyanide using acetone as a solvent, we observed that an unexpected reaction occurred between acetone, the isocyanide and the benzimidazole derivative, resulting in the formation of diazepine derivatives, with DMAD as spectator reagent. Thus we turned our attention to exploring the observed reaction and examined the substrate scope. The variation allowed us to obtain isocyanide-based quinoxaline products without the use of a catalyst. This Chapter describes the synthesis and characterisation of the diazepines and quinoxalines, and proposes possible mechanistic pathways for their formation.

## 6.2 Preparation of benzimidazole derivatives using 1,2-phenylenediamines

Benzimidazoles are considered as useful intermediates and active fragments for organic and inorganic synthesis. [225] Several synthetic approaches for the preparation of benzimidazole have been previously reported in literature. The first reported benzimidazole, 2,5(2,6)dimethylbenzimidazole, was obtained by the reduction of 2-nitro-4-methylacetanilide in 1872 by Hoebrecker.[226] Wright[225] and Kadhim[227] described the reaction of 1,2-phenylenediamine derivatives with either thiourea or urea in alcohol solution with an equal amount of HCl to give 1*H*-benzo[*d*]imidazole-2(3*H*)-thione or 1*H*-benzo[*d*]imidazol-2(3*H*)-one, respectively. Guida and coworkers[228] also reported preparation of 1*H*-benzo[*d*]imidazol-2(3*H*)-imine from *o*-phenylenediamine and cyanogen bromide.

Based on the above methods, the benzimidazole derivatives were first prepared using three different alcoholic solvents: methanol, ethanol and isopropanol, as shown in Scheme 6.1. This was done to investigate the effect of the alcohol solvent on the reaction time and percentage yield. In addition, acetonitrile and acetone were also investigated as solvents, but they afforded inferior yields.




| 6.1: $R_1$ | $R_2$ |
|---|---|
| 6.1a: H | H |
| 6.1b: $CH_3$ | H |
| 6.1c: $CO_2CH_3$ | H |
| 6.1d: F | H |
| 6.1e: Cl | H |
| 6.1f: Br | H |
| 6.1g: $CH_3$ | $CH_3$ |
| 6.1h :Cl | Cl |

| 6.2: X |
|---|
| 6.2a: O |
| 6.2b: N |
| 6.2c: S |

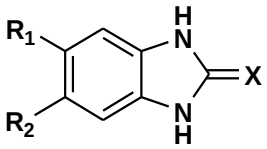
| 6.3: $R_1$ | $R_2$ | X |
|---|---|---|
| 6.3a: H | H | O |
| 6.3b: $CH_3$ | H | O |
| 6.3c: $CO_2CH_3$ | H | O |
| 6.3d: F | H | O |
| 6.3e: Cl | H | O |
| 6.3f: Br | H | O |
| 6.3g: H | H | S |
| 6.3h: H | H | N |
| 6.3i: $CO_2CH_3$ | H | S |
| 6.3j: $CH_3$ | $CH_3$ | O |
| 6.3k: Cl | Cl | O |

**Scheme 6.1**: Preparation of 1*H*-benzo[*d*]imidazol-2(3*H*)-one derivatives (**6.3a-i**) under reflux in different solvents.

1,2-Phenylenediamine derivatives **6.1**, HCl and either urea **6.2a**, guanidine hydrochloride **6.2b** or thiourea **6.2c** were reacted under reflux in alcohol solvent for 3-8 h, while being monitored

by TLC. The precipitates formed from the reactions were removed by filtration and washed with isopropanol to obtain solid benzimidazole derivatives **6.3a-i**, which gave properties comparable to those reported in the literature as shown in Table 6.1.

**Table 6.1**: Isolated yields of 1*H*-benzo[*d*]imidazol-2(3*H*)-one derivatives (**6.3a-i**)




**6.3**

| **Compound: Mp ºC:(found) Lit*** | **$R_1$** | **$R_2$** | **X** | **MeOH Yield% (time)** | **EtOH Yield% (time)** | **iPrOH Yield% (time)** |
|---|---|---|---|---|---|---|
| **6.3a**:(274-276) 275-279[227]* | H | H | O | 73 (3 h) | 84 (3 h) | 76 (3 h) |
| **6.3b**:(291-293) 292[229]* | $CH_3$ | H | O | 61 (8 h) | 86 (3 h) | 71(3 h) |
| **6.3c**:(311-313) 312-313[230]* | $CO_2CH_3$ | H | O | 67 (8 h) | 80 (5 h) | 75(8 h) |
| **6.3d**:(299-301) 300[231]* | F | H | O | 74 (8 h) | 81 (3 h) | 75 (3 h) |
| **6.3e**:(323-325) 324-326[229]* | Cl | H | O | 71 (8 h) | 75 (3 h) | 77 (3 h) |
| **6.3f**:(335-337) 336-337[232]* | Br | H | O | 70 (8 h) | 83 (3 h) | 79 (3 h) |
| **6.3g**:(115-117)114-118[227]* | H | H | S | 72 (3 h) | 81 (3 h) | 74 (3 h) |
| **6.3h**:(181-183) 182-184[228]* | H | H | N | 83 (3 h) | 75 (3 h) | 78 (3 h) |
| **6.3i**: (284-287) 285[233]* | $CO_2CH_3$ | H | S | 75 (8 h) | 82 (5 h) | 80 (8 h) |
| **6.3j**:(394-396)l395.[234]* | $CH_3$ | $CH_3$ | O | 61 (8 h) | 76(8 h) | 73 (8 h) |
| **6.3k**:(397-399) 398[233].* | Cl | Cl | O | 60 (8 h) | 70(8 h) | 70 (8 h) |

Lit*: reported melting point

Mp: melting point

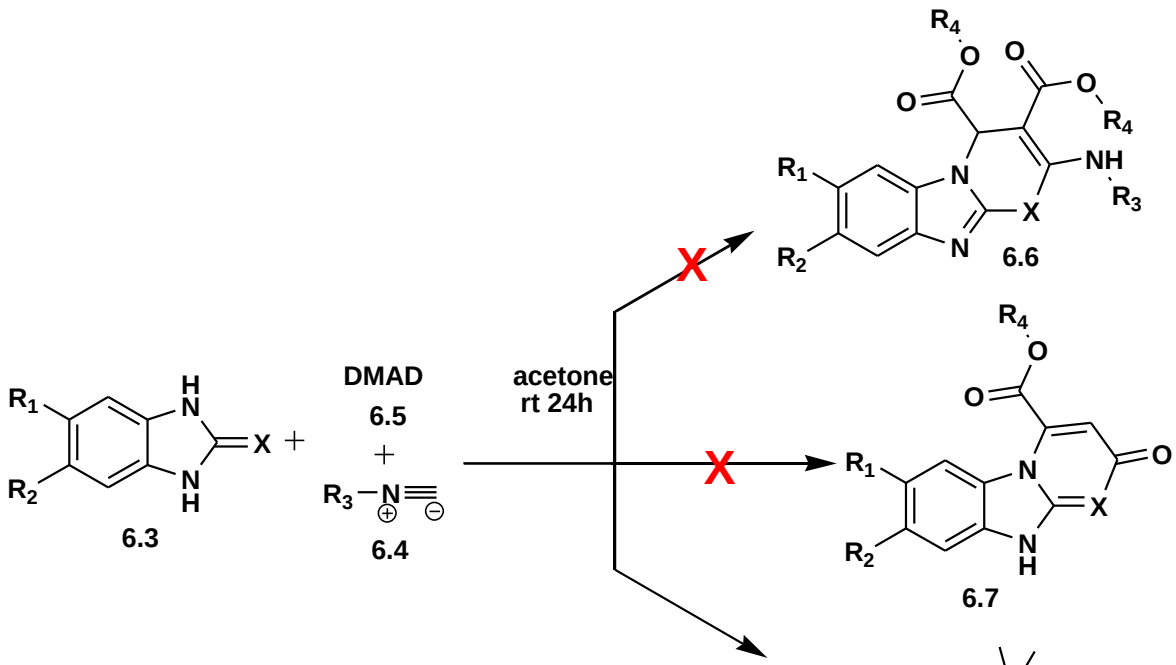
The yields of the products were higher when using ethanol and isopropanol as solvents with higher boiling points of 78.37 ºC and 82.5 ºC when compared to methanol with a boiling point of 64.7 ºC. The shortest reaction time observed was 3 h, for *o*-phenylenediamine and urea or guanidine. The longest reaction time (8 h) was required for all di-substituted 1,2-phenylenediamine derivatives, and the mono-substituted ester.

*6.2.1 Analysis of 1H-benzo[d]imidazol-2(3H)-one derivatives (**6.3a-k**) by NMR spectroscopy*

The $^{1}$H NMR spectra of benzimidazole compounds **6.3a-k** exhibited one broad singlet signal in the region of δ =7.11- 5.45 ppm corresponding to the two NH groups. $^{13}$C NMR spectra showed characteristic signals at δ=165.9-159.7 ppm corresponding to C=O for **6.3a-e** and **6.3i-j**, δ 158.2 ppm corresponding to N=C for **6.3h** and δ=183.9 ppm and δ=183.9 ppm corresponding to C=S for **6.3g** and **6.3i**, respectively.

## 6.3 Unexpected synthesis of diazepine derivatives from benzimidazoles, isocyanide derivatives and acetone.

Reaction of benzimidazole **6.3**, isocyanide **6.4** and DMAD **6.5** in acetone was anticipated to result in one or both of two possible products **6.6** or **6.7**, however, unexpectedly products **6.8a-h** were obtained, as shown in Scheme 6.2. These results show that the solvent acetone **6.9** participated in the reaction as a reagent to form a diazepine-based scaffold, while DMAD remained unreacted. Of interest, is that the reaction was able to produce **6.8** using benzimidazoles **6.3a** or **6.3g-h** containing either oxygen, nitrogen or sulfur at the **X** position. We had considered the possibility that DMAD could cyclise with the benzimidazole derivative to form compounds **6.7** (Scheme 6.2, X=S), excluding the isocyanide, as this was in line with the work of Zeng and co-workers,[184] who reacted 1*H*-benzo[*d*]imidazole-2(3*H*)-thione and DMAD using methanol as the solvent. However, neither compound **6.6** nor **6.7** was observed, only compound **6.8** was isolated from the reactions (Scheme 6.2).



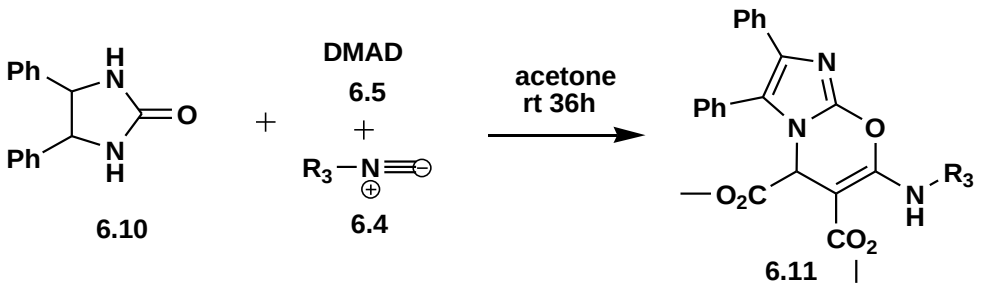

| 6.3: $R_1$ | $R_2$ | X |
|---|---|---|
| 6.3a: H | H | O |
| 6.3b: $CH_3$ | H | O |
| 6.3c: $CO_2CH_3$ | H | O |
| 6.3d: F | H | O |
| 6.3e: Cl | H | O |
| 6.3f: Br | H | O |
| 6.3g: H | H | S |
| 6.3h: H | H | N |
| 6.3i: $CO_2CH_3$ | H | S |
| 6.3j: $CH_3$ | $CH_3$ | O |
| 6.3k: Cl | Cl | O |

6.4a: *t*-Bu
6.4b: tetramethylbutyl
6.4c: cyclohexyl
6.4d: tosylmethyl

| 6.8: $R_1$ | $R_2$ | $R_3$ | Yield % |
|---|---|---|---|
| 6.8a: H | H | *t*-Bu | 84 |
| 6.8b: H | H | tetramethylbutyl | 80 |
| 6.8c: H | H | cyclohexyl | 84 |
| 6.8d: H | H | tosylmethyl | 71 |
| 6.8e: $CH_3$ | $CH_3$ | *t*-Bu | 74 |
| 6.8f: $CH_3$ | $CH_3$ | tertramethylbutyl | 67 |
| 6.8g: $CH_3$ | H | *t*-Bu | 19 |
| 6.8h: F | H | *t*-Bu | trace |

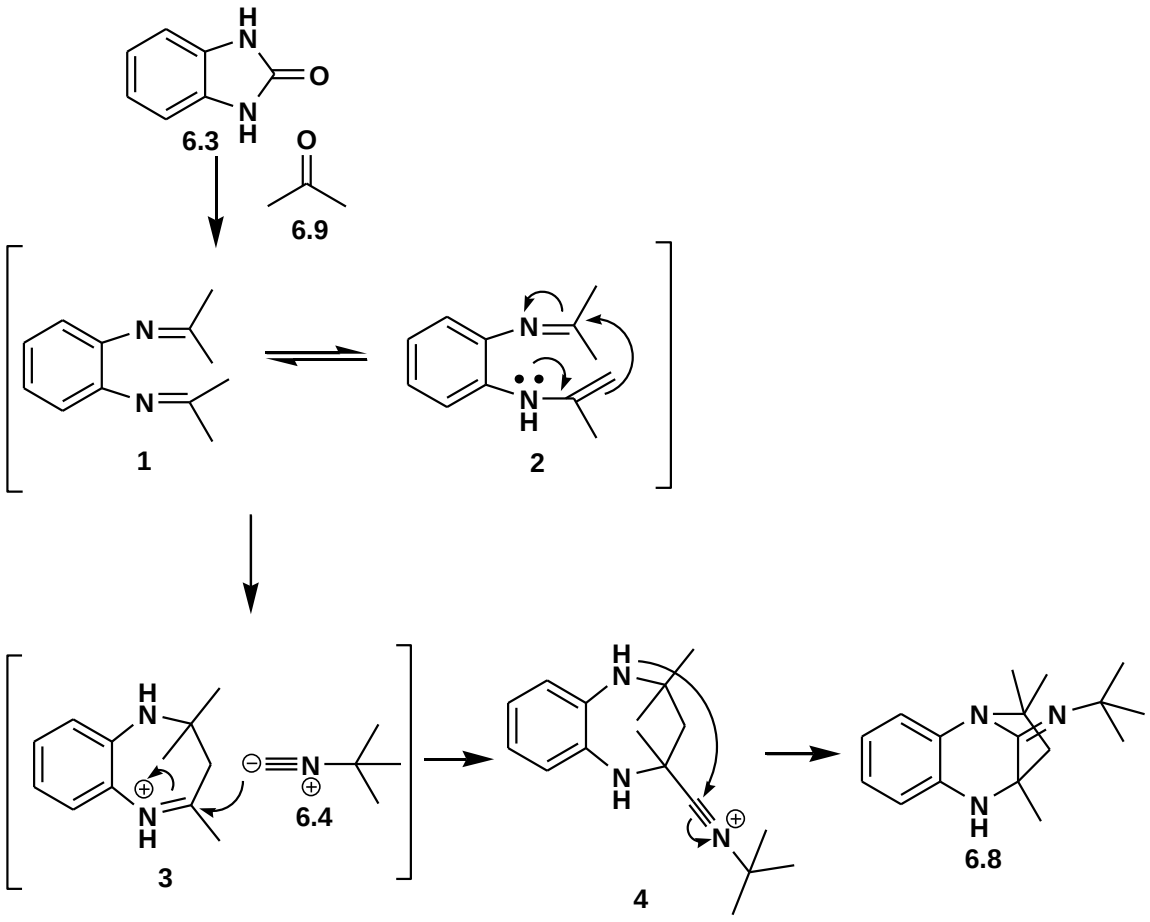
**Scheme 6.2**: Synthesis of products **6.8a-h**

Interestingly, when Adib and co-workers[235] reacted 4,5-diphenyl-1,3-dihydro-2*H*-imidazol-2-one **6.10** , an imidazole derivative rather than a benzimidazole derivative, with isocyanide and DMAD, they obtained the expected 5*H*-imidazo-[2,1-*b*][1,3]oxazine derivatives **6.11** in acetone as shown in Scheme 6.3.



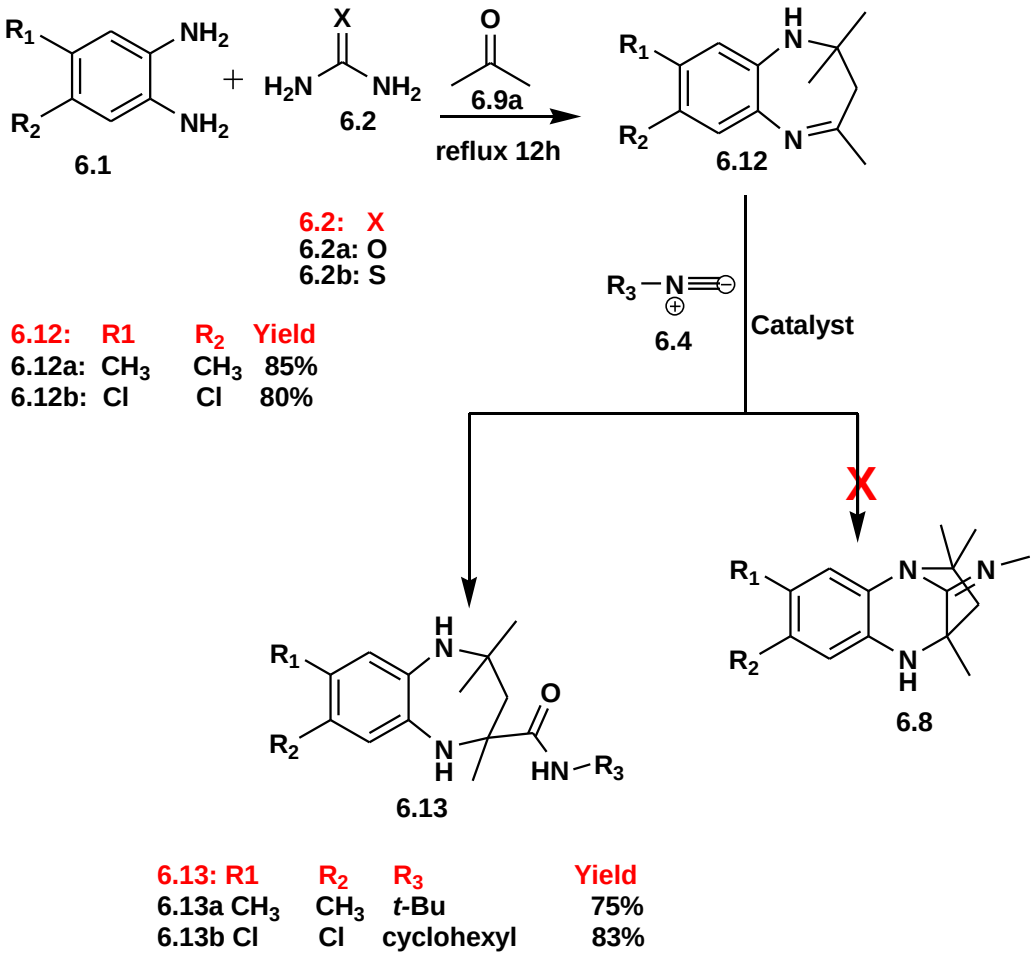

**Scheme 6.3**: Synthesis of 2,3-diphenyl-5*H*-imidazo[2,1-*b*][1,3]oxazine

Possible explanations for the formation of diazepines **6.8** is still under investigation, however, we propose and speculate the reaction is occurring as shown in Scheme 6.4. The mechanistic pathway suggests condensation of acetone with benzimidazole derivative **6.3** to give intermediate **1**, followed by internal cyclisation at event **2** leading to the formation of the intermediate at event **3**. The nucleophilic addition of isocyanide **6.4** at event **3**, followed by intramolecular cyclisation may lead to formation of **6.8**.




**Scheme 6.4**: Proposed reaction mechanisms for the formation of **6.8a**

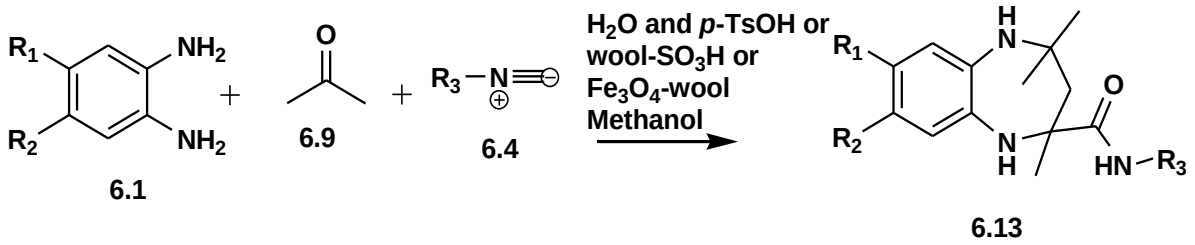
In an attempt to investigate the mechanism of the formation of compounds **6.8**, we prepared diazepine scaffold **6.12**, a proposed intermediate, under refluxing conditions using urea and thiourea, as shown in Scheme 6.5. These kinds of scaffolds **6.12a**[236] and **6.12b**[237,238,239] are well exploited and reported by Landge and Török[238] using a solvent-free solid acid catalysed method; by Varala and coworkers[239] using ceric ammonium nitrate catalyst and by Shaabani and coworkers[240] using ammonium chloride as a green catalyst. When we put to test the reaction of isocyanide **6.4**, acetone **6.9** and diazepine **6.12** in order to achieve diazepine product **6.8** using *p*-TsOH or montmorillonite K-10 clay as catalysts we instead obtained products **6.13a-b** in good yield as shown in Scheme 6.5. We further tried different conditions such as refluxing and different solvents including acetone, acetonitrile ethanol and methanol with no success in obtaining diazepine scaffold **6.8**.




**Scheme 6.5**: Synthesis of product **6.13**.

Shaabani and coworkers[240,241,242] used two different approaches to synthesise **6.13b** and derivatives: an *in-situ* approach of diazepine **6.12** with isocyanide **6.4** in the presence of water

using ammonium chloride as a catalyst and a 3-CR reaction of phenylenediamine **6.1** with isocyanide **6.4** in the presence of acetone **6.9** using *p*-TsOH and water or wool-$SO_3H$ or $Fe_3O_4$-wool as a catalyst in methanol solutions as shown in Scheme 6.6.




**Scheme 6.6**: Synthesis of **6.13** by Shaabani and coworkers using 3-CR

*6.3.1. The effect of activating and deactivating substituent groups on yield of* ***6.8a-h***

Diazepine derivatives **6.8** were isolated in different yields based on the nature of the isocyanides and benzimidazole functional groups, as shown in Scheme 6.2. The highest yields for this set of compounds were obtained from 1*H*-benzo[*d*]imidazol-2(3*H*)-imine derivatives without substituents on the aromatic ring **6.8a-c** from *tert*-butyl, tetramethylbutyl and cyclohexyl isocyanide substituents, respectively. However, using TosMIC and 1*H*-benzo[*d*]imidazol-2(3*H*)-imine gave a slightly lower, but still reasonable yield. The 7,8-dimethyl substituted compounds gave **6.8e-f** in moderate yields, whilst methyl mono-substituted **6.8g** was obtained in poor yield (Scheme 6.2 The weakly deactivating fluorine group gave only a trace yield, while mono-substituted bromine, chlorine and ester derivatives subjected to the same reaction conditions resulted in no conversion. This may suggest that formation of derivatives **6.8** is inhibited by deactivating groups on the aromatic ring.

To check the reactivity of ketone in the formation of the products, 3-pentanone **6.9b** was used to replace acetone, in the hopes that a similar product could be obtained, however the replacement of acetone by 3-pentanone **6.9b** resulted in no conversion (Scheme 6.2). This might suggest that two methyl terminal groups on the acetone are more favoured to cyclise and form diazepine **6.8** when compared to the 3-pentanone, which is more hindered. It is worth mentioning that the unsymmetrical starting materials **6.3** (only $R_1$ or $R_2 \neq H$) may give rise to the possibility of regioisomers resulting from swapping of the positions of the substituent. For example product **6.8g** from the starting material **6.3b** contains methyl and H groups at position

$R_1$ and/or $R_2$. However, after the reaction only one of the two possible regioisomers was isolated, but we were unable to establish unequivocally which of the two we had obtained.

*6.3.2 The structure elucidation of **6.8a-h**, **6.12a-b** and **6.13** by FTIR, NMR, HRMS and X-ray crystallography techniques*

The obtained structures of diazepines **6.8a-h** were analysed by FTIR and NMR spectroscopy, HRMS (**6.8a-g**) and single X-ray crystallographic analysis (**6.8a**).

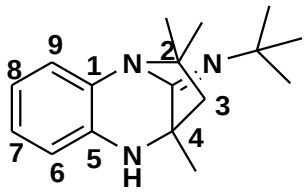



**Figure 6.1**. Structure of diazepine **6.8a**

By way of a representative example, spectroscopic data for compound **6.8a** (Figure 6.1) is discussed. The $^1$H NMR spectrum showed multiplet peaks corresponding to four aromatic CH groups (positions 6-9) at δ = 6.69-6.52, a broad NH singlet at δ = 3.90, two doublets at δ 2.04-2.00 ppm and 1.79-1.76 corresponding to the $CH_2$ at position 3, a singlet from $CH_3$ at δ = 1.39, one singlet for three *tert*-butyl $CH_3$ groups at δ =1.28, and two $CH_3$ singlets at δ =1.16 and δ =1.08. The $^{13}$C NMR spectrum of **6.8a** showed 15 distinct resonances in accordance with the proposed structure. The mass spectrum of **6.8a** showed the molecular ion $[M+H]^+$ peak at m/z 272.2119 which is consistent with the mass of the proposed product for which the calculated value is 272.2121. The FTIR spectrum displayed characteristic absorption bands for the imine group at 1696 $cm^{-1}$ and for the amine group at 2922 $cm^{-1}$. Unambiguous evidence for the structure of **6.8a** was obtained from single-crystal X-ray analysis as shown in Figure 6.2.

Generally the rest of the series **6.8b-h** showed similar patterns: FTIR spectra of this set of compounds showed characteristic absorption bands for N-H in the range of 3348 $cm^{-1}$ to 2851 $cm^{-1}$, while the N=C stretching band appeared at a range of 1723 $cm^{-1}$ to 1601 $cm^{-1}$. The $^1$H NMR spectra of compounds **6.8b-h** each exhibited one singlet signal in the region of δ 3.90-3.25 ppm corresponding to the NH, two doublets at δ 2.09-1.56 ppm corresponding to $CH_2$ and three singlet signals between δ 1.45-0.82 ppm corresponding to three $CH_3$ groups. $^{13}$C NMR spectra show characteristic signals in the region of δ 168.7-157.7 ppm corresponding to N=C and $CH_2$ signals (confirmed by a $^{13}$C DEPT 135 experiment) at δ 52.3-57.4 ppm. ESI-LCMS was used to confirm the masses of the synthesised compounds **6.8a-g**. Chromatograms

exhibited the protonated parent molecular ion $[M+1]^+$, fragmentation patterns and decomposition patterns (Table 6.2).

FTIR spectra of compounds **6.12a-b** (Scheme 6.4) showed characteristic absorption bands for N-H in the range of 3314 $cm^{-1}$ to 2914 $cm^{-1}$, while the N=C stretching band appeared at a range of 1733 $cm^{-1}$ to 1633 $cm^{-1}$. The $^1H$ NMR spectra of compounds **6.12a-b** exhibited one singlet signal in the region of δ 5.35-4.43 ppm corresponding to the NH, a singlet δ 2.26 ppm corresponding to $CH_2$ and two singlet signals between δ 2.22-1.23 ppm corresponding to three $CH_3$ groups. $^{13}C$ NMR spectra showed characteristic signals in the region of δ 173.2-170.0 ppm corresponding to N=C and $CH_2$ signals (identified by a $^{13}C$ DEPT 135 experiment) at δ 45.6-45.3 ppm. ESI-LCMS was used to confirm synthesis of compounds **6.12a-b**. Chromatograms exhibited the protonated parent molecular ion $[M+1]^+$ peaks: **6.10a** ($C_{14}H_{21}N_2^+$ 217.1699 found 217.1702) and **6.13b** ($C_{12}H_{15}Cl_2N_2^+$ 257.0607 found 257.0612).

The $^1H$ NMR spectrum of **6.13a** showed an NH broad peak doublet at δ = 7.21 and 7.19, a singlet from two aromatic CH groups at δ = 6.39, a broad NH singlet at δ = 2.39 and δ = 1.21, two doublets at δ = 2.15 ppm and δ = 1.53 corresponding to the $CH_2$, a singlet from $CH_3$ at δ = 1.29, one singlet for three *tert*-butyl $CH_3$ groups at δ =1.25, and two $CH_3$ singlets at δ =1.14 and δ =1.06. The $^{13}C$ NMR spectrum of **6.13a** showed 17 distinct resonances in accordance with the proposed structure. The mass spectrum of **6.13a** showed the molecular ion $[M+H]^+$ peak at m/z 318.2521 which is consistent with the proposed molecular structure for which the calculated value is 318.2540. The FTIR spectrum displayed characteristic absorption bands for the carbonyl group at 1642 $cm^{-1}$ and for the amine groups at 3380 $cm^{-1}$, 3286 $cm^{-1}$ and 2926 $cm^{-1}$.

**Table 6.2** The chemical formulae, and calculated and measured molecular ions of **6.8a-g**

| Compound | Chemical Formula [M+1] | Calculated m/z | Measured m/z |
|---|---|---|---|
| **6.8a** | $C_{17}H_{26}N_3^+$ | 272.2121 | 272.2119 |
| **6.8b** | $C_{21}H_{34}N_3^+$ | 328.2747 | 328.2731 |
| **6.8c** | $C_{19}H_{28}N_3^+$ | 298.2278 | 298.2278 |
| **6.8d** | $C_{21}H_{26}N_3O_2S^+$ | 384.1740 | 384.1742 |

| Compound | Chemical Formula [M+1] | Calculated m/z | Measured m/z |
|---|---|---|---|
| **6.8e** | $C_{19}H_{30}N_3^+$ | 300.2434 | 300.2436 |
| **6.8f** | $C_{23}H_{38}N_3^+$ | 356.3060 | 356.3057 |
| **6.8g** | $C_{18}H_{28}N_3^+$ | 286.2278 | 286.2278 |

X-ray analysis unambiguously confirmed the structure of **6.8a** to be 2-methyl-*N*-(2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[*b*][1,4]diazepin-10-ylidene)propan-2-amine, as shown in Figure 6.2.

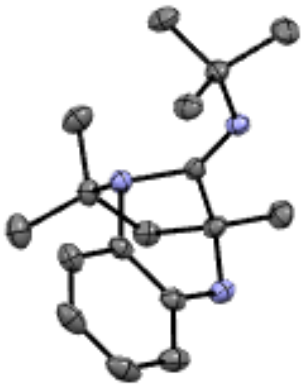


**Figure: 6.2**: Single-crystal X-ray structure (ORTEP) of **6.8a**

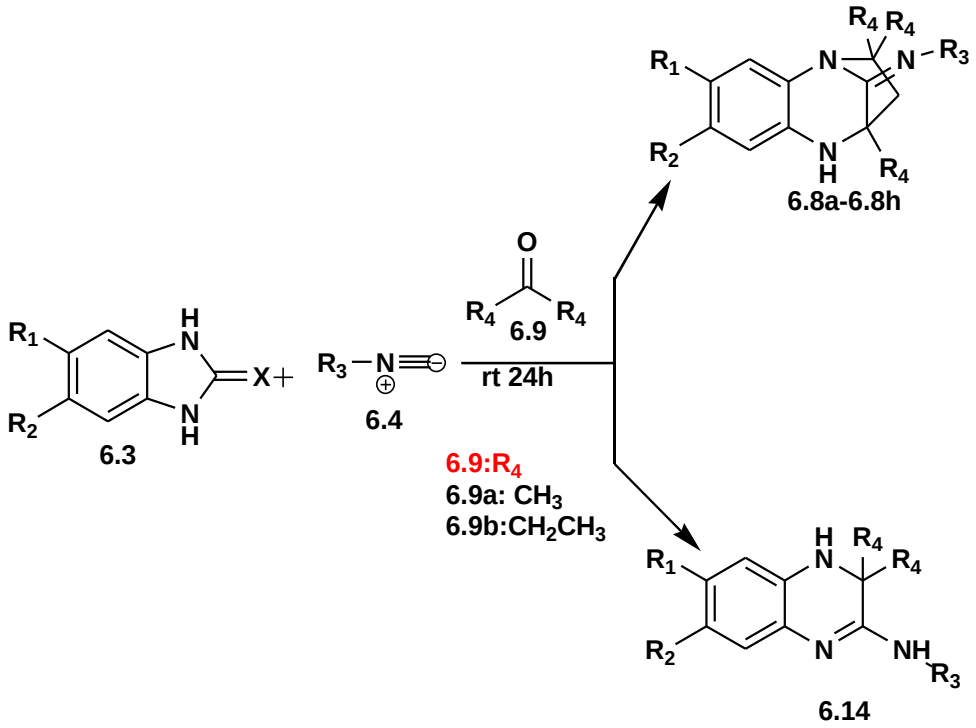
In total eight novel 2-methyl-*N*-(2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[*b*][1,4]diazepin-10-ylidene)propan-2-amine derivatives **6.8a-h** were synthesised and to the best of our knowledge the method and the compounds have not been reported before. Compound **6.11a** was found to be novel, whereas **6.11b** was reported by Shaabani and coworkers.[240,241,242]

## 6.4. Catalyst-free synthesis of novel quinoxaline-based compounds from benzimidazoles, isocyanide derivatives and acetone /3-pentanone (6.14a-i)

The investigation into the preparation of compounds **6.8** continued with the aim of confirming the role and influence of DMAD as applied in the reactions previously discussed in Section

6.3. Based on the previous findings DMAD was observed to have a spectator role, therefore reactions were repeated and DMAD was omitted from the starting materials, affording the same products **6.8a-h** in similar yields. On the contrary, for benzimidazoles bearing deactivating groups such as chlorine, fluorine and an ester, which under the conditions shown in Scheme 6.2 were unable to produce diazepine derivatives **6.8**, the omission of DMAD surprisingly resulted in the formation of quinoxaline-based products **6.14** (Scheme 6.7). These compounds were obtained without the assistance of a catalyst. This class of quinoxalines has been reported by Kolla and Lee,[243] Edayadulla and Lee[244] and Jehbez and Safaei,[245] using ethylenediaminetetraacetic acid (EDTA), $CeO_2$-nanoparticles and zirconium tetrachloride as catalysts, respectively. Shaabani and coworkers[246] reported the use of di-substituted 4,5-dimethyl-1,2-phenylenediamine and 4,5-dichloro-*o*-phenylenediamine to synthesise quinoxalines using *p*-TsOH as a catalyst. The synthesis of these quinoxaline-based compounds was monitored by TLC and the quinoxaline products **6.14a-g** were obtained and purified by column chromatography. The reactions were repeated using 3-pentanone to replace acetone which resulted in only **6.14h-i**.

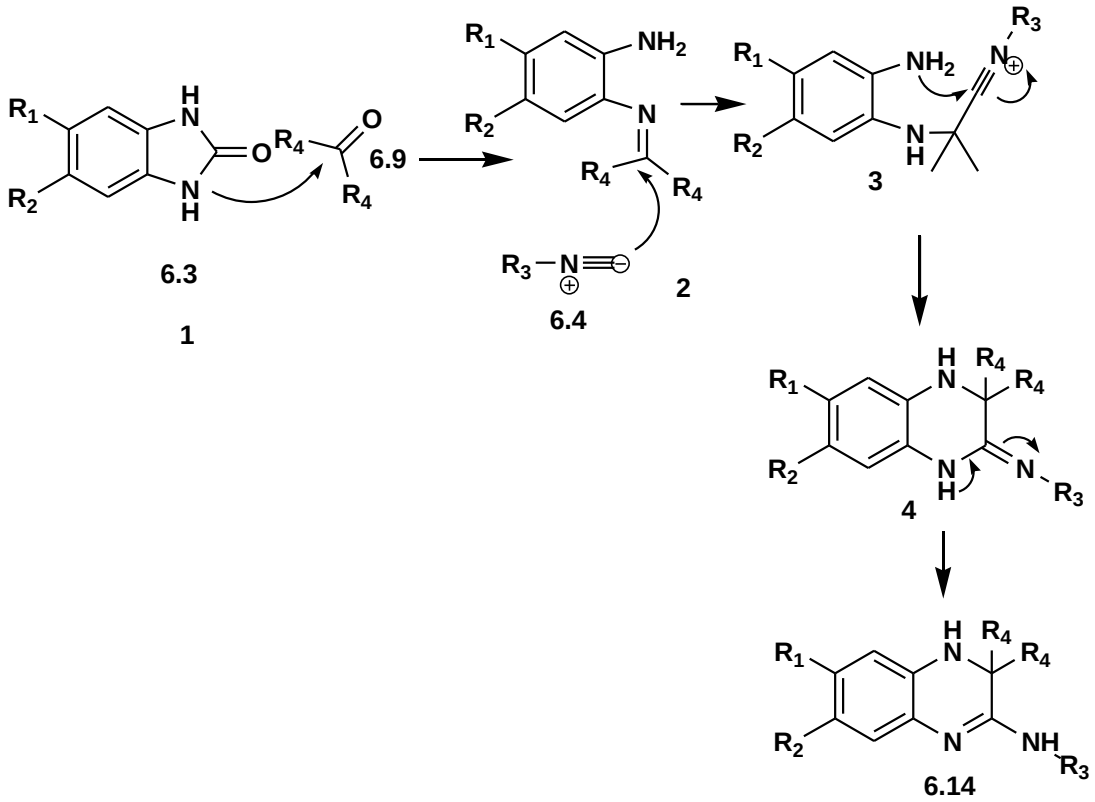
While reactions with benzimidazoles to produce quinoxaline-based compounds were successful without the use of a catalyst, attempts to use 1,2-phenylenediamine derivatives (with isocyanides and acetone) were unsuccessful.




| 6.3: $R_1$ | $R_2$ | X | 6.14: $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|---|---|
| 6.3a: H | H | O | 6.14a:$CO_2CH_3$ | H | *t*-Bu |
| 6.3b: $CH_3$ | H | O | 6.14b:$CO_2CH_3$ | H | tetramethylbutyl |
| 6.3c: $CO_2CH_3$ | H | O | 6.14c: F | H | tetramethylbutyl |
| 6.3d: F | H | O | 6.14d: H | F | tetramethylbutyl |
| 6.3e: Cl | H | O | 6.14e: Cl | H | tetramethylbutyl |
| 6.3g: H | H | S | 6.14f: Cl | Cl | *t*-Bu |
| 6.3h: H | H | N | 6.14g: Cl | Cl | tetramethylbutyl |
| 6.3i: $CO_2CH_3$ | H | S | 6.14h: H | H | *t*-Bu |
| 6.3j: $CH_3$ | $CH_3$ | O | 6.14i: Cl | Cl | *t*-Bu |
| 6.3k: Cl | Cl | O | | | |

**Scheme 6.7**: Synthesis of products **6.14a-i** and **6.8** without catalysts.

The mechanism for the formation of quinoxalines **6.14** is still under investigation. A proposed possible mechanism for the formation of **6.14** under the conditions shown in Scheme 6.8 (see Kolla and Lee[243] using EDTA). The carbonyl group from acetone is an electrophilic centre at event **1**, which could lead to formation of intermediate at event **2**. The nucleophilic addition of isocyanide **6.4** to intermediate at event **3** takes place, followed by intramolecular cyclisation to afford intermediate **4** which isomerised to **6.14**.



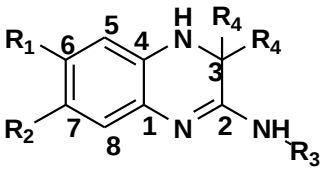
**Scheme 6.8:** Possible mechanism for the formation of quinoxalines **6.14**

*6.4.1 The effect of deactivating substituents on the yield of **6.14a-i**.*

The novel quinoxaline-based products **6.14a-i** were isolated in different yields based on the nature of the functional groups from the isocyanides and benzimidazole derivatives, as shown in Table 6.4. The highest yields for this set of compounds using acetone were obtained from the di-substituted 5,6-dichloro-1*H*-benzo[*d*]imidazol-2(3*H*)-one **6.3** to form **6.14f** and **6.14g** from *tert*-butyl and tetramethylbutyl, respectively. Based on the yields obtained it is evident that mono-substituted deactivating groups at either position 6 or 7 such as ester, fluorine and chlorine gave lower and average yields, while the deactivating bromine group reaction was never successful. Interestingly the dichloro substituted products **6.14f** and **6.14g** gave good yield. Using 3-pentanone, isocyanide **6.4a** and 1*H*-benzo[*d*]imidazol-2(3*H*)-one **6.3a** gave higher yields while the deactivating 5,6-dichloro-1*H*-benzo[*d*]imidazol-2(3*H*)-one **6.3k** gave lowest yields. Under the conditions stipulated in Scheme 6.2 compounds **6.14a-e** were not favoured because of the deactivating groups either at position 6 or 7). Once again, it needs to be mentioned that the unsymmetrical starting materials **6.3** may give rise to the possibility of isomers. For example, we obtained one regioisomer out of the two possible isomers of product **6.14a**, **6.14b and 6.14e** which contains H and/or ester/Cl groups at position $R_1$ and/or $R_2$.

However, when using **6.3d** containing F and H at positions $R_1$ and/or $R_2$ we were able to isolate two regioisomers after the reaction, but we were unable to distinguish them (**6.14c** and **6.14d)**.

**Table 6.3**: Isolated yields of **6.14a-i**




| Compound | $R_1$ | $R_2$ | $R_3$ | $R_4$ | Acetone Yield% (time) | 3-Pentanone Yield% (time) |
|---|---|---|---|---|---|---|
| **6.14a** | $CO_2CH_3$ | H | *t*-Bu | $CH_3$ | 46 (24 h) | - |
| **6.14b** | $CO_2CH_3$ | H | tetramethylbutyl | $CH_3$ | 52 (24 h) | - |
| **6.14c** | F | H | tetramethylbutyl | $CH_3$ | 30 (24 h) | - |
| **6.14d** | H | F | tetramethylbutyl | $CH_3$ | 22 (24 h) | - |
| **6.14e** | Cl | H | tetramethylbutyl | $CH_3$ | 46(24h) | - |
| **6.14f** | Cl | Cl | *t*-Bu | $CH_3$ | 74 (24 h) | - |
| **6.14g** | Cl | Cl | tetramethylbutyl | $CH_3$ | 76 (24 h) | - |
| **6.14h** | H | H | *t*-Bu | $CH_2CH_3$ | 76 (24 h) | 76 (24 h) |
| **6.14i** | Cl | Cl | *t*-Bu | $CH_2CH_3$ | 19 (24 h) | 19 (24 h) |

No reaction (-)

*6.4.2 The elucidation of products **6.14a-i** by FTIR, NMR, HRMS and Single X-ray Crystallography techniques for **6.14f-g**.*

The synthesised structures **6.14a-i** were analysed by FTIR and NMR spectroscopy and HRMS. By way of a representative example, spectroscopic data is discussed for quinoxaline compound **6.14a** (Figure 6.3).

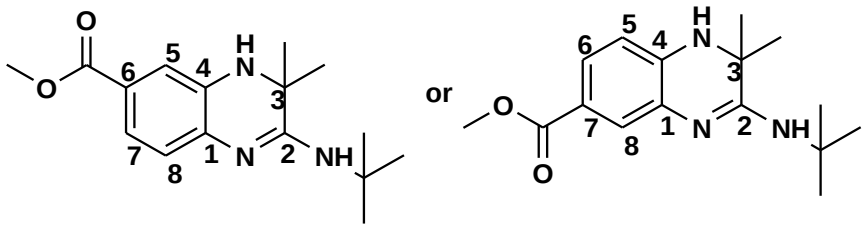



**Figure 6.3**. Structure of quinoxaline **6.14a**

The $^{1}$H NMR spectrum showed a doublet at δ = 7.51-7.49 with a *J* value of 8 Hz corresponding to an aromatic CH, a doublet between δ = 7.28- 7.27 with a *J* value of 1.6Hz corresponding to CH, a doublet appeared at δ = 7.08-7.06 with a *J* value of 8.4 Hz corresponding to an aromatic CH, two singlets were found at δ = 4.46 and δ = 3.60 corresponding to two NH groups, a singlet at δ = 3.89 was assigned to the methoxy group, a singlet from the *tert*-butyl group appeared at δ = 1.51 and a singlet from two methyl groups was observed at δ = 1.31. The $^{13}$C NMR spectrum of **6.14a** showed 13 distinct resonances in accordance with the proposed structure. The mass spectrum of **6.14a** showed the molecular ion $[M+H]^+$ peak at m/z 290.1865 which is consistent with the mass of the proposed product for which the calculated value is 290.1863. The FTIR spectrum displayed characteristic absorption bands for the carbonyl group at 1693 $cm^{-1}$, imine group at 1616 $cm^{-1}$ and for the amine group at 2901 $cm^{-1}$.

Generally the rest of the series **6.14b-i** showed similar patterns:the FTIR spectra of this set of compounds showed characteristic absorption bands for N-H in the range of 3456 $cm^{-1}$ to 2901 $cm^{-1}$, while the N=C stretching band appeared between 1770 $cm^{-1}$ and 1605 $cm^{-1}$. The $^{1}$H NMR spectra of compounds **6.14b-i** exhibited two singlet signals in the region of δ 4.52-3.25 ppm corresponding to the NH groups and two singlet signals at δ 1.52-0.97 ppm corresponding to two methyl groups. $^{13}$C NMR spectra showed characteristic signals in the region of δ 159.4-154.9 ppm corresponding to N=C and two $CH_3$ signals in the region of δ 29.3-8.3 ppm. ESI-LCMS was used to confirm the masses of synthesised compounds **6.14b-i**, which exhibited the protonated parent molecular ions $[M+1]^+$ as shown in Table 6.4.

Table 6.4: Chemical formulae, and calculated and measured molecular ions of **6.14a-i**

| Compound | Chemical Formula [M+1] | Calculated m/z | Measured m/z |
|---|---|---|---|
| **6.14a** | $C_{16}H_{24}N_3O_2^+$ | 290.1863 | 290.1865 |
| **6.14b** | $C_{20}H_{32}N_3O_2^+$ | 346.2489 | 346.2492 |
| **6.14c** | $C_{18}H_{29}FN_3^+$ | 306.2340 | 306.2348 |
| **6.14d** | $C_{18}H_{29}FN_3^+$ | 306.2340 | 306.2345 |
| **6.14e** | $C_{18}H_{29}ClN_3^+$ | 322.2045 | 322.2055 |
| **6.14f** | $C_{14}H_{20}Cl_2N_3^+$ | 300.1029 | 300.1039 |
| **6.14g** | $C_{18}H_{28}Cl_2N_3^+$ | 356.1655 | 356.1660 |
| **6.14h** | $C_{16}H_{26}N_3^+$ | 260.2121 | 260.2125 |
| **6.14i** | $C_{16}H_{24}Cl_2N_3^+$ | 328.1342 | 328.1343 |

X-ray analysis unambiguously confirmed the structure of **6.14f** to be (*E*)-*N*-(6,7-dichloro-3,3-dimethyl-3,4-dihydroquinoxalin-2(1*H*)-ylidene)-2-methylpropan-2-amine and **6.14g** to be (*E*)-*N*-(6,7-dichloro-3,3-dimethyl-3,4-dihydroquinoxalin-2(1*H*)-ylidene)-2,4,4-trimethylpentan-2-amine, as shown in Figure 6.4.

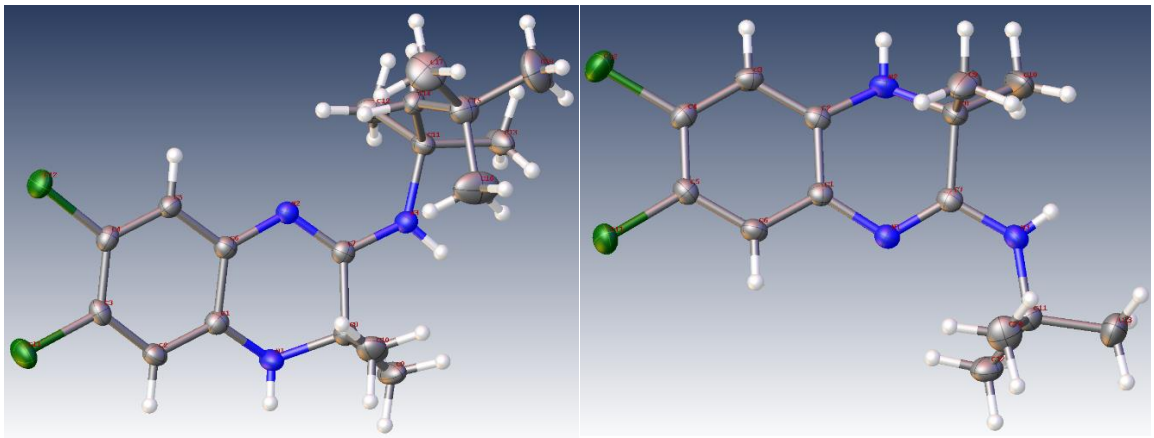
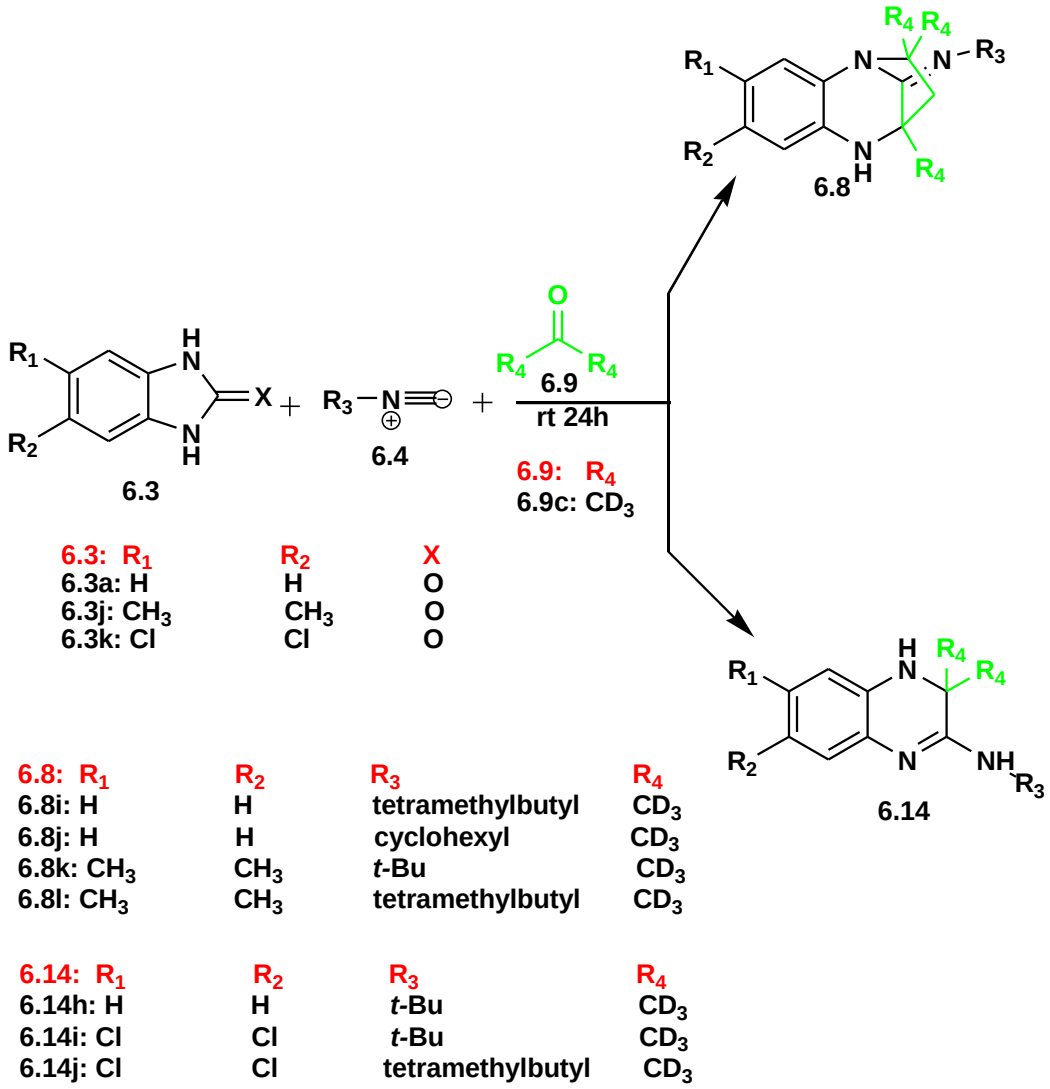


**Figure 6.4**: Single-crystal X-ray structure (ORTEP) of **6.14g (**left**)** and **6.14f (**right**)**

A total of nine (*E*)-*N*-(3,3-dimethyl-3,4-dihydroquinoxalin-2(1*H*)-ylidene)-2-methylpropan-2-amine derivatives compounds **6.14** were synthesised, and to best of our knowledge, these compounds are novel and the method used for their preparationhas never been reported before.

### 6.5 Synthesis of diazepines and quinoxalines using deuterated-acetone (acetone-$d_6$)

In order to elucidate the role of acetone in the reactions shown in Schemes 6.2 and 6.4, deuterated acetone was used in the reactions instead of normal acetone. The deuterated acetone was used to probe the reactivity towards the formation of the diazepine scaffold and trace the atom involvement in the final diazepine **6.8** and quinoxaline **6.14** products. The anticipation was that deuterated acetone should mimic the normal acetone to produce heavier isotopes of the diazepines **6.8** and quinoxalines **6.14**. Due to the expense of acetone-$d_6$ fewer (seven) reactions were set up and the same reaction protocol previously described in Scheme 6.5 was followed (Scheme 6.9). The first set of reactions involved 1*H*-benzo[*d*]imidazol-2(3*H*)-one **6.3a** and *t*-Bu-, tetramethylbutyl- and cyclohexyl isocyanides. The second set of reactions involved benzimidazoles **6.3h** and **6.3i** with only two isocyanide derivatives (*t*-Bu and tetramethylbutyl). Four deuterated diazepines **6.8i-l** and three deuterated quinoxalines **6.14h-j** were obtained.



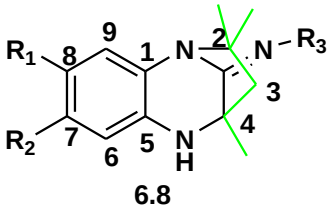

**Scheme 6.9**: Synthesis of deuterated diazepines **6.8i-l** and deuterated quinoxalines **6.14h-j** *(Green-deuterated-atoms)*

*6.5.1 The effect of acetone-d6 on yield of diazepines **6.8** and quinoxalines **6.14**.*

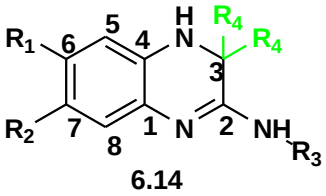
The use of acetone-$d_6$ instead of normal acetone yielded partially deuterated diazepines **6.8i-l** in average yield. The acetone-$d_6$ was relatively less reactive compared to normal acetone as shown in Scheme 6.2. In addition, acetone-$d_6$ was completely inert to forming the diazepine-frame when 1*H*-benzo[*d*]imidazol-2(3*H*)-one **6.3a** and *tert*-butyl isocyanide **6.4** were reacted, instead resulting in formation of deuterated (*E*)-*N*-(3,3-dimethyl-3,4-dihydroquinoxalin-2(1*H*)-ylidene)-2-methylpropan-2-amine **6.14h**. In contrast, acetone-$d_6$ was relatively reactive in forming the quinoxaline-based isotopes **6.14h-j**, with comparable yields to normal acetone

obtained for the two reactions tested. The highest yield was of **6.14j** from reaction of deactivating di-substituted 5,6-dichloro-1*H*-benzo[*d*]imidazol-2(3*H*)-one **6.3k**.

**Table 6.5**: Isolated yields of diazepine-isotopes **6.8i-l** and quinoxaline isotopes **6.14h-j**




6.8




6.14

| Compound | $R_1$ | $R_2$ | $R_3$ | $R_4$ | Acetone-$d_6$ Yield% (Time) |
|---|---|---|---|---|---|
| **6.8i** | H | H | tetramethylbutyl | $CD_3$ | 57 (24 h) |
| **6.8j** | H | H | Cyclohexyl | $CD_3$ | 61 (24 h) |
| **6.8k** | $CH_3$ | $CH_3$ | *t*-Bu | $CD_3$ | 73 (24 h) |
| **6.8l** | $CH_3$ | $CH_3$ | tetramethylbutyl | $CD_3$ | 61 (24 h) |
| **6.14h** | H | H | *t*-Bu | $CD_3$ | 70 (24 h) |
| **6.14i** | Cl | Cl | *t*-Bu | $CD_3$ | 72 (24 h) |
| **6.14j** | Cl | Cl | tetramethylbutyl | $CD_3$ | 80 (24 h) |

*6.5.2 Analysis of deuterated diazepines and quinoxaline derivatives by FTIR, NMR and HRMS*

Compounds **6.8i-l** and **6.14h-j** were synthesised in deuterated acetone and analysed by FTIR and NMR spectroscopy and HRMS. The FTIR spectra of compounds **6.8i-l** showed characteristic absorption bands for N-H in the range of 3312 $cm^{-1}$ to 2854 $cm^{-1}$, while the N=C stretching band appeared in the range of 1699 $cm^{-1}$ to 1610 $cm^{-1}$. The $^1H$ NMR spectra of compounds **6.8i-l** exhibited one singlet signal in the region of δ 3.87-3.68 ppm corresponding to the NH group. $^{13}C$ NMR spectra showed characteristic signals in the region of δ 168.5-151.5 ppm corresponding to the N=C group. The FTIR spectra of compounds **6.14h-j** showed characteristic absorption bands for N-H in the range of 3460 $cm^{-1}$ to 2901 $cm^{-1}$, while the N=C stretching band appeared in the range of 1770 $cm^{-1}$ to 1605 $cm^{-1}$. The $^1H$ NMR spectra of

compounds **6.14i-l** exhibited two singlet signals for NH in the region of δ 4.36-3.43 ppm. $^{13}C$ NMR spectra showed characteristic signals in the region of δ 158.4-157.6 ppm corresponding to the N=C group. ESI-LCMS was used to confirm the masses of the synthesised deuterated derivatives **6.8i-l** and **6.14h-j**. Chromatograms exhibited the protonated molecular ion and/or included deuterated-protons $[M+1]^+$ from the isotopes. By way of example, the following results were observed: compound **6.8j** ($C_{19}H_{28}N_3^+$ 298.2278 plus 11-D found isotope 309.2884), **6.14h** ($C_{14}H_{22}N_3^+$ 232.1808 plus 6-D found isotope 238.2188) **6.14i** ($C_{14}H_{20}Cl_2N_3^+$ 300.1029 plus 6-D found isotope 306.1372) and **6.12j** ($C_{18}H_{28}Cl_2N_3^+$ 356.1655 plus 6-D found isotope 362.2024).

A total of seven deuterated derivatives of diazepines (**6.8i-l**) and quinoxalines (**6.14h-j**) were synthesised and to best of our knowledge these compounds are novel.

X-ray crystallographic analysis confirmed the structure of **6.8k** to be 2-methyl-*N*-(2,2,4,7,8-pentamethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[*b*][1,4]diazepin-10-ylidene)propan-2-amine, as shown in Figure 6.5.

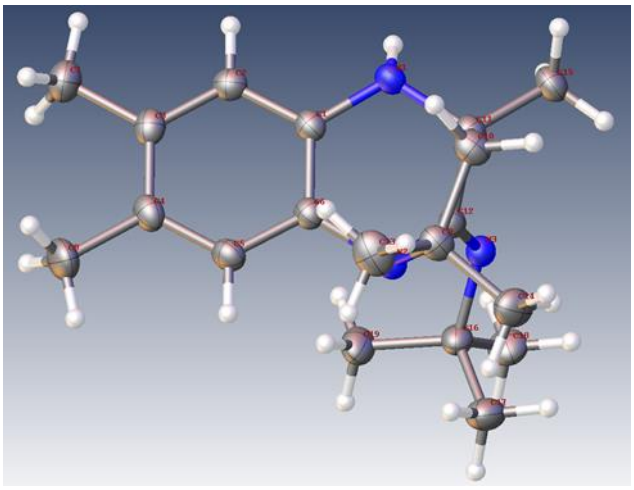
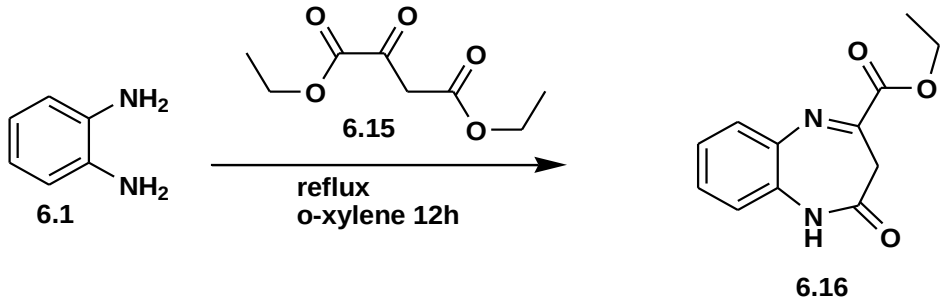


**Figure 6.5**: Single-crystal X-ray structure (ORTEP) of **6.8k-D**.

## 6.6 Synthesis of methyl 4-oxo-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxylate derivatives 6.13 from DMAD/DEtAD/DTAD and 1,2-phenylenediamines/ benzimidazoles.

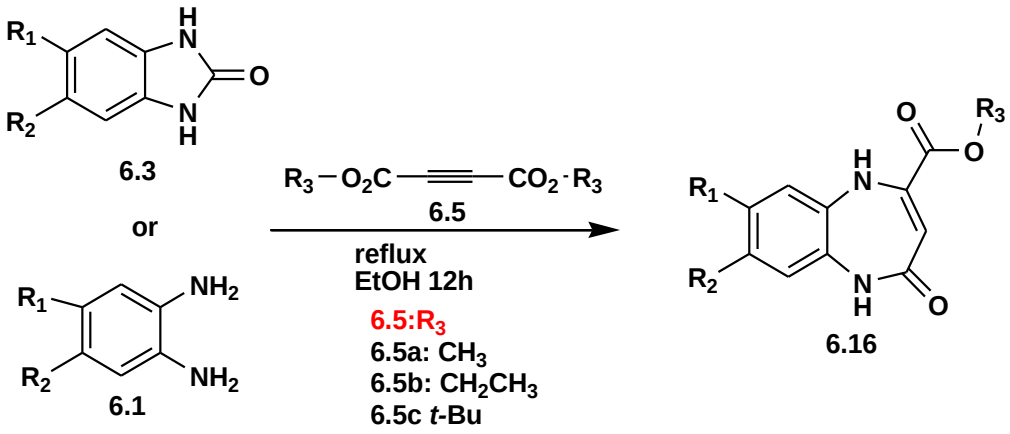
The reactivity of benzimidazole derivatives and 1,2-phenylenediamines was further investigated under reflux conditions using dialkyl acetylenedicarboxylates as Michael acceptors. Solomko and coworkers[247] have reported the preparation of the similar ethyl 2,3-

dihydro-2-oxo-1*H*-1,5-benzodiazepine-4-carboxylate from *o*-phenylenediamine and oxaloacetic ester in *o*-xylene under reflux as shown in Scheme 6.10.




**Scheme 6.10**: Synthesis of ethyl 2,3-dihydro-2-oxo-1*H*-1,5-benzodiazepine-4-carboxylate

When DMAD **6.4** treated with benzimidazole or1,2-phenylenediamines resulted in formation of 4-oxo-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxylate derivatives **6.16** under reflux condition with ethanol as shown in Scheme 6.11. The reaction involves Michael addition/intramolecular cyclization to form interesting fused [6-7] diazepine scaffolds. The reactions were cooled and the solvents were removed under reduced pressure to obtain the residue that was purified by column chromatography using hexane/ ethyl acetate (1:1) as eluent to obtain the novel products. Alternatively, the precipitate was washed with cold ethanol and filtered to give solid products. Diazepine compounds **6.16** were obtained in moderate to good yields.




| 6.1: $R_1$ | $R_2$ |
|---|---|
| 6.1a: H | H |
| 6.1b: $CH_3$ | H |
| 6.1c: $CO_2CH_3$ | H |
| 6.1d: F | H |
| 6.1e: Cl | H |
| 6.1f: Br | H |
| 6.1g: $CH_3$ | $CH_3$ |
| 6.1h :Cl | Cl |
| 6.1i: $NO_2$ | H |

| 6.16: $R_1$ | $R_2$ | $R_3$ |
|---|---|---|
| 6.16a: H | H | $CH_3$ |
| 6.16b: H | H | $CH_2CH_3$ |
| 6.16c: H | H | *t*-Bu |
| 6.16d: $NO_2$ | H | $CH_3$ |
| 6.16e: $NO_2$ | H | $CH_2CH_3$ |
| 6.16f: $NO_2$ | H | *t*-Bu |
| 6.16g: Cl | Cl | $CH_3$ |
| 6.16h: Cl | Cl | $CH_2CH_3$ |
| 6.16i: Cl | Cl | *t*-Bu |
| 6.16j: $CH_3$ | $CH_3$ | $CH_3$ |

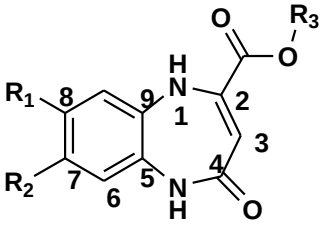
**Scheme 6.11:** Synthesis of methyl 4-oxo-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxylate derivatives **6.16**.

*6.6.1. The effect of activating and deactivating groups on synthesis of methyl 4-oxo-4,5-dihydro-1H-benzo[b][1,4]diazepine-2-carboxylate derivatives* ***6.16***

The methyl 4-oxo-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxylate derivatives were obtained in different yields based on the different nature of the functional groups from 1,2-phenylenediamines (yield A) and benzimidazoles (yield B) as shown in Table 6.7. Benzimidazole **6.3** gave lower yields compared to reactions of 1,2-phenylenediamines **6.1** with free primary amines. The highest yields for this set of compounds was obtained for **6.16d** with a strong deactivating and electron-withdrawing nitro group at position 8**,** whereas the lowest yield was obtained for **6.16j**, a di-substituted activating and electron-donating methyl group at position 7 and 8. The weakly deactivating di-substituted chlorine atoms at position 7 and 8 gave good yields. The reactions of mono-substituted substrates **6.1** or **6.3** with deactivating groups at either position 7 or 8 (Br, F, Cl and $CO_2CH_3$) were not successful, and neither was reaction of the substrate mono-substituted at position 7 or 8 with the activating $CH_3$ group. Reactions were more favoured with a strong deactivating mono-substituent (nitro), rather than an activating group (methyl) at position 7 or 8. As expected, the unsymmetrical starting

material **6.1i** may give rise to the possibility of two regioisomers of product **6.16d-f**, however, after the reactions we obtained only one regioisomer of product **6.16d-f** but we were unable to establish unequivocally which one was present.

**Table 6.6**: Isolated yields of methyl 4-oxo-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxylate derivatives (**6.16a-j**)




| compound | $R_1$ | $R_2$ | $R_3$ | Yield% A | Yield% B |
|---|---|---|---|---|---|
| **6.16a** | H | H | $CH_3$ | 83 | 72 |
| **6.16b** | H | H | $CH_2CH_3$ | 79 | 68 |
| **6.16c** | H | H | *t*-Bu | 75 | 62 |
| **6.16d** | $NO_2$ | H | $CH_3$ | 92 | - |
| **6.16e** | $NO_2$ | H | $CH_2CH_3$ | 85 | - |
| **6.16f** | $NO_2$ | H | *t*-Bu | 80 | - |
| **6.16g** | Cl | Cl | $CH_3$ | 83 | 71 |
| **6.16h** | Cl | Cl | $CH_2CH_3$ | 76 | 64 |
| **6.16i** | Cl | Cl | *t*-Bu | 75 | 66 |
| **6.16j** | $CH_3$ | $CH_3$ | $CH_3$ | 62 | 60 |

No reaction (-)

*6.6.2 The structure elucidation of **6.16a-j** by FTIR, NMR spectroscopy and HRMS techniques*

By way of a representative example, spectroscopic data is discussed for compound **6.16a** (Figure 6.6)

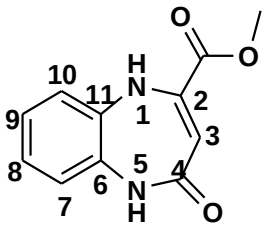



**Figure 6.6**. Structure of **6.16a**

The $^{1}H$ NMR spectrum showed two singlet at δ 11.74 and 11.03 corresponding to two NH groups. Multiplet peaks were found around δ 7.40-7.39 and δ 7.05-7.03 corresponding to four aromatic CH protons. A singlet CH peak at δ 5.52 from position 3 and a singlet methyl peak at δ 3.68 were also observed. The $^{13}C$ NMR spectrum of **6.16a** showed 11 distinct resonances in accordance with the proposed structure. The mass spectrum of **6.16a** showed the molecular ion $[M+H]^+$ peak at m/z 219.0752 which is consistent with the mass of the proposed product. The FTIR spectrum displayed characteristic absorption bands for the carbonyl group at 1686 and 1614 $cm^{-1}$ and for the amine group at 3215 $cm^{-1}$ and 2902 $cm^{-1}$.

Generally the rest of the series **6.16b-j** showed similar patterns. The FTIR spectra of these compounds showed characteristic absorption bands for N-H in the range of 3226 $cm^{-1}$ to 2881 $cm^{-1}$, while the N=C stretching band appeared in the range of 1737 $cm^{-1}$ to 1602 $cm^{-1}$. The $^{1}H$ NMR spectra of compounds **6.16b-j** exhibited two singlet signals in the region of δ 12.19-10.97 ppm corresponding to the two NH groups and a singlet at around δ 5.58-5.42 ppm corresponding to CH. $^{13}C$ NMR spectra showed characteristic signals in the region of δ 169.7-155.4 ppm corresponding to two C=O groups and CH signals at δ 87.8-82.6 ppm. ESI-LCMS was used to confirm the masses of the synthesised compounds **6.16b-j**, as shown in Table 6.7. Chromatograms exhibited the protonated parent molecular ion $[M+1]^+$/ $[M+Na]^+$.

**Table 6.7**: The chemical formulae, and calculated and measured molecular ions of **6.16a-j**

| Compound | Chemical Formula [M+1] | Calculated m/z | Measured m/z |
|---|---|---|---|
| **6.16a** | $C_{11}H_{11}N_2O_3^+$ | 219.0764 | 219.0752 |
| **6.16b** | $C_{12}H_{13}N_2O_3^+$ | 233.0921 | 233.0920 |
| **6.16c** | $C_{14}H_{17}N_2NaO_3^+$ | 283.1059 | 283.1051 |
| **6.16d** | $C_{11}H_{10}N_3O_5^+$ | 264.0615 | 264.0611 |
| **6.16e** | $C_{12}H_{12}N_3O5^+$ | 278.0771 | 278.0762 |
| **6.16f** | $C_{14}H_{15}N_3NaO_5^+$ | 328.0909 | 328.1013 |
| **6.16g** | $C_{11}H_9Cl_2N_2O_3^+$ | 286.9985 | 286.9982 |
| **6.16h** | $C_{12}H_{11}Cl_2N_2O_3^+$ | 301.0141 | 301.0139 |
| **6.16i** | $C_{14}H_{14}Cl_2N_2NaO_3^+$ | 351.0279 | 351.0366 |
| **6.16j** | $C_{13}H_{15}N_2O_3^+$ | 247.1077 | 247.1075 |

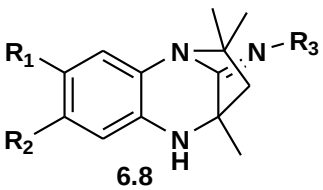
A total of ten (10) methyl 4-oxo-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxylate derivatives (**6.16a-j**) were synthesised, and to the best of our knowledge these compounds have not been reported previously.

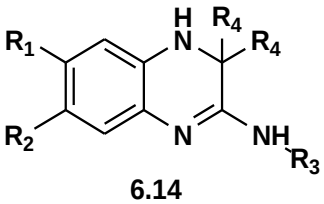
### 6.7 Biological evaluations of diazepine and quinoxaline derivatives

The toxicity of the diazepines and quinoxalines was evaluated in the MT-4 cell line at 100 µM. Auranofin was used as a cytotoxic control compound starting at 100 µM in a serial dilution series and DMSO was used as a background control. From the first set of diazepines **6.8a-g**, compound **6.8g** was the only compounds that had a $CC_{50}$ value above 200 µM and is considered to be relatively non-toxic as recorded in Table 6.8. Compounds **6.8a**, **6.8d** and **6.8e** have $CC_{50}$ values above 100 µM and are considered to be relatively non-toxic. Three compounds with $CC_{50}$ values below 100 µM **6.8a** (41 µM), **6.8b** (86 µM) and **6.8f** (26 µM) can be considered to be relatively toxic.

The quinoxaline derivatives **6.14a-i** had only one compound, **6.14h**, with a $CC_{50}$ value above 200 µM, while three compounds **6.14a**, **6.14c** and **6.14i** had $CC_{50}$ values above 100 µM and are therefore relatively non-toxic. Out of nine compounds, more than half of the compounds **6.14b** (28 µM), **6.14d** (35 µM), **6.14e** (31 µM), **6.14f** (12 µM) and **6.14g** (21 µM) had $CC_{50}$ values below 100 µM and are considered to be relatively toxic. The diazepines **6.16a-e** and **6.16g-j** were found to have $CC_{50}$ values above 200 µM and are considered to be relatively non-toxic. Compound **6.16f** was found to have a $CC_{50}$ value of 166 µM and is therefore relatively non-toxic.

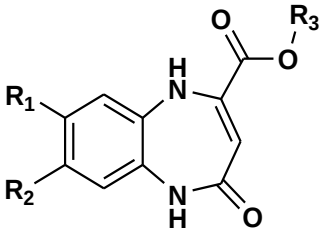
**Table 6.8**: $CC_{50}$ values of synthesised diazepine and quinoxaline derivatives

| Structures | Number | $CC_{50}$ | % Antiviral inhibition (infected MT4) |
|---|---|---|---|
|  6.8 | **6.8a** | 175 µM | -0.85 |
| | **6.8b** | 41 µM | |
| | **6.8c** | 86 µM | |
| | **6.8d** | 126 µM | -9.62 |
| | **6.8e** | 109 µM | |
| | **6.8f** | 26 µM | |
| | **6.8g** | > 200 µM | -966 |




6.14

| | | |
|---|---|---|
| **6.14a** | 196 µM | 0.25 |
| **6.14b** | 28 µM | |
| **6.14c** | 178 µM | 7.12 |
| **6.14d** | 35 µM | |
| **6.14e** | 31 µM | |
| **6.14f** | 12 µM | |
| **6.14g** | 21 µM | |
| **6.14h** | > 200 µM | 7.39 |
| **6.14i** | 171 µM | 3.38 |




6.16

| | | |
|---|---|---|
| **6.16a** | > 200 µM | 7.98 |
| **6.16b** | > 200 µM | 2.98 |
| **6.16c** | > 200 µM | 8.41 |
| **6.16d** | > 200 µM | 14.05 |
| **6.16e** | > 200 µM | 1.94 |
| **6.16f** | 166 µM | 96.96 |
| **6.16g** | > 200 µM | 35.60 |
| **6.16h** | > 200 µM | 3.19 |
| **6.16i** | > 200 µM | 96.535 |
| **6.16j** | > 200 µM | 2.50 |

Finally the non-toxic comounds were subjected to antiviral testing in infected MT4 cells. The tests were carried out in a cell-based assay and each compound was analysed at a single dose of 100 µM using Dolutegravir and Raltegravir as control compounds. Based on the outcome the highest percentage inhibition activity of 96% was found for compound **6.16i** and **6.16f**. Compound **6.16i** contains two chlorine groups at $R_1$ and $R_2$ with a *tert*-butyl group at $R_3$while **6.16f** contains a nitro group at either $R_1$ or $R_2$ with a *tert*-butyl group at. The third best activity was observed for **6.16g**, containing two chlorine groups at $R_1$ and $R_2$ with a methyl group at R3, with 35% inhibition.

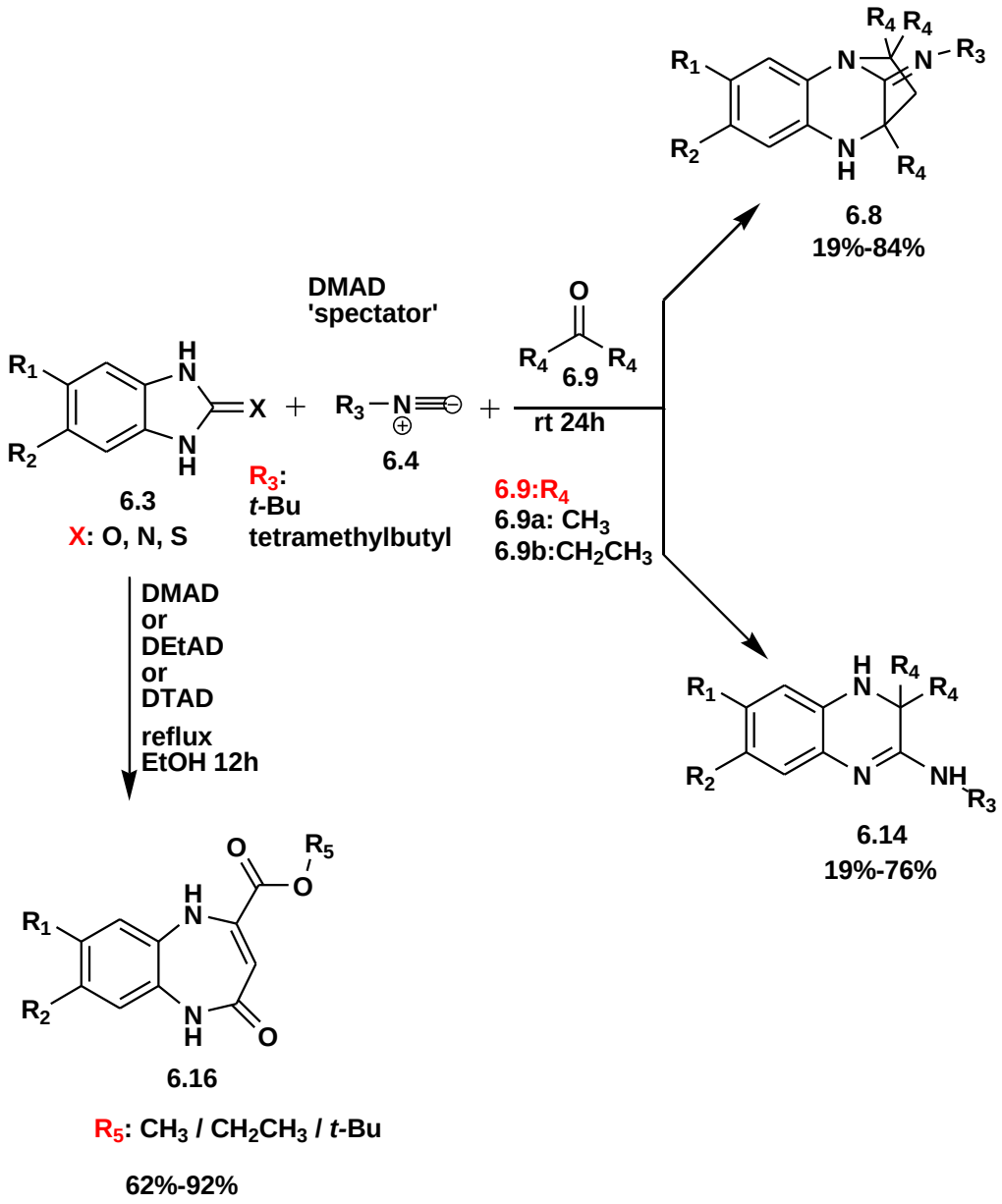
The antimicrobial activities of these sets of compounds showed no significant activity against the bacterial strains.

### 6.8. Conclusions

Two different series of compounds **6.8** and **6.14** were prepared *via* 3-CR in order to develop drug-like compounds. The diazepine compounds **6.8** were achieved from benzimidazoles **6.3**, isocyanides **6.4**, acetone **6.9a** and DMAD (spectator) from trace to good yields as shown in Scheme 6.12. The same reactions were repeated using deuterated acetone and gave four isotopes in moderate yields. The generated diazepine scaffolds **6.8** were only achieved when

the benzimidazole substituents were electron donating or were hydrogen atoms. The reactions of benzimidazoles with deactivating groups such as Br, Cl, F and $CO_2CH_3$ as substituents at $R_1$ and $R_2$ were not successful to obtain diazepines **6.8**. The omission of DMAD from the initial method resulted in quinoxaline **6.14** derivatives from benzimidazoles **6.3** with only deactivating groups such as Cl, F and $CO_2CH_3$ as substituents on $R_1$ and $R_2$. The same method was also repeated using deuterated acetone to give three (3) quinoxaline **6.14** isotopes in moderate to good yields. When using benzimidazole **6.3** with hydrogen or methyl as substituents at positions $R_1$ and $R_2$ we only achieved diazepine **6.8** products, whereas when using deactivating groups such as Cl, F and $CO_2CH_3$ we achieved quinoxalines **6.14**.

Interestingly, the acetone-D gave the quinoxaline **6.14h** isotope instead of the diazepine **6.8a** isotope. Direct reaction of 1,2-phenylenediamine **6.1** with isocyanide **6.4** and acetone **6.9** was not successful for producing either diazepine-based or quinoxaline-based scaffolds. The two component reaction of 1,2-phenylenediamine **6.1** or benzimidazole **6.3** with electron deficient alkynes (DMAD, DEtAD and DTAD) **6.5** yielded diazepine **6.16** derivatives. Benzimidazoles **6.3** gave lower yields as compared to 1,2-phenylenediamine **6.1**. Thirty four novel of the synthesised diazepine-based and quinoxaline-based compounds were found to be novel. Eighteen of the synthesised compounds were found to cause 50% cell reduction at a concentration above 100 μM. We were delighted to find that three compounds **6.16i**, **6.16g** and **6.16f**, showed significant antiviral activity against HIV-1. Unfortunately none of the compounds showed any significant antimicrobial activity.




**Scheme 6.12:** The summary of reaction pathways to formation of **6.8**, **6.14** and **6.16**.

### 6.9. Future work

Future work will entail structural modification of substituents $R_1$ and $R_2$ on the aromatic site to synthesise more derivatives and explore the 3-CR to produce diazepines **6.8** and quinoxalines **6.14** without using a catalyst. In addition, functional group inter-conversion of the ester group on **6.16** as shown in Figure 6.6 will be applied. We will further investigate the role of DMAD in the reaction towards the product formation of **6.8** and **6.14;** and other possible routes to synthesise compounds **6.8**. The modification of **6.16** will be done based on good results

obtained from antiviral activities, whereas compounds **6.8** and **6.14** may be subjected to other biological evaluations such as Tuberculosis and Cancer studies.

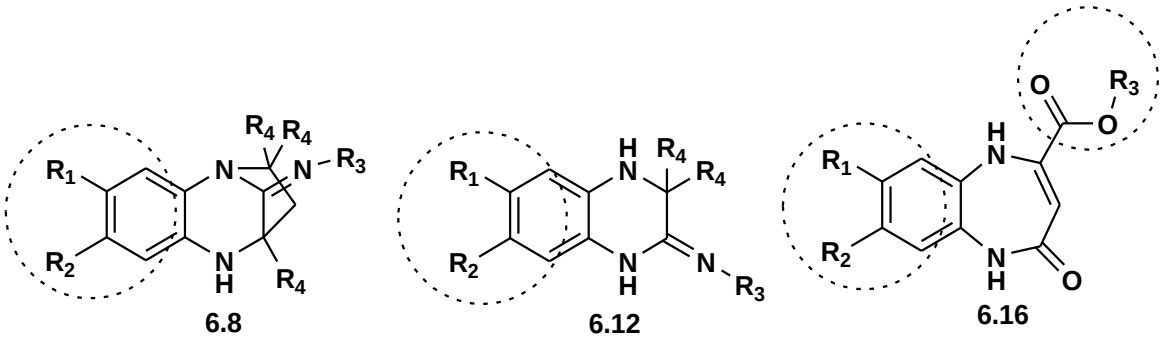



**Figure 6.6:** Possible structural modifications of the diazepine- and quinoxaline-based compounds.

# CHAPTER 7

# General conclusions

## 7.1 Strategy and approaches employed

Computational modelling was done as one of the strategies to identify bioactive scaffolds with the ability to disrupt the interaction between IN and LEDGF/p75. This led us to the synthesis of hexahydroquinoline compounds and identification of fused heterocyclic compounds bearing five-six, six-six and six-seven fused ring scaffolds.

Reactions where three or more reagents (starting materials) react to form one product that comprise of all or almost all the atoms of the starting materials are knowns as multicomponent reactions (MCRs). This approach was employed for synthetic purposes to develop bioactive heterocyclic scaffolds. MCRs possess the capability to rapidly generate molecular diversity and structural complexity, hence are considered useful for the preparation of natural products and the development of drugs in pharmaceutical processes.

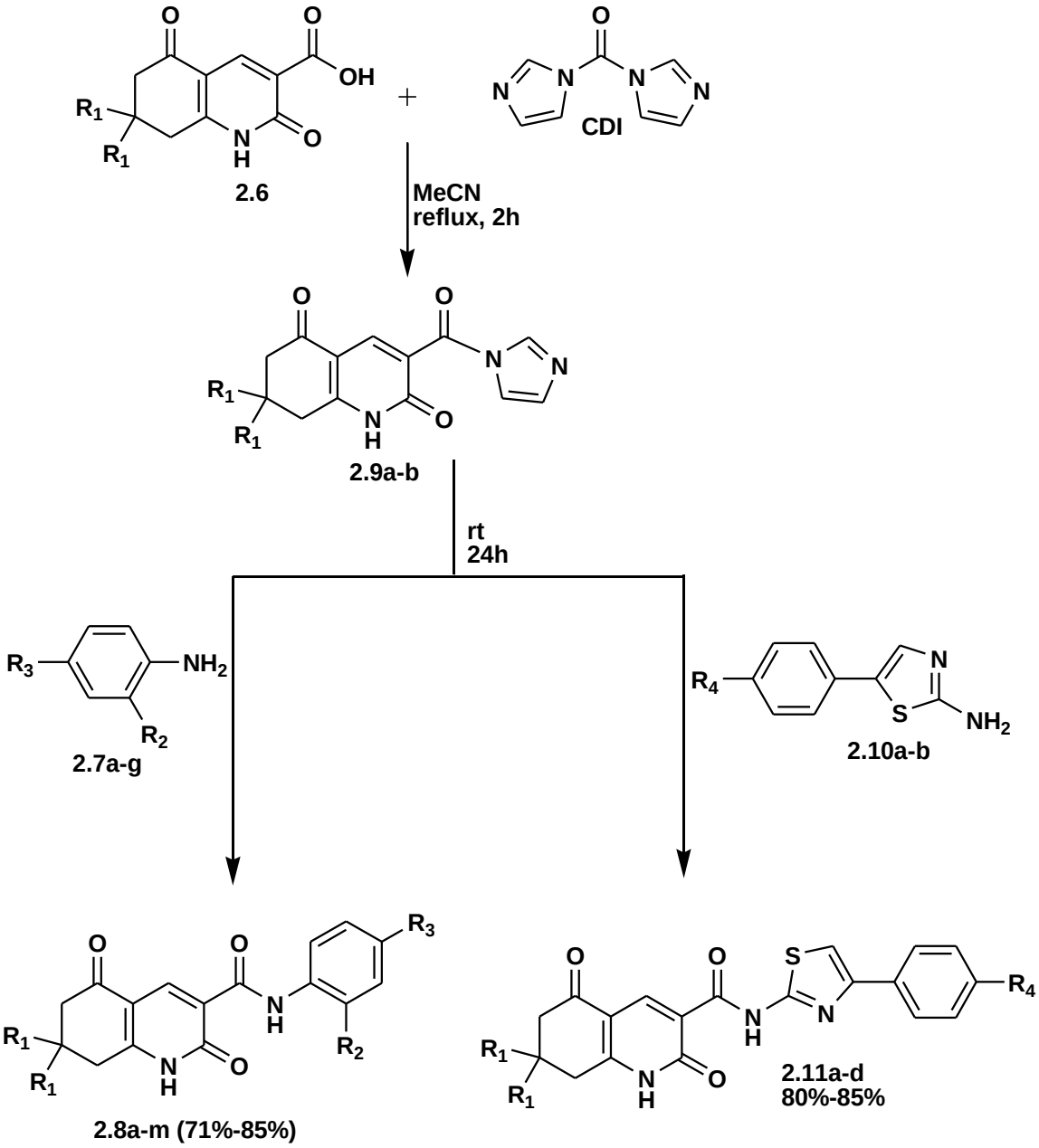
This thesis is concerned with the synthesis of fused-ring heterocyclic scaffolds (with possible biological applications) *via* 3-CR as follows:

a) Reaction of electron deficient alkynes (such as DMAD and DEtAD) and isocyanides to generate zwitterion adducts *in-situ* was explored. These zwitterions were found to react with 5-membered ring thiazole, triazole and pyrrolidine derivatives, or six-membered ring thiazines. An attempt to replace the environmentally unfriendly solvent dichloromethane with a greener solvent such as acetone, isopropanol or ethanol resulted in average to low yields when synthesising pyrimidines and thiazines; b) An alternative route to the synthesis of thiazole-pyrimidines *via* 3-CR (using thiourea, α-haloketone and DMAD) was investigated; c) A catalyst-free and solvent-free 3-CR synthetic route to diazepines and quinoxalines was demonstrated.

## 7.2 Synthesis of hexahydroquinoline compounds

Based on the availability of reagents and a facile synthetic route, one of the ChemBridge compounds containing the hexahydroquinoline scaffold was identified from a pool of the potential compounds *via* computational modelling. The compound *N*-(4-chlorophenyl)-7,7-

dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide was synthesised together with its derivatives as shown in Scheme 7.1. The reaction of 2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid derivatives (**2.6a-b**), with CDI and anilines **2.7** gave rise to 7,7-dimethyl-2,5-dioxo-*N*-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide derivatives (**2.8a-m** and **2.11a-d**) in good yields.




| 2.8: | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 2.8a: | $CH_3$ | H | Cl |
| 2.8b: | $CH_3$ | H | Br |
| 2.8c: | $CH_3$ | H | $CH_3$ |
| 2.8d: | $CH_3$ | $CH_3$ | $CH_3$ |
| 2.8e: | $CH_3$ | OH | H |
| 2.8f: | $CH_3$ | $CH_3O$ | $CH_3O$ |
| 2.8g: | $CH_3$ | H | H |
| 2.8h: | H | H | Cl |
| 2.8i: | H | H | Br |
| 2.8j: | H | H | $CH_3$ |
| 2.8k: | H | OH | H |
| 2.8l: | H | $CH_3O$ | $CH_3O$ |
| 2.8m: | H | H | H |

| 2.7: | $R_2$ | $R_3$ |
|---|---|---|
| 2.7a: | H | Cl |
| 2.7b: | H | Br |
| 2.7d: | $CH_3$ | $CH_3$ |
| 2.7e: | OH | H |
| 2.7f: | $OCH_3$ | $OCH_3$ |
| 2.7g: | H | H |
| 2.7h | Br | Br |
| 2.7i | Cl | Cl |
| 2.7j | F | F |
| 2.7k | $NO_2$ | H |

| 2.10: | $R_4$ |
|---|---|
| 2.10a: | F |
| 2.10b: | $OCH_3$ |

| 2.11: | $R_1$ | $R_4$ |
|---|---|---|
| 2.11a: | $CH_3$ | F |
| 2.11b: | $CH_3$ | $OCH_3$ |
| 2.11c: | H | F |
| 2.11d: | H | $OCH_3$ |

**Scheme .7.1:** Synthesis of **2.8a-m** and **2.11a-d**

### 7.3 The synthesis of pyrimidines, thiazines and their analogues

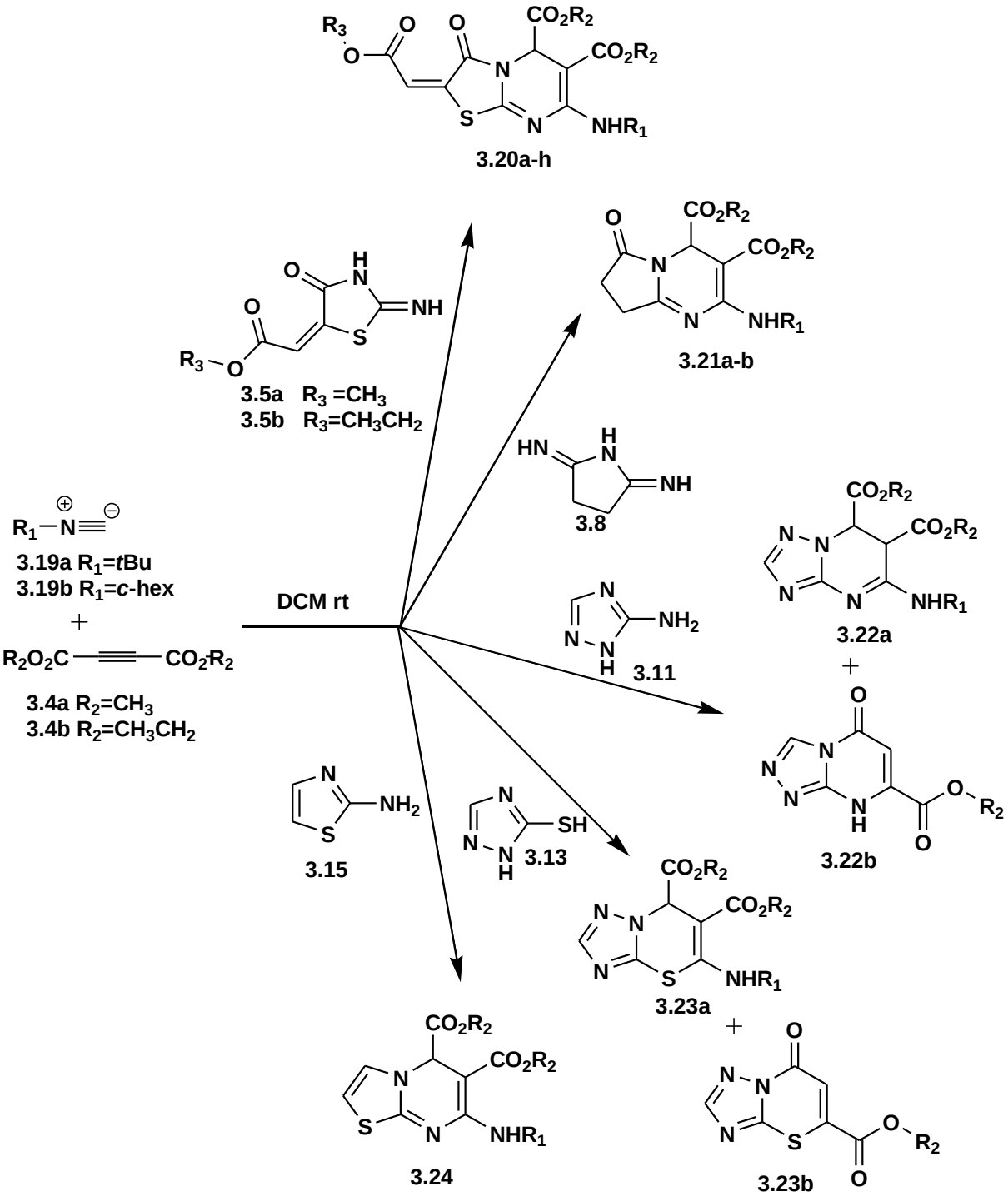
The use of a zwitterion adduct for the synthesis of pyrimidine derivatives (**3.20a-h**, **3.21a-b**, **3.22a**, **3.23a** and **3.24**) was successfully accomplished using 5-membered rings containing an acidic proton such as thiazole, triazole-, and pyrrolidine derivatives as shown in Scheme 7.2. The reactivity of the 5-membered substrate towards the zwitterion adducts was found to be influenced by the following factors:

(1) The order of addition or reactants, reaction conditions and temperature: The addition of DMAD to the 5-membered ring compound in dry dichloromethane at room temperature (rt) followed by slow addition of isocyanide gave 71% as the highest yield for **3.20d** using Method A. When isocyanide was added to DMAD at 0 ºC and then followed by the 5-membered ring substrate the highest yield obtained was 86% for **3.20d** from Method B.

(2) The $pK_a$ of the 5-membered ring substrate: for example when substrate **3.5a-b** with a $pK_a$ of 0.5 was tested to react with the zwitterion adduct this resulted in good yield 75% - 87%, while substrates **3.11** and **3.13** with $pK_a$ values of 9.2 and 11.1 gave 30% and 24% yield, respectively. In addition, substrate **3.11** and **3.13** resulted in co-products, from the direct reaction of the DMAD and the 5-membered ring in the presence of isocyanide.

(3) Solvent: Dichloromethane, a polar aprotic solvent with low dielectric constant was favoured to give good yields, as compared to the less favoured acetone, ethanol and isopropanol.

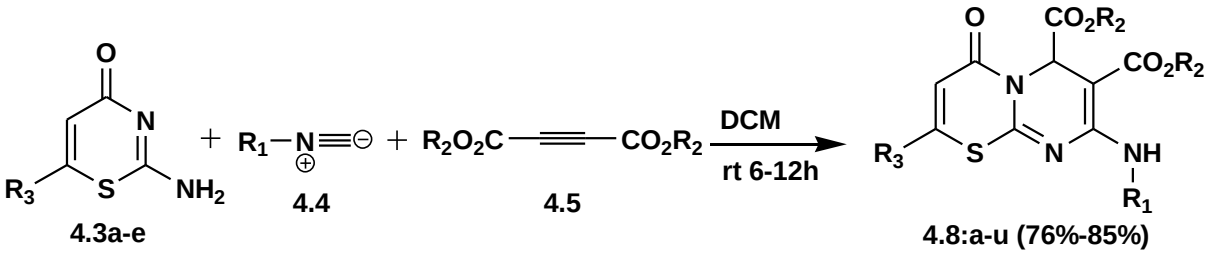
Thirteen novel pyrimidine derivatives were obtained by direct reaction of the zwitterion adduct and their respective 5-membered substrates and only two co-products, **3.22b**[104] and **3.23b**,[105] were found to be previously reported. This aspect of the work has been published in Tetrahedron Letters, **2020**, *61*, 151796.




**Scheme 7.2:** Synthesis of highly functionalised pyrimidines *via* one pot 3-CR.

After successful synthesis of these pyrimidine derivatives, we applied six-membered thiazine substrates with a $pK_a$ range from -1.8 to -2.2, which contain an acidic proton (represented in Scheme 7.3). Dichloromethane was favoured as a solvent, giving good to high yields compared to acetone, ethanol and isopropanol. Acetone gave average yields that were better than those

seen for ethanol and isopropanol. The reactions proceeded smoothly and the thiazine substrates were found to be suitable to form the desired products in good yields (75% – 85%). Thiazines were found to be suitable to react with zwitterion adducts affording a total of twenty-one novel thiazine-based compounds.




**4.4 $R_1$**
**4.4a: *t*-Bu**
**4.4b: tetramethylbutyl**
**4.4c: cyclohexyl**

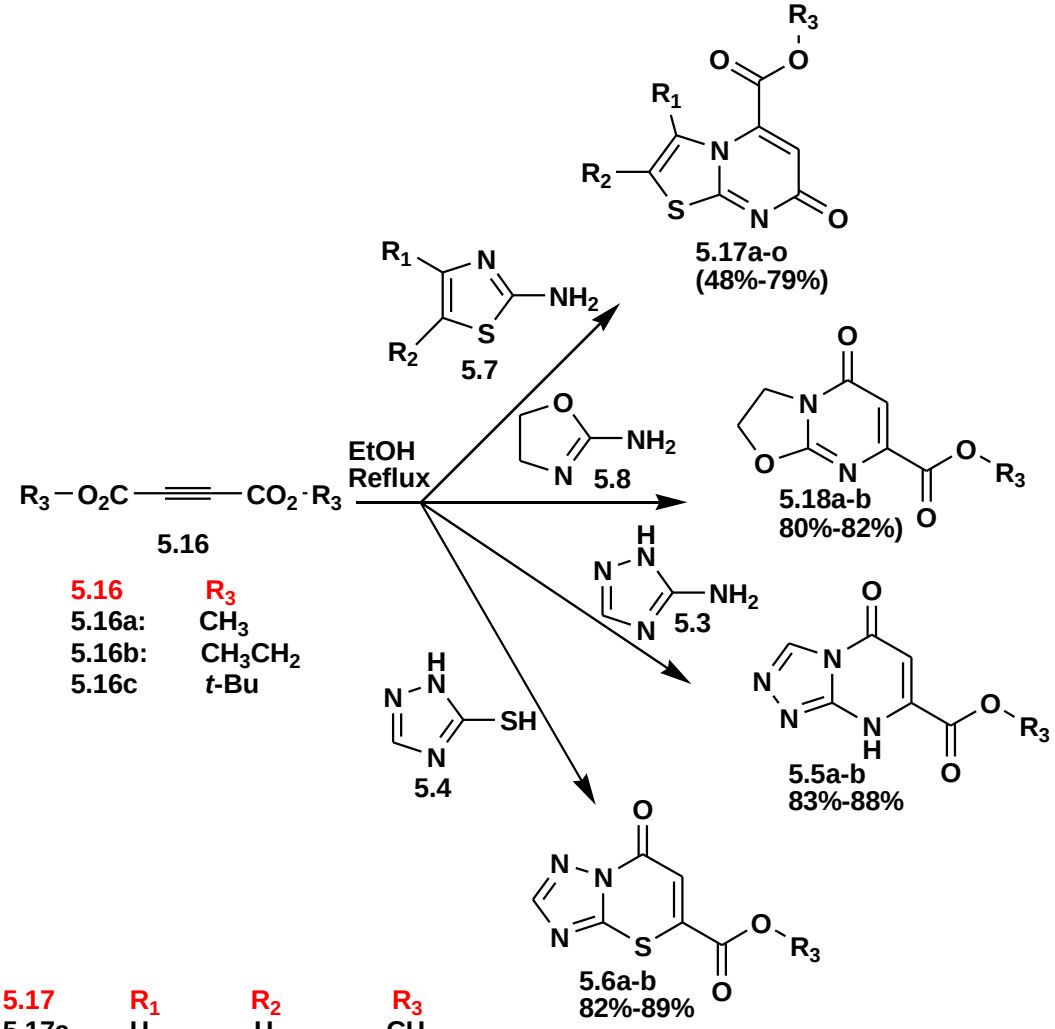
**4.5 $R_2$**
**4.5a: $CH_3$**
**4.5b: $CH_2CH_3$**

| 4.8: | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 4.8a | *t*-Bu | $CH_3$ | H |
| 4.8b | *t*-Bu | $CH_2CH_3$ | H |
| 4.8c | *t*-Bu | $CH_3$ | $CH_3$ |
| 4.8d | *t*-Bu | $CH_2CH_3$ | $CH_3$ |
| 4.8e | tetramethylbutyl | $CH_3$ | $CH_3$ |
| 4.8f | tetramethylbutyl | $CH_2CH_3$ | $CH_3$ |
| 4.8g | cyclohexyl | $CH_3$ | $CH_3$ |
| 4.8h | cyclohexyl | $CH_2CH_3$ | $CH_3$ |
| 4.8i | *t*-Bu | $CH_3$ | $CH_2CH_3$ |
| 4.8j | *t*-Bu | $CH_2CH_3$ | $CH_2CH_3$ |
| 4.8k | tetramethylbutyl | $CH_3$ | $CH_2CH_3$ |
| 4.8l | tetramethylbutyl | $CH_2CH_3$ | $CH_2CH_3$ |
| 4.8m | *t*-Bu | $CH_3$ | $CH_2CH_2CH_3$ |
| 4.8n | *t*-Bu | $CH_2CH_3$ | $CH_2CH_2CH_3$ |
| 4.8o | tetramethylbutyl | $CH_3$ | $CH_2CH_2CH_3$ |
| 4.8p | tetramethylbutyl | $CH_2CH_3$ | $CH_2CH_2CH_3$ |
| 4.8q | *t*-Bu | $CH_3$ | $C_6H_6$ |
| 4.8r | *t*-Bu | $CH_2CH_3$ | $C_6H_6$ |
| 4.8s | tetramethylbutyl | $CH_3$ | $C_6H_6$ |
| 4.8t | tetramethylbutyl | $CH_2CH_3$ | $C_6H_6$ |
| 4.8u | cyclohexyl | $CH_3$ | $C_6H_6$ |

**Scheme 7.3**: Synthesis of dimethyl 8-(*tert*-butylamino)-4-oxo-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate derivatives (**4.8a-u**).

Bearing in mind the co-products obtained from the direct reaction of DMAD and the nucleophilic 5-membered-ring substrates **3.11** and **3.13**, in the presence of isocyanide, we tested the reactivity of DMAD towards the prepared 5-membered ring and thiazines. The reactions were carried out under refluxing ethanol, and all the substrates with low $pK_a$ values i.e. thiazole **3.5a-b** and thiazine **4.3a-e** were found to be non-reactive towards DMAD, as expected, resulting in recovery of the starting materials (Scheme 7.2 and 7.3).

Nucleophilic substrates **5.3**, **5.4**, **5.7a-h** and **5.8**, as shown in Scheme 7.4, react with DMAD\DEtAD to give the desired pyrimidine products in average to good yields of products **5.17a-o** which ranged from 48% to 79%.




| 5.16 | $R_3$ |
|---|---|
| 5.16a: | $CH_3$ |
| 5.16b: | $CH_3CH_2$ |
| 5.16c | *t*-Bu |

| 5.17 | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 5.17a | H | H | $CH_3$ |
| 5.17b | $CH_3$ | H | $CH_3$ |
| 5.17c | $CH_3$ | $CH_3$ | $CH_3$ |
| 5.17d | Ph | H | $CH_3$ |
| 5.17e | Ph-$CH_3$ | H | $CH_3$ |
| 5.17f | Ph-F | H | $CH_3$ |
| 5.17g | Ph-Cl | H | $CH_3$ |
| 5.17h | H | H | $CH_3CH_2$ |
| 5.17i | $CH_3$ | H | $CH_3CH_2$ |
| 5.17j | $CH_3$ | $CH_3$ | $CH_3CH_2$ |
| 5.17k | Ph | H | $CH_3CH_2$ |
| 5.17l | Ph-$CH_3$ | H | $CH_3CH_2$ |
| 5.17m | Ph-F | H | $CH_3CH_2$ |
| 5.17n | Ph-Cl | H | $CH_3CH_2$ |
| 5.17o | H | H | *t*-Bu |

| 5.7 | $R_1$ | $R_2$ |
|---|---|---|
| 5.7a | H | H |
| 5.7b | $CH_3$ | H |
| 5.7c | $CH_3$ | $CH_3$ |
| 5.7d | Ph | H |
| 5.7e | Ph-$CH_3$ | H |
| 5.7f | Ph-F | H |
| 5.7g | Ph-Cl | H |
| 5.7h | Ph-$CH_3O$ | H |

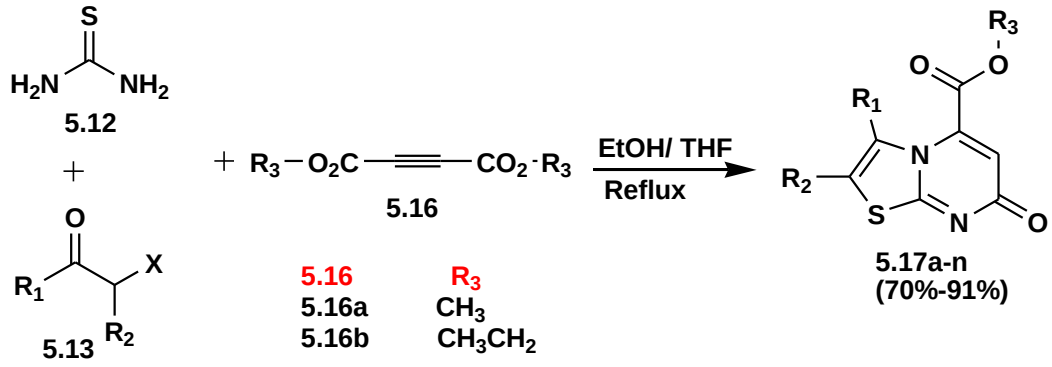
| 4.5 | $R_3$ |
|---|---|
| 5.5a: | $CH_3$ |
| 5.5b: | $CH_3CH_2$ |

| 5.5 | $R_3$ |
|---|---|
| 5.6.b: | $CH_3$ |
| 5.6.d: | $CH_3CH_2$ |

| 5.18 | $R_3$ |
|---|---|
| 5.18a: | $CH_3$ |
| 5.18b: | $CH_3CH_2$ |

**Scheme 7.4:** Synthesis of **5.5**, **5.6**, **5.17** and **5.18** *via* 2-CR.

In order to improve the yields of thiazole-pyrimidines **5.17a-n** we applied a 3-CR method that incorporates thiourea **5.12**, α-haloketone **5.13** and DMAD **5.16** to give **5.17**; we reacted thiourea **5.12** with chloroacetaldehyde **5.13a** in the presence of DMAD **5.16a**, using a 1:1 solution of ethanol and THF under reflux for at least 12 h, which gave rise to methyl 7-oxo-7*H*-thiazolo[3,2-*a*]pyrimidine-5-carboxylate **5.17a** in 87% yield, an improvement over the 72% obtained *via* 2-CR, as shown in Scheme 7.5.




| 5.13 | $R_1$ | $R_2$ | X |
|---|---|---|---|
| 5.13a | H | H | Cl |
| 5.13b | $CH_3$ | H | Cl |
| 5.13c | $CH_3$ | $CH_3$ | Cl |
| 5.13d | Ph | H | Br |
| 5.13e | Ph-$CH_3$ | H | Br |
| 5.13f | Ph-F | H | Cl |
| 5.13g | Ph-Cl | H | Br |
| 5.13h | Ph-$OCH_3$ | H | Br |
| 5.13i | Ph-$NO_2$ | H | Br |

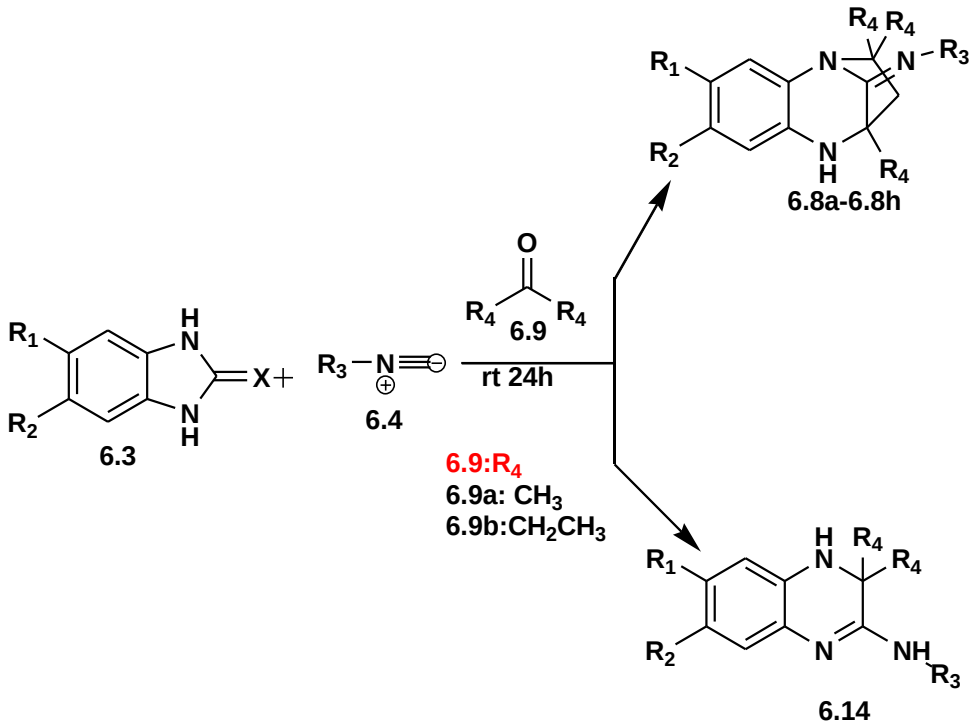
| 5.17 | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 5.17a | H | H | $CH_3$ |
| 5.17b | $CH_3$ | H | $CH_3$ |
| 5.17c | $CH_3$ | $CH_3$ | $CH_3$ |
| 5.17d | Ph | H | $CH_3$ |
| 5.17e | Ph-$CH_3$ | H | $CH_3$ |
| 5.17f | Ph-F | H | $CH_3$ |
| 5.17g | Ph-Cl | H | $CH_3$ |
| 5.17h | H | H | $CH_3CH_2$ |
| 5.17i | $CH_3$ | H | $CH_3CH_2$ |
| 5.17j | $CH_3$ | $CH_3$ | $CH_3CH_2$ |
| 5.17k | Ph | H | $CH_3CH_2$ |
| 5.17l | Ph-$CH_3$ | H | $CH_3CH_2$ |
| 5.17m | Ph-F | H | $CH_3CH_2$ |
| 5.17n | Ph-Cl | H | $CH_3CH_2$ |

**Scheme 7.5.** Synthesis of **5.17a-n** *via* 3-CR.

## 7.4 Synthesis of diazepines and quinoxalines under catalyst-free and solvent-free conditions, *via* 3-CR.

The reaction of benzimidazole **6.3**, isocyanide **6.4** and acetone **6.9** without the use of catalyst or solvent resulted in formation of novel diazepines **6.8** and quinoxalines **6.14** as shown in Scheme 7.6. The reaction to either form the diazepine **6.8** or quinoxaline **6.14** scaffold, was influenced by the benzimidazole substituents: for example when $R_1$ and $R_2$ of the benzimidazole **6.3** corresponded to H or two activating methyl groups, the resulting scaffold was diazepine **6.8** in reasonable to good yields ranging from 67% -84%. When $R_1$ and $R_2$ of

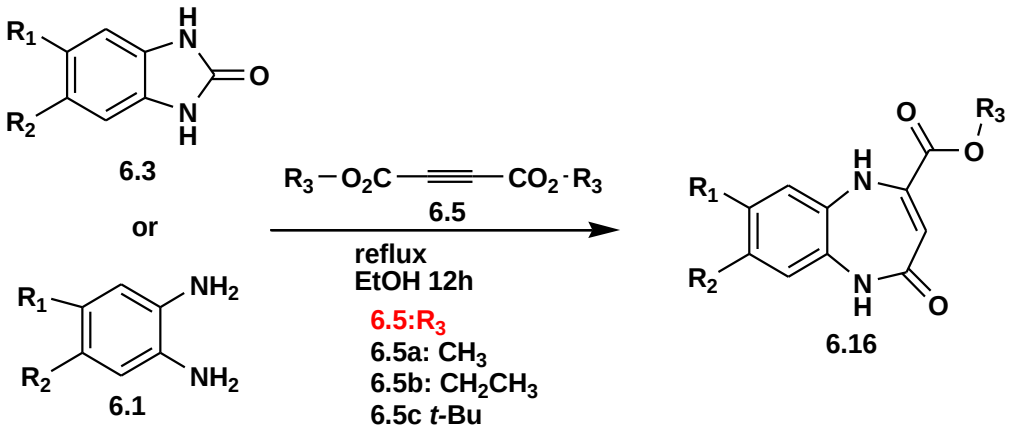
the benzimidazole **6.3** contained deactivating groups such as chlorine, fluorine or an ester, the resulting scaffold was quinoxaline **6.14**. From the quinoxalines **6.14** it was evident that the disubstituted chlorine gave a good yield, while mono-substituted deactivating groups (ester, fluorine or chlorine) at either position $R_1$ or $R_2$ resulted in low to average yields. To investigate the involvement of acetone, we further tested the reaction using 3-pentanone or deuterated acetone. The 3-pentanone gave a quinoxaline scaffold when $R_1$ and $R_2$ of the benzimidazole corresponded to H or two chlorine atoms. The deuterated acetone gave the diazepine scaffold when hydrogen atoms or two activating methyl groups were substituents of the benzimidazole at $R_1$ and $R_2$. When the dichloro substituted benzimidazole was tested it resulted in a quinoxaline scaffold. When deuterated acetone, *tert*-butyl isocyanide and benzimidazole with hydrogen atoms at $R_1$ and $R_2$ were put to the test they resulted in a quinoxaline **6.2** scaffold instead of the anticipated diazepine **6.8** scaffold. A total of twenty-six novel diazepines **6.8** and quinoxalines **6.14** were obtained.




| 6.3: $R_1$ | $R_2$ | X | 6.14: $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|---|---|
| 6.3a: H | H | O | 6.14a:$CO_2CH_3$ | H | *t*-Bu |
| 6.3b: $CH_3$ | H | O | 6.14b:$CO_2CH_3$ | H | tetramethylbutyl |
| 6.3c: $CO_2CH_3$ | H | O | 6.14c: F | H | tetramethylbutyl |
| 6.3d: F | H | O | 6.14d: H | F | tetramethylbutyl |
| 6.3e: Cl | H | O | 6.14e: Cl | H | tetramethylbutyl |
| 6.3g: H | H | S | 6.14f: Cl | Cl | *t*-Bu |
| 6.3h: H | H | N | 6.14g: Cl | Cl | tetramethylbutyl |
| 6.3i: $CO_2CH_3$ | H | S | 6.14h: H | H | *t*-Bu |
| 6.3j: CH3 | CH3 | O | 6.14i: Cl | Cl | *t*-Bu |
| 6.3k: Cl | Cl | O | | | |

**Scheme 7.6**: Synthesis of **6.8a-h** and **6.14a-i** without catalyst.

Phenylenediamine **6.1** or benzimidazole **6.3** were treated with DMAD\DEtAD\DTAD, under refluxing ethanol to obtain diazepine **6.16** scaffolds as shown in Scheme 7.7. Out of ten synthesised diazepines **6.16**.




| 6.1: $R_1$ | $R_2$ |
|---|---|
| 6.1a: H | H |
| 6.1b: $CH_3$ | H |
| 6.1c: $CO_2CH_3$ | H |
| 6.1d: F | H |
| 6.1e: Cl | H |
| 6.1f: Br | H |
| 6.1g: $CH_3$ | $CH_3$ |
| 6.1h :Cl | Cl |
| 6.1i: $NO_2$ | H |

| 6.16: $R_1$ | $R_2$ | $R_3$ |
|---|---|---|
| 6.16a: H | H | $CH_3$ |
| 6.16b: H | H | $CH_2CH_3$ |
| 6.16c: H | H | *t*-Bu |
| 6.16d: $NO_2$ | H | $CH_3$ |
| 6.16e: $NO_2$ | H | $CH_2CH_3$ |
| 6.16f: $NO_2$ | H | *t*-Bu |
| 6.16g: Cl | Cl | $CH_3$ |
| 6.16h: Cl | Cl | $CH_2CH_3$ |
| 6.16i: Cl | Cl | *t*-Bu |
| 6.16j: $CH_3$ | $CH_3$ | $CH_3$ |

**Scheme 7.7:** Synthesis of methyl 4-oxo-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxylate derivatives **6.16**.

### 7.5 Biological evaluations of all newly synthesised compounds

The synthesised compounds were subjected to the following biological evaluations (in collaboration):

a) Antimicrobial tests using Gram-positive bacterial species: *Staphylococcus aureus* and *Bacillus cereus* and two Gram negative bacterial species *Escherichia coli* and *Pseudomonas aeruginosa* with Minimum Inhibitory Concentration (MIC) assay determined by the iodonitrotetrazolium (INT) as indicator, b) HIV-1 antiviral test using MT-4 cells and c) cytotoxicity studies were done by using the MT-4 cell line at 100 μM.

The synthesised hexahydroquinolines in Chapter 2 were evaluated using an Alphascreen assay for determination of percentage inhibition on the interaction of HIV-1-IN and LEDGF/p75 proteins, multimerization, cytotoxicity, antiviral and antimicrobial activities were also preliminarily tested. From the initial seventeen hexahydroquinoline-3-carboxamide (**2.8** and

**2.11**) compounds screened for IN-LEDGF/p75 inhibition, only two compounds showed more than 50% inhibition at a dose concentration of 100 μM of test compound: **2.8k** with 53% inhibition, and **2.8e** with inhibition of 52%. The multimerization evaluation results showed that this set of compounds did not significantly induce multimerization in the HIV-1 IN Alpha Screen assay. After toxicity testing this set of compounds were considered to be relatively non-toxic. None of this compound showed any significant antimicrobial activities.

In Chapter 3, twelve of the synthesised pyrimidines were found to be relatively non-toxic, whereas three of them were considered to be toxic. None of the synthesised compounds showed any significant activity against the bacterial strains tested. In Chapter 4 only two compounds were considered relatively non-toxic and none of them showed any significant antimicrobial activity. In Chapter 5 twelve compounds were found to be relatively non-toxic out of twenty-one tested compounds and in Chapter 6 eighteen compounds out of twenty-six were found to be non-toxic. We were delighted that three compounds from Chapter 6 had significant antiviral activity. None of these compounds exhibited any significant antimicrobial properties.

### 7.6 Final remarks

The application of MCRs for development and synthesis of heterocyclic scaffolds in synthetic chemistry promotes safe practices (green solvents, solvent-free, mild conditions and room temperature), facile methods and economically cheaper approaches (solvent-free, catalyst-free and including cheap and safe acetone).

MCRs additionally manifest considerable potential for awareness of green production processes and protocols.[20,248] Compared to sequential multistep reactions, MCRs contribute to minimising chemical waste in many chemical processes.[22,23 ,24]

# Chapter 8

# Experimental procedures

## 8.1General information

### *8.1.1 Solvents and reagents*

All commercially available reagents were supplied by RadChem or Sigma Aldrich and used without further purification. Dry solvents were used directly from an LC-Tech SP-1 Solvent Purification System stored under argon. All solvents used for chromatographic purposes were supplied by Sigma Aldrich and/or RadChem and were used without further distillation.

### *8.1.2 Analysis of data*

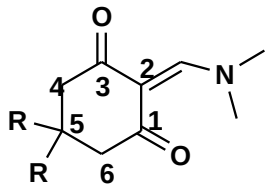
NMR: spectra were recorded at 298 K on a 400 MHz Bruker Avance Spectrometer equipped with a BBI 5 mm probe. Chemical shifts are reported in ppm, and are referenced internally to residual solvent resonances [7.26 ($CDCl_3$) and 2.50 (DMSO-$d_6$) for $^1$H NMR; 77.16 ($CDCl_3$) 40.45 (DMSO-$d_6$) for $^{13}$C NMR]. For mass spectrometry (LC-MS/MS), high-resolution spectra were obtained on an LC-MS system. Fourier-transform Infrared spectra (FTIR) were recorded using a Perkin Elmer FT-IR Spectrometer Spectrum Two. All signals are reported on the wavenumber scale ($\nu$/cm$^{-1}$). Single X-ray Crystallography was done in collaboration were applicable.

### *8.1.3. Nomenclature and compound numbering*

The system of numbering the compounds within the discussions are used for purposes of discussion and are not meant to reflect the systematic numbering of these compounds. The nomenclature is based on the IUPAC system.

## 8.2 Synthetic methods: Chapter 2

*8.2.1 General method for the synthesis of 2-((dimethylamino) methylene))- cyclohexane-1,3-dione derivatives (**2.3a-b**)*




**2.3a-b**

In a 250 ml round-bottomed flask equipped with a magnetic stirrer, a neat solution of 1,3-cyclohexanedione derivative (1 eq.) and *N*,*N*-dimethylformamide dimethyl acetal (1 eq.) was stirred for 3 h at room temperature (rt) while monitoring by TLC. The crude mixture was concentrated and dried *in vacuo* to obtain solid products.[175]

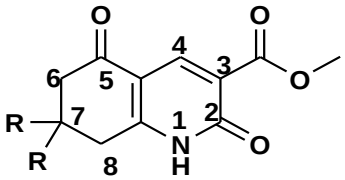
*8.2.1.1 2-((Dimethylamino)methylene)-5,5-dimethylcyclohexane-1,3-dione (**2.3a**)*

4,4-Dimethyl-1,3-cyclohexanedione (**2.1a**) (10.00 g, 71 mmol) and *N*,*N*-dimethylformamide dimethyl acetal (**2.2**) (8.50 g, 71.3 mmol) were reacted to give 2-((dimethylamino)methylene)-5,5-dimethylcyclohexane-1,3-dione (**2.3a**). **Physical characteristics**: yellow solid; Yield: 12.81 g, 92%; **Mp**: 93-95 ºC (Lit.[175] 94 ºC); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.94 (1H, s, CH), 3.33 (3H, s, N-$CH_3$), 3.12 (3H, s, N-$CH_3$), 2.29 (4H, s, $CH_2$-C-$CH_2$), 0.99 (6H, s, $CH_3$-C-$CH_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 195.7 (2 × C=O), 161.8 (CHN), 108.2 (C-2), 52.1 (N-$CH_3$), 48.5 (C-5, 44.8 (N-$CH_3$), 30.9 (2 X $CH_3$), 28.5 (2 × $CH_2$).

*8.2.1.2 2-((Dimethylamino) methylene)- cyclohexane-1,3-dione (**2.3b**)*

1,3-Cyclohexanedione (**2.1b**) (10 g, 89.20 mmol) and *N*,*N*-dimethylformamide dimethyl acetal (**2.2**) (10.63 g, 89.2 mmol) were reacted to give 2-((dimethylamino) methylene)- cyclohexane-1,3-dione (**2.3b)**. **Physical characteristics**: brown solid; Yield: 13.51 g, 90%; **Mp**: 114-116 ºC (Lit.[175] 116ºC); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.01 (1H, s, CH), 3.35 (3H, s, N-$CH_3$), 3.13 (3H, s, N-$CH_3$), 2.42 (4H, t, *J* = 6.4 Hz, H-4 and H-6), 1.93-1.87 (2H, m, H-5); **$^{13}$C NMR** (101 MHz, CDCl3) δ 196.2 (2 X C=O), 162.3 (C-H), 109.4 (C-2), 48.6 (N-$CH_3$), 44.7 (N-$CH_3$), 38.2 (2 x $CH_2$, C-4 and C-6), 19.6 (C-5).

*8.2.2 General method for the synthesis of methyl 7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate derivatives (**2.5a-b**)*




**2.5a-b**

In a 250 ml round-bottomed flask equipped with a stirrer bar, methyl cyanoacetate (**2.4**) (10 eq.) was added to a solution of 2-(dimethylaminomethylene)-cyclohexane-1,3-dione derivative (**2.3a-b**) (1 eq.) in anhydrous methanol (60 ml). The reaction mixture was heated under reflux for 12 h while monitoring by TLC. The crude solid product was collected by filtration, washed thoroughly with diethyl ether (15 ml) and recrystallized from ethanol (15 ml) to obtain pure product.

*8.2.2.1 Methyl 7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate (**2.5a**)*

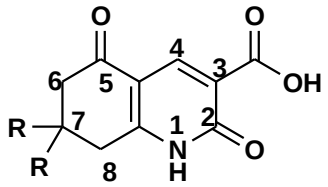
Methyl cyanoacetate (**2.4**) (51.53 g, 520 mmol) and 2-((dimethylamino)methylene)-5,5-dimethylcyclohexane-1,3-dione (**2.3a**) (10,17 g, 52 mmol) were reacted to give methyl 7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate (**2.5a**). **Physical characteristics**: yellow solid; Yield: 11.31 g, 87%; **Mp**: 244-246 ºC (Lit.[174] 243-245 ºC); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 12.55 (1H, s, NH) 8.39 (1H, s, CH), 3.77 (3H, s, $CH_3$-O), 2.77 (H-6, 2H, s, $CH_2$), 2.39 (H-8, 2H, s, ($CH_2$), 1.03 (6H, s, 2 × $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 192.9, 164.4, 160.3 (3 × C=O), 159.7 (Ar-C), 141.0 (C-4), 117.4 (Ar-C), 110.9 (Ar-C), 51.8 (O-$CH_3$), 49.9 (C-6, $CH_2$), 32.5 (C-7, $CH_3$), 27.7 (2 × $CH_3$).

*8.2.2.2 Methyl 2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate (**2.5b**)*

Methyl cyanoacetate (**2.4**) (85.12 g, 859 mmol) and 2-((dimethylamino)methylene) cyclohexane-1,3-dione (14.36 g, 85.9 mmol**)** were reacted to give methyl 2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate (**2.5b**); **Physical characteristics**: yellow solid; Yield: 17.21 g, 90%; **Mp**: 273-275 ºC (Lit.[174] 272-274 ºC); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 12.58 (1H, s, NH), 8.39 (1H, s, CH), 3.76 (3H, s, $CH_3$-O), 2.87 (H-6, 2H, t, $J$ = 6.0 Hz, $CH_2$), 2.47 (H-8, 2H, t, $J$ = 6.0 Hz, $CH_2$), 2.06-1.99 (H-7, 2H, m, $CH_2$); **$^{13}$C NMR** (101 MHz, DMSO-

$d_6$) δ 193.1, 164.4, 161.9 (3 × C=O), 159.2 (Ar-C), 141.4 (C-H), 117.5 (Ar-C), 111.9 (Ar-C), 51.8 (O-$CH_3$), 38.9 (C-6, $CH_2$), 26.6 (C-8, $CH_2$), 20.4 (C-7, $CH_2$).

*8.2.3 General method for the synthesis of 2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid derivatives (**2.6a-b**)*




**2.6a-b**

In a 250 ml round-bottomed flask equipped with a stirrer bar, containing a solution of 2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate derivative (**2.5**) (1 eq.) in methanol (25 ml) and water (25 ml) was added sodium hydroxide (4 eq.). The resulting reaction mixture was then heated under reflux for 3 h while monitoring by TLC. The reaction was then cooled to 0°C and hydrochloric acid (1M) was slowly added until the solution became acidic. Ethyl acetate (20 ml) and water (20 ml) were added and the aqueous layer was extracted with ethyl acetate twice (2 × 15 ml). The combined organic layers were concentrated and dried *in vacuo* to obtain solid products.

*8.2.3.1 7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**2.6a**)*

7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3- carboxylate (10.00 g, 40 mmol) (**2.5**) and sodium hydroxide (6.40 g, 160 mmol) were reacted to give 7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid 7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**2.6a**); **Physical characteristics**: white solid; Yield: 8.01 g, 85%; **Mp**: 298-300 ºC (Lit.[249] 299 ºC); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 13.72 (1H, br s, OH), 13.49 (1H, br s, NH), 8.55 (1H, s, CH), 2.86 (2H, s, ($CH_2$), 2.45 (2H, s, ($CH_2$), 1.04 (6H, s, 2 X $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 193.1, 164.9, 164.4 (3 × C=O), 160.3 (Ar-C), 141.5 (C-H), 115.4 (Ar-C), 113.8 (Ar-C), 49.9 (C-6, $CH_2$), 32.6 (C-7), 27.7 (2 X $CH_3$).

*8.2.3.2 2,5-Dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**2.6b**)*

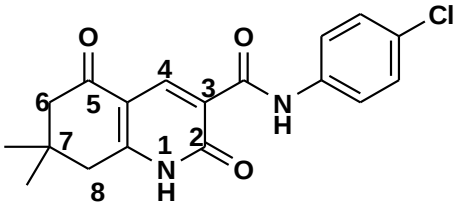
Methyl 2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate (**2.5**) (10.00 g, 45 mmol) and sodium hydroxide (9.04 g, 226 mmol) were reacted to give 2,5-dioxo-1,2,5,6,7,8-

hexahydroquinoline-3-carboxylic acid (**2.6b**); **Physical characteristics**: Brown solid; Yield: 8.01 g, 79%; **Mp**: 297-299 ºC (Lit.[249] 298 ºC); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 13.75 (1H, br s, OH), 13.49 (1H, br s, N-H) 8.57 (1H, s, CH), 2.97 (H-6, 2H, t, $J$ =6.0 Hz, $CH_2$), 2.54 (H-8, 2H, t, $J$ = 6.4 Hz, $CH_2$), 2.11-2.05 (H-7, 2H, m, $CH_2$); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 193.3, 164.5 , 164.40 (3 × C=O), 162.0 (Ar-C), 141.7 (C-H), 115.3 (Ar-C), 114.7 (Ar-C), 36.5 (C-6, $CH_2$), 26.8 (C-8, $CH_2$), 20.3 (C-7, $CH_2$).

*8.2.4 General method for the synthesis of 7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide derivatives using CDI as a coupling reagent (**2.8a-m, 2.11a-d**).*

In a two-necked 100 ml round-bottomed flask equipped with a stirrer bar a solution of the appropriate 2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid derivative (**2.6**) (1 eq.) and carbonyldiimidazole (CDI, 1.3 eq.) in acetonitrile (MeCN, 25 ml) at 50 ºC were reacted for 2 h under argon. The reaction was cooled to room temperature (rt) and aniline (**2.7**)/thiazol-2-amine derivatives (**2.10**) (1 eq.) was added to the solution under inert argon conditions. The resulting reaction mixture was stirred at rt for 24 h while monitoring by TLC. The precipitated solid was isolated by filtration and washed with deionised water (8 ml) and heptane (8 ml), and dried to obtain the pure product.

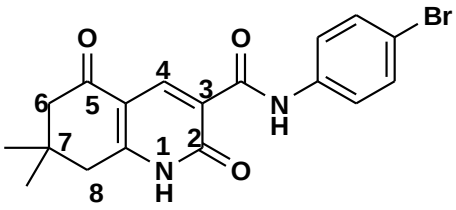
*8.2.4.1 N-(4-chlorophenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8a**)*




7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**2.6a**) (0.51 g, 2.17 mmol), CDI (0.46 g, 2.82 mmol) and 4-chloroaniline (**2.7a**) (0.28 g, 2.17 mmol) were reacted to give *N*-(4-chlorophenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8a**); **Physical characteristics**: white solid; Yield: 0.61 g, 82%; **Mp**: 347-349 ºC; **R$_f$**: 0.7, hexane/ethyl acetate (1:1); **$^1$H NMR** (400 MHz, DMSO-$d_6$ ) δ 13.15 (1H, s, N-H), 11.70 (1H, s, Ar-N-H), 8.71 (1H, s, CH), 7.73 (2H, d, $J$ = 8.4 Hz, ArH), 7.41 (2H, d, $J$ = 8 Hz,

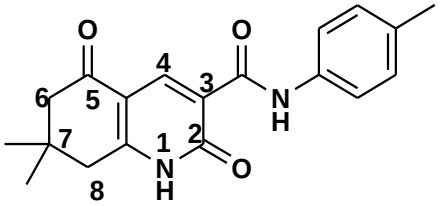
ArH), 2.85 (2H, s, $CH_2$), 2.44 (2H, s, $CH_2$), 1.04 (6H, s, 2 × $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 193.2, 163.4, 161.1 (3 X C=O), 159.2 (Ar-C), 140.2 (Ar-CH), 137.1 (Ar-C), 128.9 (Ar-CH), 127.5 (Ar-C), 121.4 (Ar-CH), 118.1 (Ar-C), 113.0 (Ar-C), 49.9 (C-6, $CH_2$), 32.6 (C-7), 27.7 (2 X $CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 2971, 2864, 1679, 1621, 1598, 1550, 1485, 1383, 1302, 1234; **HRMS** (ESI-TOF) m/z: $[M+H]^+$ Calculated for $C_{18}H_{18}ClN_2O_3^+$ 345.1000, found 345.1012.

*8.2.4.2 N-(4-bromophenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8b**)*



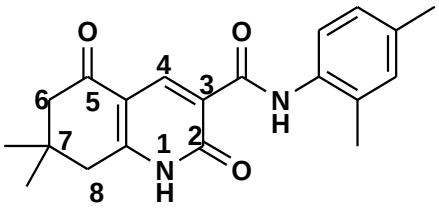

7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**2.6a**) (0.15 g, 0.64 mmol), CDI (0.13 g, 0.83 mmol) and 4-bromoaniline (**2.7b**) (0.11 g, 0.64 mmol) were reacted to give *N*-(4-bromophenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8b**); **Physical characteristics**: white solid; Yield: 0.18 g, 72%, **Mp**: 234-236 ºC; **R$_f$** :0.7, hexane/ethyl acetate (1:1); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 13.14 (1H, br s, N-H), 11.71 (1H, s, Ar-N-H), 8.72 (1H, s, CH), 7.68 (2H, d, *J* = 8.8 Hz, ArH), 7.54 (2H, d, *J* = 8.8 Hz, ArH), 2.85 (2H, s, H-6), 2.44 (, 2H, s,H-8), 1.04 (6H, s, 2×$CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 192.9, 163.3, 160.2 (3 × C=O), 159.8 (Ar-C), 140.1 (Ar-CH), 138.6 (Ar-C), 131.9 (Ar-C), 130.3 (Ar-CH), 121.5 (Ar-CH), 117.2 (Ar-CH), 111.6 (Ar-CH), 50.0 (C-6, $CH_2$), 32.6 (C-7) , 27.7 (2 × $CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3145, 2954, 1700, 1682, 1655, 1606, 1584, 1534, 1481, 1399; **HRMS** (ESI-TOF) m/z: $[M+H]^+$ Calculated for $C_{18}H_{18}BrN_2O_3^+$ 391.0495, found 391.0481.

*8.2.4.3 7,7-Dimethyl-2,5-dioxo-N-(p-tolyl)-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8c**)*



7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**2.6a**) (0.15 g, 0.64 mmol), CDI (0.134 g, 0.83 mmol) and *p*-toluidine (**2.7c**) (0.110 g, 0.64 mmol) were reacted to give 7,7-dimethyl-2,5-dioxo-N-(*p*-tolyl)-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8c**); **Physical characteristics**: white solid ; Yield: 0.21 g, 76%; **Mp**: 340-342 ºC; **$R_f$** 0.7, hexane/ethyl acetate (1:1); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 13.10 (1H, br s, N-H), 11.55 (1H, s, Ar-N-H), 8.72 (1H, s, CH), 7.58 (2H, d, *J* = 7.6 Hz, ArH), 7.18 (2H, d, *J* = 7.6 Hz, Ar), 2.84 (2H, s, $CH_2$), 2.42 (2H, s, $CH_2$), 2.28 (3H, s, Ar-$CH_3$) 1.04 (6H, s, 2 × $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 193.2, 163.4, 160.7 (3 × C=O), 158.9 (Ar-C), 140.0 (Ar-CH), 135.7 (Ar-C) , 133.0 (Ar-C), 129.5 (Ar-CH), 119.7 (Ar-CH) , 118.4 (Ar-C), 112.9 (Ar-C), 50.0 (C-6, $CH_2$), 32.7 (C-7), 27.7 (2 × $CH_3$), 20.5 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3131, 2871, 1690, 1629, 1596, 1574, 1550, 1516, 1485, 1382; **HRMS** (ESI-TOF) m/z: $[M+H]^+$ Calculated for $C_{19}H_{21}N_2O_3^+$ 325.1547, found 325.1578.

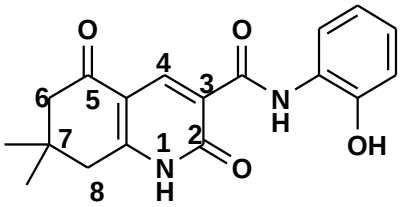
*8.2.4.4 N-(2,4-Dimethylphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8d**)*




7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**2.6a**) (0.25 g, 1.10 mmol), CDI (0.22 g, 1.38 mmol) and 2.4 dimethylaniline (**2.7d**) (0.13 g, 1.1 mmol) were reacted to give *N*-(2,4-dimethylphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8d**); **Physical characteristics**: white solid; Yield: 0.28 g, 78%; **Mp**:343-345 ºC; **$R_f$** :0.7, hexane/ethyl acetate (1:1): **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 13.06 (1H, br s, N-H), 11.48 (1H, s, Ar-N-H), 8.74 (1H, s, Ar-H), 8.12 (1H, d, *J* = 8.0 Hz, Ar-H), 7.06 (1H, s, Ar-H), 7.01 (1H, d, *J* = 8.4 Hz, Ar-H), 2.85 (2H, s, $CH_2$), 2.44 (2H, s, $CH_2$), 2.25 and 2.28 (6H, 2 × s, Ar-$CH_3$), 1.04 (6H, s, 2× $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 193.3, 163.7, 160.5 (3 × C=O), 158.9 (Ar-C), 140.1 ((Ar-CH), 134.2 (Ar-C), 132.8 (Ar-C), 130.9 (Ar-C), 127.3 (Ar-C), 126.7 (Ar-C), 120.9 (Ar-C) , 118.6 (Ar-C) , 112.9 (Ar-C) , 50.1 (C-6, $CH_2$), 32.7 (C-7), 27.7 (2$CH_3$), 20.4 ($CH_3$), 17.7 ($CH_3$) ; **FT-IR $\nu_{max}$/cm$^{-1}$**: 3131, 2942,

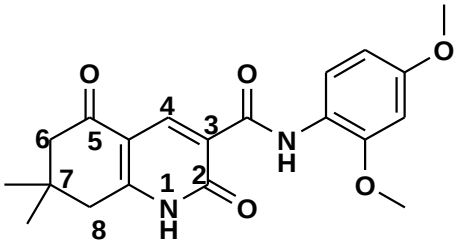
1674, 1621, 1593, 1543, 1483, 1458, 1426, 1389, 1303; **HRMS** (ESI-TOF) m/z: $[M+H]^+$ Calculated for $C_{20}H_{23}N_2O_3^+$ 339.1703, found 339.1711.

*8.2.4.5 N-(2-Hydroxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8e**)*




7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**2.6a**) (0.25 g, 1.10 mmol), CDI (0.22 g, 1.38 mmol) and 2-hydroxyaniline (**2.7e**) (0.116 g, 1.1 mmol) were reacted to give *N*-(2-hydroxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8e**); **Physical characteristics**: white solid; Yield: 0.28 g, 80%; **Mp**: 329-331 ºC; $\mathbf{R_f}$ :0.6, hexane/ethyl acetate (1:1); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 12.96 (1H, br s, N-H), 11.71 (1H, s, Ar-N-H), 10.03 (1H, s, O-H) 8.74 (1H, s, Ar-H), 8.38 (1H, d, *J* = 7.6 Hz, Ar-H), 6.93-6.87 (2H, m, Ar-H), 6.79 (1H, t, *J* = 7.2 Hz, Ar-H) 2.84 (2H, s, $CH_2$), 2.43 (2H, s, $CH_2$), 1.04 (6H, s, 2 × $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 193.3, 163.1, 160.6 (3 × C=O), 158.9 (Ar-C), 146.6 (Ar-C), 140.0 (Ar-CH), 126.9 (Ar-C), 123.9 (Ar-CH), 120.2 (Ar-C), 119.1 (Ar-CH), 118.7 (Ar-C), 114.6 (Ar-CH),112.7 (Ar-C), 50.0 (C-6, $CH_2$), 32.6 (C-7), 27.7 (2 × $CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3038, 2961, 1670, 1623, 1602, 1575, 1537, 1499, 1442,1417, 1391; **HRMS** (ESI-TOF) m/z: $[M+H]^+$ Calculated for $C_{18}H_{19}N_2O_4^+$ 327.1339 found 327.1335.

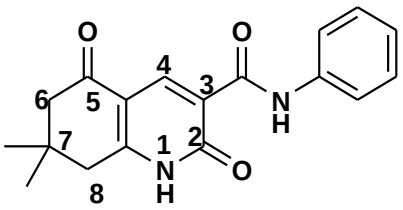
*8.2.4.6 N-(2,4-Dimethoxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.12f**)*




7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**2.6a**) (0.25 g, 1.10 mmol), CDI (0.22 g, 1.38 mmol) and 2.4 dimethoxy aniline (**2.7f**) (0.16 g, 1.1 mmol) were reacted to give *N*-(2,4-dimethoxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8f**); **Physical characteristics**: black solid; Yields: 0.32

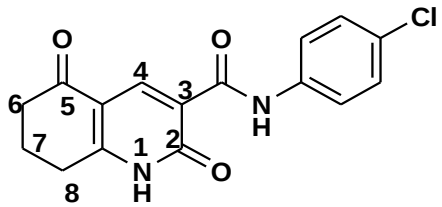
g, 82%; **Mp**: 282-284 ºC; **$R_f$** :0.6, hexane/ethyl acetate (1:1): **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 12.95 (1H, br s, N-H), 11.67 (1H, s, Ar-N-H), 8.72 (1H, s, CH), 8.32 (1H, d, *J* = 8.4 Hz, ArH), 6.66 (1H, s, ArH), 6.53 (1H, d, *J* = 8.4 Hz, ArH), 3.87 (3H, s, Ar-O-$CH_3$), 3.77 (3H, s, Ar-O-$CH_3$), 2.84 (2H, s, $CH_2$), 2.43 (2H, s, $CH_2$), 1.04 (6H, s, 2 × $CH_3$); **$^{13}$C NMR (**101 MHz, DMSO-$d_6$) δ 193.2, 163.1, 160.2 (3 × C=O), 158.7 (Ar-C), 156.2 (Ar-CH), 149.7 (Ar-C), 139.8 (Ar-CH), 121.2 (Ar-C), 120.7 (Ar-CH), 118.6 (Ar-C), 112.7 (Ar-C), 104.2 (Ar-C), 98.8 (Ar-CH), 56.0 (O-$CH_3$), 55.3 (O-$CH_3$), 50.0 (C-7, $CH_2$), 32.6 (C-8, $CH_2$), 30.7 (C-7) , 27.7 (2 × $CH_3$) ; **FT-IR $\nu_{max}$/cm$^{-1}$**: 3266, 2961, 1670, 1623, 1602, 1575, 1537, 1499, 1442,1417,1391; **HRMS** (ESI-TOF) m/z: $[M+H]^+$ Calculated for $C_{20}H_{23}N_2O_5^+$ 371.1600, found 371.1604.

*8.2.4.7 7,7-Dimethyl-2,5-dioxo-N-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8g**)*



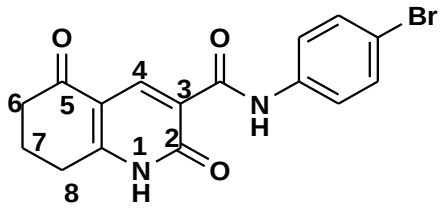

7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**2.6a**) (0.25 g, 1.1 mmol), CDI (0.22 g, 1.38 mmol) and aniline hydrochloride (**2.7g**) (0.14 g, 1.10 mmol) were reacted to give 7,7-dimethyl-2,5-dioxo-*N*-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8g**); **Physical characteristics**: white solid; Yield: 0.26 g, 79%; **Mp**: 332-334 ºC (Lit.[176] 332 ºC); **$R_f$** 0.7, hexane/ethyl acetate (1:1); **$^1$H NMR** (400 MHz,DMSO-$d_6$) δ 13.12 (1H, br s, N-H), 11.61 (1H, s, Ar-N-H), 8.72 (1H, s, Ar-H), 7.68 (2H, d, *J* = 7.2 Hz, Ar-H), 7.36 (2H, d, *J* = 7.6 Hz, Ar-H), 7.11 (1H, t, *J* = 6.4 Hz, Ar-H), 2.83 (2H, s, $CH_2$), 2.42 (2H, s, $CH_2$), 1.03 (6H, s, 2 × $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 193.2, 163.4, 160.9 (3 × C=O), 159.0 (Ar-C), 140.1 (Ar-CH), 138.2 (Ar-C), 129.1 (Ar-C), 123.9 (Ar-C), 119.7 (Ar-C), 118.3 (Ar-C), 112.9 (Ar-C), 49.9 (C-6, $CH_2$), 38.9 (C-7), 27.7 (2 × $CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3139,2866, 1679, 1618, 1596, 1555, 1485, 1442, 1421, 1302; **HRMS** (ESI-TOF) m/z: $[M+H]^+$ Calculated for $C_{18}H_{19}N_2O_3^+$ 311.1390, found 311.1419.

*8.2.4.8 N-(4-Chlorophenyl)-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8h**)*




2,5-Dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**2.6b**) (0.4 g, 1.93 mmol), CDI (0.41 g, 2.51 mmol) and 4-chloroaniline (**2.7a**) (0.25 g, 1.93 mmol) were reacted to give ***N***-(4-chlorophenyl)-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8h**); **Physical characteristics**: white solid; Yield: 0.49 g, 80%, **Mp**: 200-202 ºC; **$R_f$** 0.4, hexane/ethyl acetate (1:1): **$^1$H NMR** (400 MHz, DMSO-*$d_6$*) δ 13.11 (1H, s, N-H), 11.71 (1H, s, Ar-N-H), 8.73 (1H, s, CH), 7.69 (1H, d, *J* = 8.8 Hz, ArH), 7.55 (1H, d, *J* = 8.8 Hz, ArH), 2.95 (2H, t, *J* = 6.0 Hz, H-6), 2.09-2.04 (2H, m, H-7); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 193.3, 163.0, 161.1 (3 × C=O), 160.9 (Ar-C), 140.6 (Ar-C), 137.5 (Ar-CH) , 131.8 (Ar-C), 121.7 (Ar-C), 118.0 (Ar-C), 115.5 (Ar-C), 113.9 (Ar-C), 36.6 (C-6, $CH_2$), 26.5 (C-8, $CH_2$), 20.4 (C-7, $CH_2$); **FT-IR $\nu_{max}$/cm**): 3157, 2954, 1700, 1683, 1654, 1606, 1584, 1481, 1381, 1380.; **HRMS** (ESI-TOF) m/z: $[M+H]^+$ Calculated for $C_{16}H_{14}ClN_2O_3^+$ 317.0687, found 317.0688.

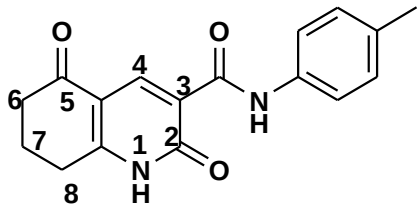
*8.2.4.9 N-(4-Bromophenyl)-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8i**)*




2,5-Dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**2.6b**) (0.40 g, 1.93 mmol), CDI (0.41 mg, 2.51 mmol) and 4-bromoaniline (**2.7b**) (0.33 g, 1.93 mmol) were reacted to give *N*-(4-bromophenyl)-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8i**); **Physical characteristics**: brown solid; Yield: 0.56 g, 86%, **Mp**: 342-344 ºC; **$R_f$** 0.35, hexane/ethyl acetate (1:1); **$^1$H NMR** (400 MHz, DMSO-*d6*) δ 13.11 (1H, s, N-H), 11.72 (1H, s, Ar-N-H), 8.74 (1H, s, Ar-H), 7.75 (2H, d, *J* = 8.4 Hz, ArH), 7.43 (2H, d, *J* = 8.4 Hz, ArH), 2.93 (2H, t, *J* = 6.0 Hz, H-6), 2.11-2.05 (2H, m, H-7); **$^{13}$C NMR** (101 MHz, DMSO-*$d_6$*) δ 193.4, 163.0, 161.1 (3 × C=O), 160.9 (Ar-C), 140.6 (Ar-CH), 137.1 (Ar-C), 128.9 (Ar-CH), 127.5 (Ar-C) , 121.4

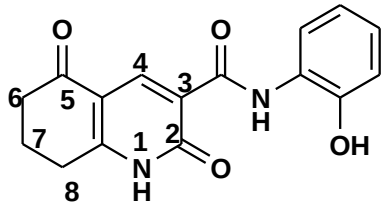
(Ar-CH), 118.0 (Ar-C), 113.9 (Ar-C), 36.6 (C-6, $CH_2$), 26.6 (C-8, $CH_2$), 20.5 (C-7, $CH_2$); **FT-IR $\nu_{max}$/cm**: 3145, 2954 , 1700, 1682, 1655, 1606, 1584, 1534, 1481, 1399; **HRMS** (ESI-TOF) m/z: $[M+H]^+$ Calculated for $C_{16}H_{14}BrN_2O_3^+$ 363.0182, found 363.0164.

*8.2.4.10 2,5-Dioxo-N-(p-tolyl)-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8j**)*




2,5-Dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**2.6b**) (0.40 g, 1.93 mmol), CDI (0.41 g, 2.51 mmol) and *p*-toluidine (**2.7c**) (0.21 g, 1.93 mmol) were reacted to give 2,5-dioxo-*N*-(*p*-tolyl)-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8j**); **Physical characteristics**: grey solid; Yield: 0.42 g, 71%, **Mp**: 330-332 ºC; **$R_f$** 0.4, hexane/ethyl acetate (1:1) : **$^1$H NMR** (400 MHz, ) δ 13.06 (1H, s, N-H), 11.57 (1H, s, Ar-N-H), 8.73 (1H, s, CH), 7.58 (2H, d, *J* = 8.4 Hz, ArH), 7.17 (2H, d, *J* = 7.6 Hz, ArH), 2.93 (2H, t, *J* = 6.8 Hz, H-6), 2.27 (3H, s, Ar-$CH_3$); 2.08-2.03 (2H, m, H-7); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 193.4, 163.1, 160.7 (3 × C=O), 160.7 (Ar-C), 140.3 (Ar-CH), 135.7 (Ar-C), 132.9 (Ar-C), 129.5 (Ar-CH), 119.7 (Ar-CH), 118.3 (Ar-C), 113.9 (Ar-C), 36.6 (C-6, $CH_2$), 26.5 (C-7, $CH_2$), 20.5 ($CH_3$); **FT-IR $\nu_{max}$/cm**: 3131, 2871, 1679, 1629, 1596, 1574, 1550, 1516, 1485, 1382; **HRMS** (ESI-TOF) m/z: $[M+H]^+$ Calculated for $C_{17}H_{17}N_2O_3^+$ 297.1234, found 297.1240.

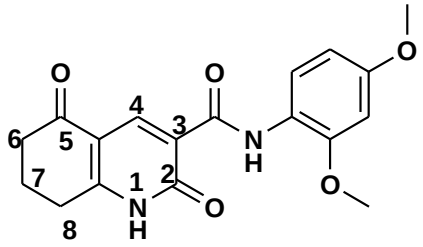
*8.2.4.11 N-(2-Hydroxyphenyl)-2,5-dioxo-1,2,5,6,7,8 hexahydroquinoline-3-carboxamide (**2.8k**)*




2,5-Dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**2.6b**) (0.4 g, 1.93 mmol), CDI (0.41 g, 2.51 mmol) and 2-hydroxyaniline (**2.7e**) (0.21 g, 1.93 mmol) were reacted to give *N*-(2-hydroxyphenyl)-2,5-dioxo-1,2,5,6,7,8 hexahydroquinoline-3-carboxamide (**2.8k)**; **Physical characteristics**: grey solid; Yield: 0.44 g, 76%, **Mp**: 324-326 ºC; **$R_f$** 0.3, hexane/ethyl acetate (1:1): **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 12.89 (1H, br s, N-H), 11.72 (1H, s, Ar-N-H), 10.04

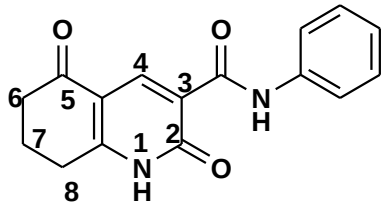
(1H, s, OH), 8.75 (1H, s, Ar-H), 8.37 (1H, d, *J* = 7.6 Hz, ArH), 6.90 (2H, d, *J* = 9.6 Hz, ArH), 6.79 (1H, t, *J* = 7.2 Hz, ArH) 2.92 (2H, t, *J* = 5.6 Hz, H-6), 2.09-2.03 (2H, m, H-7), **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 193.4, 162.7, 160.7 (3 × C=O), 160.6 (Ar-C), 146.6 (Ar-C), 140.4 (Ar-CH), 126.9 (Ar-C) , 123.9 (Ar-CH), 120.2 (Ar-CH), 119.1 (Ar-CH), 118.6 (Ar-C), 114.6 (Ar-CH), 113.7 (Ar-C), 36.6 (C-6, $CH_2$), 26.5 (C-8, $CH_2$), 20.5 (C-7, $CH_2$); **FT-IR $\nu_{max}$/cm** : 3038, 2955, 2829, 1658, 1597, 1570, 1493, 1384; **HRMS** (ESI-TOF) m/z: $[M+H]^+$ Calculated for $C_{16}H_{15}N_2O_4^+$ 299.1026, found 299.1050.

*8.2.4.12 N-(2,4-Dimethoxyphenyl)-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8l**)*



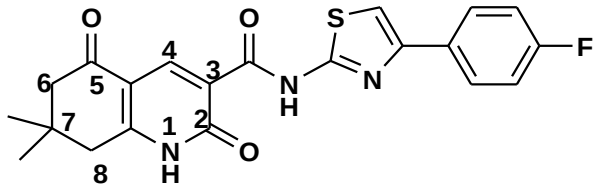

2,5-Dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**2.6b**) (0.40 g, 1.93 mmol), CDI (0.41 mg, 2.51 mmol) and 2,4-dimethoxy aniline (**2.7i**)(0.29 g, 1.93 mmol) were reacted to give *N*-(2,4-dimethoxyphenyl)-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8l**); **Physical characteristics**: grey solid; Yield: 0.50 g, 83%, **Mp**: 339-341 ºC; **$R_f$** 0.29, hexane/ethyl acetate (1:1); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 12.69 (1H, s, N-H), 11.56 (1H, s, Ar-N-H), 8.76 (1H, s, CH), 8.31 (1H, d, *J* = 8.4 Hz, ArH), 6.66 (1H, s, ArH), 6.54 (1H, d, *J* = 8.4 Hz, ArH), 3.88 (3H, s, Ar-O-$CH_3$), 3.78 (3H, s, Ar-O-$CH_3$), 3.06 (2H, t, *J* = 5.6 Hz, H-6), 2.95 (2H, t, *J* = 7.6 Hz, H-8), 2.11-2.06 (2H, m, H-7); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 192.7, 162.4, 159.9 (3 × C=O), 159.8 (Ar-C), 156.0 (Ar-CH), 149.7 (Ar-C), 139.8 (Ar-CH), 121.2 (Ar-C), 120.6 (Ar-CH), 118.7 (Ar-C), 113.4 (Ar-C), 104.4 (Ar-C), 98.9 (Ar-CH), 55.9 (O-$CH_3$), 55.1 (O-$CH_3$), 36.2 (C-6, $CH_2$), 26.2 (C-8, $CH_2$), 20.2 (C-7, $CH_2$) ; **FT-IR $\nu_{max}$/cm$^{-1}$**: 3266, 3119, 2961, 1670, 1623, 1602, 1575, 1499, 1442,1417,1391; **HRMS** (ESI-TOF) m/z: $[M+H]^+$ Calculated for $C_{18}H_{19}N_2O_5^+$ 343.1288, found 343.1275.

*8.2.4.13 2,5-Dioxo-N-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8m**)*



2,5-Dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**2.6b**) (0.40 g, 1.93 mmol), CDI (0.41 g, 2.51 mmol) and aniline hydrochloride (**2.7g**) (0.25 g, 1.93 mmol) were reacted to give 2,5-dioxo-*N*-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.8m**); **Physical characteristics**: grey solid; Yield: 0.44 g, 81%, **Mp**: 332-334 ºC (Lit.[176] 339 ºC); **$R_f$** 0.4, hexane/ethyl acetate (1:1); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 13.11 (1H, s, N-H), 11.64 (1H, s, Ar-N-H), 8.74 (1H, s, CH), 7.69 (2H, d, *J* = 7.6 Hz, ArH), 7.36 (2H, t, *J* = 7.2, Hz, ArH), 7.12 (1H, t, *J* = 6.8 Hz, ArH), 2.94 (2H, t, *J* = 6.4 Hz, H-6), 2.09-2.03 (2H, m, H-7); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 193.4, 163.1, 160.9 (3 × C=O), 160.8 (Ar-C), 140.4 (Ar-CH), 138.2 (Ar-C), 129.1 (Ar-C), 123.9(Ar-C), 119.7 (Ar-C), 118.2 (Ar-C) , 113.9(Ar-C), 36.6 (H-6, $CH_2$), 26.5 (H-8, $CH_2$), 20.5 (H-7, $CH_2$); **FT-IR $\nu_{max}$/cm$^{-}$**: 3026, 2957,1676, 1640, 1600, 1480, 1425; **HRMS** (ESI-TOF) m/z: $[M+H]^+$ Calculated for $C_{16}H_{15}N_2O_3^+$ 283.1077, found 283.1113.

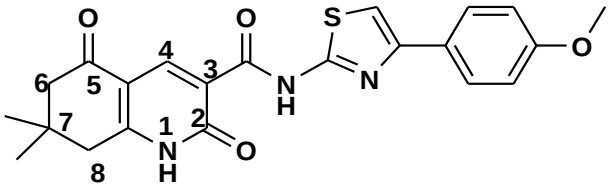
*8.2.4.14 N-(4-(4-Fluorophenyl)thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8 hexahydroquinoline-3-carboxamide (**2.11a**)*



7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**2.6a**) (0.25 g, 1.1 mmol), CDI (0.22 g, 1.38 mmol) and 4-(4-fluorophenyl)thiazol-2-amine (**2.10a**) (0.21 g, 1.10 mmol) were reacted to give *N*-(4-(4-fluorophenyl)thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8 hexahydroquinoline-3-carboxamide (**2.11a**); **Physical characteristics**: yellow solid; Yield: 0.35 g, 80%; **Mp**: 347-349 ºC; **$R_f$** 0.80, hexane/ethyl acetate (1:1); **$^1$H NMR** (400 MHz,DMSO-$d_6$) δ 13.32 (1H, br s, N-H), 12.90 (1H, s, N-H), 8.76 (1H, s, H-4), 7.97 (2H, t, *J* = 8.0 Hz, Ar-H), 7.69 (1H, Ar-H), 7.26 (2H, t, *J* = 8.8 Hz, ArH), 2.88 (2H, s, H-6), 2.46 (2H,

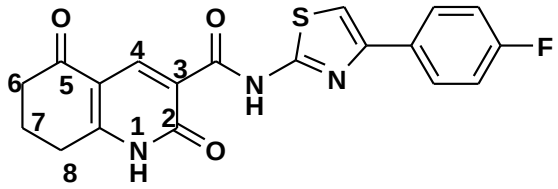
s, H-8), 1.06 (6H, s, 2 × $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 193.0, 185.8 (C=O), 162.1 (d, $J_{CF}$ = 248.0 Hz, Ar-C), 160.6 (C=O), 160.1 (Ar-C), 156.8 (Ar-C), 148.2 (Ar-C), 140.8 (Ar-CH), 130.6 (Ar-C), 127.8 (d, $J_{CF}$ = 8.0 Hz, Ar-CH), 115.9 (Ar-C), 115.5 (d, $J_{CF}$ = 21.1 Hz, Ar-CH), 113.3 (Ar-C), 108.8 (Ar-CH), 49.9 (C-6, $CH_2$), 32.6 (C-7), 27.6 (2 × $CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3110, 2965, 2928, 1673, 1620, 1573, 1545, 1485, 1386, 1301; **HRMS** (ESI-TOF) m/z: $[M+H]^+$ Calculated for $C_{21}H_{19}FN_3O_3S^+$ 412.1126, found 412.1144.

*8.2.4.15 N-(4-(4-Methoxyphenyl)thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.11b**)*



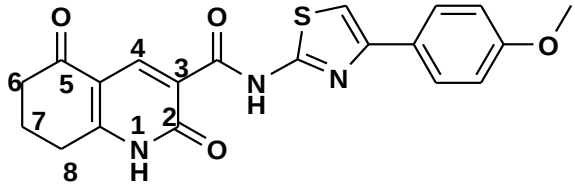

7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**2.6a**) (0.25 g, 1.1 mmol), CDI (0.22 g, 1.38 mmol) and 4-(4-methoxyphenyl)thiazol-2-amine (**2.10b**) (0.22 g, 1.10 mmol) were reacted to give N-(4-(4-methoxyphenyl)thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.11b**); **Physical characteristics**: yellow solid; Yield: 0.36 g, 82%; **Mp**: 348-350 ºC; **$R_f$** 0.7, hexane/ethyl acetate (1:1); **$^1$H NMR** (400 MHz,DMSO-$d_6$) δ 13.11 (1H, br s, N-H), 12.89 (1H, s, N-H), 8.75 (1H, s, Ar-H), 7.86 (2H, , d, $J$ = 8.4 Hz, Ar-H), 7.57 (1H, s, Ar-H), 6.99 (2H, d, $J$ = 8.4 Hz, Ar-H), 3.79 (3H, s, O-$CH_3$), 2.87 (2H, s, H-6), 2.49 (2H, s, H-8), 1.05 (6H, s, 2 × $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 193.1, 163.34, 160.8 (3 × C=O), 160.1 (Ar-C), 159.1 (Ar-C), 156.5 (Ar-C), 149.2 (Ar-C), 140.74 (Ar-CH), 127.1 (Ar-CH), 126.8 (Ar-C), 116.1(Ar-C), 114.1 (Ar-CH), 113.3(Ar-C), 106.9 (Ar-CH), 55.2 (O-$CH_3$), 49.9 (C-6, $CH_2$), 32.6 (C-7), 27.7 (2 × $CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3119, 2930, 1624, 1573, 1540, 1486, 1453, 1418, 1303; **HRMS** (ESI-TOF) m/z: $[M+H]^+$ Calculated for $C_{22}H_{22}N_3O_4S^+$ 424.1326 found 424.1345.

*8.2.4.16 N-(4-(4-Fluorophenyl)thiazol-2-yl)-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.11c**)*



2,5-Dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**2.6b**) (0.25g, 120 mmol), CDI (0.25 mg, 170 mmol) and 4-(4-fluorophenyl)thiazol-2-amine (**2.10a**) (0.23 mg, 120 mmol) were reacted to give *N*-(4-(4-fluorophenyl)thiazol-2-yl)-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.11c**); **Physical characteristics**: white solid; Yield: 0.38 g, 83%; **Mp**: 341-343 ºC; **$R_f$** 0.76, hexane/ethyl acetate (1:1); **$^1$H NMR** (400 MHz,DMSO-$d_6$) δ 13.28 (1H, br s, N-H), 13.05 (1H, s, N-H), 8.76 (1H, s, CH), 7.97 (2H, d, *J* = 7.2 Hz, Ar-H), 7.69 (1H, s, Ar-H), 7.26 (2H, d, *J* = 8.0 Hz, Ar-H ), 2.96 (2H, t, *J* = 9.2 Hz, H-6), 2.54 (2H, t, *J* = 6.4 Hz, H-8), 2.12-2.05 (2H, m, H-7); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 193.4 (C=O), 187.7 (C=O), 162.3 (d, $J_{CF}$ = 243.4 Hz, Ar-C), 156.8(C=O), 148.0 (Ar-C), 142.6 (Ar-C), 140.7 (Ar-CH), 133.7 (Ar-C), 130.6 (Ar-C), 127.8 (d, $J_{CF}$ = 9.1 Hz, Ar-CH), 115.7 (Ar-C), 115.6 (d, $J_{CF}$ = 21.1, Hz, Ar-CH), 114.3 (Ar-C), 108.7 (Ar-CH), 36.6 (C-6, $CH_2$), 26.9 (C-8, $CH_2$), 20.4 (C-7, $CH_2$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3131, 2955, 2873, 1770, 1682, 1651, 1606, 1583, 1533, 1470, 1399; **HRMS** (ESI-TOF) m/z: $[M+H]^+$ Calculated for $C_{19}H_{15}FN_3O_3S^+$ 384.0813, found 384.0825.

*8.2.4.17 N-(4-(4-Methoxyphenyl)thiazol-2-yl)-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.11d**)*



2,5-Dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**2.6b**) (0.25g, 120 mmol), CDI (0.25g, 170 mmol) and 4-(4-methoxyphenyl)thiazol-2-amine (**2.10b**) (0.25 mg, 120 mmol) were reacted to give *N*-(4-(4-methoxyphenyl)thiazol-2-yl)-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**2.11d**); **Physical characteristics**: yellow solid; Yield:

0.41g, 85%; Mp: 345-347 ºC; **$R_f$** 0.6 hexane/ethyl acetate (1:1); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 13.42 (1H, br s, N-H), 13.07 (1H, br s, N-H), 8.74 (1H, s, Ar-H), 7.85 (2H, d, *J* = 8.8 Hz, Ar-H), 7.54 (1H, s, Ar-H), 6.98 (2H, d, *J* = 8.4 Hz, Ar-H), 3.79 (3H, O-$CH_3$), 2.94 (2H, t, *J* = 7.2 Hz, H-6), 2.09-2.04 (2H, m, H-7); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 193.3, 189.9, 161.2 (3 × C=O), 159.3 (Ar-C), 156.5 (Ar-C), 149.2 (Ar-C), 141.1 (Ar-CH), 137.8 (Ar-C), 127.1 (Ar-CH), 126.9 (Ar-C), 115.7 (Ar-C), 114.3 (Ar-CH), 114.1 (Ar-C), 106.9 (Ar-CH), 55.1 (O-$CH_3$), 36.6 (C-6, $CH_2$), 27.0 (C-8, $CH_2$) , 20.4 (C-7, $CH_2$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3142, 2954, 1700, 1682, 1652, 1606, 1584, 1469, 1399, 1381; **HRMS** (ESI-TOF) m/z: $[M+H]^+$ Calculated for $C_{20}H_{18}N_3O_4S^+$ 396.1013 found 396.1023.

*8.2.5 Virtual screening data*

**Table 8.1:** Molecular docking results of ChemBridge ligands 2.8a and their derivatives, CX05168 and Mut101**.**

| | **Docking score (Kcal/mol)** | **Gscore (Kcal/mol)** | **XP G score** | **Glide emodel (Kcal/mol)** | **MMGBSA (Kcal/mol)** |
|---|---|---|---|---|---|
| **2.8a** | -4.333 | -4.333 | -4.333 | -58.112 | -52.529 |
| **2.8b** | -4.417 | -4.417 | -4.417 | -56.382 | -43.968 |
| **2.8c** | -4.646 | -4.646 | -4.646 | -46.220 | -43.406 |
| **2.8d** | -5.205 | -4.490 | -4.490 | -50.818 | -56.104 |
| **2.8e** | -5.264 | -5.264 | -5.264 | -52.124 | -42.325 |
| **2.8f** | -4.443 | -4.443 | -4.443 | -55.581 | -39.724 |
| **2.8g** | -4.701 | -4.701 | -4.701 | -55.075 | -44.082 |
| **2.8h** | -4.302 | -4.302 | -4.302 | -54.890 | -45.209 |
| **2.8i** | -5.055 | -5.055 | -5.055 | -53.178 | -45.952 |
| **2.8j** | -4.368 | -4.368 | -4.368 | -45.403 | -32.703 |
| **2.8k** | -5.096 | -5.096 | -5.096 | -47.228 | -34.155 |
| **2.8l** | -4.602 | -4.602 | -4.602 | -49.346 | -40.224 |
| **2.8m** | -4.441 | -4.441 | -4.441 | -45.085 | -34.6000 |
| **2.11a** | -3.396 | -3.396 | -3.396 | -55.500 | -46.722 |

| | Docking score (Kcal/mol) | Gscore (Kcal/mol) | XP G score | Glide emodel (Kcal/mol) | MMGBSA (Kcal/mol) |
|---|---|---|---|---|---|
| **2.11b** | -3.969 | -3.990 | -3.990 | -56.051 | -50.378 |
| **2.11c** | -3.073 | -3.095 | -3.095 | -55.383 | -47.773 |
| **2.11d** | -2.028 | -3.984 | -3.984 | -57.395 | -40.528 |
| **CX05168** | -5.329 | -5.339 | -5.339 | -52.558 | -51.171 |
| **Mut101** | -6.176 | -6.621 | -6.621 | -63.191 | -59.389 |

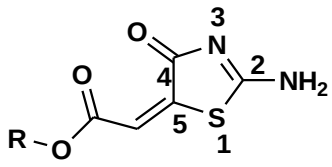
**Table 8.2:** ADME-drug-likeness parameters of synthesised hexahydroquinoline compounds using QikProp protocol.

| ENTRY | MW (g/mol) (<500) | HBD (≤5) | HBA (≤10) | QPlogBB (-3.0 to1.2) | QPlogo/w (-2 to 6.5) | QPlog HERG (>-5) |
|---|---|---|---|---|---|---|
| **2.8a** | 344.797 | 0 | 5.0 | -0.997 | 3.139 | -5.366 |
| **2.8b** | 389.248 | 0 | 5.0 | -0.992 | 3.217 | -5.397 |
| **2.8c** | 324.379 | 0 | 5.0 | -1.184 | 2.959 | -5.369 |
| **2.8d** | 338.405 | 0 | 5.0 | -1.117 | 3.255 | -5.069 |
| **2.8e** | 326.351 | 1 | 5.6 | -1.625 | 2.199 | -5.274 |
| **2.8f** | 370.404 | 0 | 6.5 | -1.317 | 2.827 | -5.247 |
| **2.8g** | 310.352 | 0 | 5.0 | -1.137 | 2.637 | -5.442 |
| **2.8h** | 316.743 | 0 | 5.0 | -1.007 | 2.472 | -5.268 |
| **2.8i** | 361.194 | 0 | 5.0 | -1.002 | 2.551 | -5.299 |
| **2.8j** | 296.325 | 0 | 5.0 | -1.194 | 2.293 | -5.273 |
| **2.8k** | 298.298 | 1 | 5.8 | -1.625 | 1.563 | -5.164 |
| **2.8l** | 342.351 | 0 | 6.5 | -1.324 | 2.161 | -5.142 |
| **2.8m** | 282.298 | 0 | 5.0 | -1.147 | 1.967 | -5.342 |
| **2.11a** | 411.450 | 0 | 6.5 | -1.171 | 3.643 | -6.154 |
| **2.11b** | 423.486 | 0 | 7.3 | -1.373 | 3.472 | -6.160 |
| **2.11c** | 383.396 | 0 | 6.5 | -1.162 | 2.945 | -6.014 |
| **2.11d** | 395.432 | 0 | 7.3 | -1.389 | 2.805 | -6.102 |

| ENTRY | MW (g/mol) (<500) | HBD (≤5) | HBA (≤10) | QPlogBB (-3.0 to1.2) | QPlogo/w (-2 to 6.5) | QPlog HERG (>-5) |
|---|---|---|---|---|---|---|
| **Mut 101** | 409.524 | 1 | 4.5 | -0.271 | 5.558 | -1.975 |
| **CX05168** | 305.376 | 1 | 3.0 | -0.386 | 4.494 | -3.034 |

## 8.3 Synthetic methods: Chapter 3

### *8.3.1 General procedure for the preparation of **3.5a** and **3.5b***




Thiourea (**3.3**) (1eq.) and absolute ethanol (30 ml) were placed in a round bottomed flask equipped with a magnetic stirrer bar. The solution was heated under reflux for 0.5 h, then 1 eq. of DMAD (**3.4a**) (1eq.) or DEtAD (**3.4b**) were slowly added through the condenser producing a milky solution, which was heated for a further 3 h. The resulting milky solution was allowed to cool to room temperature and the precipitate was filtered by suction and washed with absolute ethanol to obtain solid product.

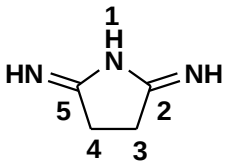
#### *8.3.1.1 (E)-Methyl 2-(2-amino-4-oxothiazol-5(4H)-ylidene)acetate (**3.5a**)*

Thiourea (**3.3**) (5.00 g, 66 mmol), ethanol (30 ml) and dimethyl acetylenedicarboxylate (**3.4a**) (9.40 g, 66 mmol) were reacted to give (*E*)-methyl 2-(2-amino-4-oxothiazol-5(4*H*)-ylidene)acetate (**3.5a**); **Physical characteristics**: White solid; Yield: 11.9 g, 97%; **Mp**: 271-273 ºC, (Lit.[107] 270-275 ºC); **$^{1}$H NMR** (400 MHz, DMSO-$d_6$) δ 9.60 (2H, s, $NH_2$), 6.64 (1H, s, CH), 3.76 (3H, s, OMe), **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 178.5 (C=O), 177.4 (C=O), 166.4 (Ar-C) 148.3 (Ar-C), 114.7 (CH), 52.52 (OMe).

#### *8.3.1.2 (E)-Ethyl 2-(2-amino-4-oxothiazol-5(4H)-ylidene)acetate (**3.6b**)*

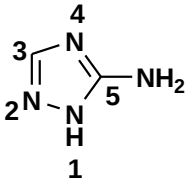
Thiourea (**3.3**) (5.00 g, 66 mmol), ethanol (30 ml) and diethyl acetylenedicarboxylate (**3.4b**) (11.17 g, 66 mmol) were reacted to give (*E*)-ethyl 2-(2-amino-4-oxothiazol-5(4*H*)-ylidene)acetate **(3.5b)** ; **Physical characteristics**: White solid; Yield: 12.5 g, 95%; **Mp**: 237-239 ºC, (Lit.[108] 238 ºC); **$^{1}$H NMR** (400 MHz, DMSO-$d_6$) δ 9.59 (2H, br s, $NH_2$), 6.61 (1H, s, CH), 4.21 (2H, q, , $J$ = 7.2 Hz, O-$CH_2$), 1.24 (3H, t, $J$ = 7.2 Hz, $CH_3$), **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 178.5 (C=O), 177.4 (C=O), 165.8 (Ar-C) 148.0 (Ar-C), 115.0 (CH), 61.3 (O-$CH_2$), 14.0 ($CH_3$).

*8.3.1.3 Preparation of pyrrolidine-2,5-diimine (**3.8**)*



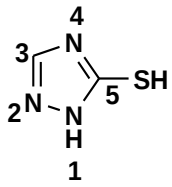

A solution of succinitrile (**3.6**) (5.00 g, 38 mmol) in dry methanol (30ml) (1eq.) was treated with excess liquid ammonium hydroxide (**3.7**) (6.60 g, 190 mmol) and the mixture was refluxed at 65ºC in a round bottomed flask for 24 h. The methanolic solution was filtered, washed with 10 ml of ethyl acetate and 10 ml diethyl ether and dried to obtain pyrrolidine-2,5-diimine (**3.8**); **Physical characteristics**: White solid crystals; Yield: 2.52 g, 70%; **Mp**: 199-201 ºC, (Lit.[185] 200 ºC); **$^{1}$H NMR** (400 MHz, DMSO-$d_6$) δ 8.08 (1H, s, NH), 7.26 (1H, s, NH), 6.72 (1H, s, NH), 2.61-2.59 and 2.31-2.25 (1H and 3H, 2 × m, $CH_2$-$CH_2$). **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 193.5 (C=NH), 187.1 (C=NH), 31.3 ($CH_2$), 28.0 ($CH_2$).

*8.3.1.4. Preparation of 1H-1,2,4-triazol-5-amine (**3.11**)*



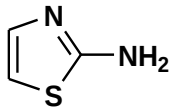

A solution of aminoguanidine bicarbonate (**3.9**) and formic acid (**3.10**) in a round bottomed flask, was stirred at rt for at least 0.5 h to complete release of $CO_2$. After addition of 20 ml of toluene, the stirred reaction mixture was heated at reflux for 24 h. After cooling to rt, the mixture was placed in an ice bath for 0.5 h, the formed solid was collected by filtration, washed with 10 ml toluene and 10 ml ethanol and dried to obtain the product 1*H*-1,2,4-triazol-5-amine (**3.11**): Aminoguanidine bicarbonate (**3.9**) (10.00 g, 74 mmol) and formic acid (**3.10**) (3.40 g, 74 mmol) were reacted to give1*H*-1,2,4-triazol-5-amine (**3.11**); **Physical characteristics**: White solid crystals; Yield: 5.2 g, 84%; **Mp**: 141-143 ºC lit. [186]142 ºC; **$^{1}$H NMR** (400 MHz, DMSO-$d_6$) δ 12.14 (1H, s, NH), 7.45 (1H, s, Ar-H), 5.78 (2H, br s, $NH_2$), **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 157.7 (Ar-C), 147.87 (Ar-C).

*8.3.1.5 Preparation of 1H-1,2,4-triazole-5-thiol (**3.13**)*




Formic acid (**3.10**) was heated in a round-bottomed flask at 100ºC for 0.5 h, and thiosemicarbazide (**3.12**) (5 eq.) was added slowly to the hot solution until it completely dissolved. To this solution, boiling water was added, which generated a milky solution which was cooled in an ice bath for 2 h to afford 2-formylhydrazinecarbothioamide intermediate, which was collected by suction filtration and then dissolved in 20% NaOH. This was heated under reflux for another 2 h, after which the reaction mixture was allowed to cool, crushed ice was added and then the solution was neutralised by dropwise addition of concentrated HCl. The solid product obtained was collected by filtration and recrystallized from ethanol. 1*H*-1,2,4-triazole-5-thiol (**3.13**): Formic acid (**3.10**) (2.50 g, 11 mmol), thiosemicarbazide (**3.12**) (5.00 g, 55 mmol), Water 1.1 ml, and (20% NaOH) were reacted to give 1*H*-1,2,4-triazole-5-thiol (**3.13**); **Physical characteristics**: White solid Yield crystals: 1.3 g, 82%; **Mp**:219-221 ºC (Lit. [187]220-222 ºC); **$^{1}$H NMR** (400 MHz, DMSO-$d_6$) δ 13.42 (2H, br s, SH and NH), 8.25 (1H, s, Ar-CH), **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 165.5 (Ar-C), 140.5 (Ar-C).

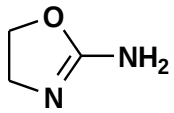
*8.3.1.6 Preparation of thiazol-2-amine (**3.15**)*




A solution of thiourea (**3.3**) (1 eq.), chloroacetaldehyde (**3.14**) (1 eq.) and ethanol (25 ml) were placed in a round bottomed flask equipped with a magnetic stirrer and heated under reflux for 12 h, while monitoring by TLC (hexane/ethyl acetate (6 : 4). The reaction mixture was filtered, washed with 10 ml ethanol and dissolved in water (25 ml). The pH of the solution was adjusted to pH 11 by addition of a $Na_2CO_3$ solution and extracted with dichloromethane (2 × 30 ml). The combined extracts was dried over sodium sulphate and after solvent evaporation the product thiazol-2-amine (3**.15**) was obtained: Thiourea (**3.3**) (2.20 g, 28 mmol) and chloroacetaldehyde (**3.14**) (3.50 g, 28 mmol) were reacted to give thiazol-2-amine (**3.15a**): **Physical characteristics**: Brown solid crystals; Yield: 2.7 g, 96%; **Mp**: 90-92 ºC (Lit.[188] 89-

90 ºC); **$^{1}$H NMR** (400 MHz, $CDCl_3$) δ 7.07 (1H, d, *J* = 3.6 Hz, Ar-H), 6.51 (1H, d, *J* = 3.6 Hz, Ar-H) 5.24 (2H, s, $NH_2$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 168.4 (Ar-C), 138.9 (Ar-CH), 108.8 (Ar-CH).

*8.3.1.7 Preparation of 2-Imino-1,3-oxazolidine 3.18*




A mixture of 2-chloroethylamine hydrochloride (**3.16**) (10.00 g, 86 mmol) and sodium cyanate (**3.17**) (5.60 g, 86 mmol) in water (25 mL) was stirred at 90 °C for 4 h. The solution was concentrated under reduced pressure and the semisolid residue extracted with hot ethanol. Anhydrous ether (10ml) was added to the combined ethanolic solutions to give white crystals product **3.18**. **Physical characteristics**: White solid; Yield: 5.9 g, 79%; **Mp**: 104-106 ºC (lit.[189] 105 ºC); **$^{1}$H NMR** (400 MHz, DMSO-*d6*) δ 9.20 (2H, br s, $NH_2$), 4.72 (2H, t, *J* = 8.4 Hz, $CH_2$), 3.77 (2H, t, *J* = 8.4 Hz, $CH_2$), **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 162.8 (C-2), 70.5 ($CH_2$), 42.9 ($CH_2$).

*8.3.2 General procedures for the synthesis of **3.20a-h**, **3.21a-b**, **3.22a-b**, **3.23a-b** and **3.24.***

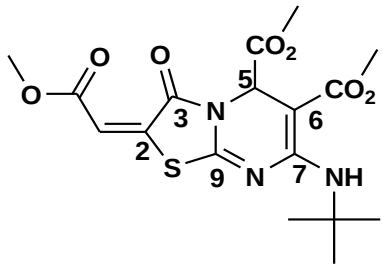
**Method A**

In a round bottomed flask equipped with a magnetic stirrer bar, DMAD or DEtAD (1eq.) was added to a mixture of compound **3.5a-b, 3.8, 3.11, 3.13** or **3.15** in dry $CH_2Cl_2$ (5 ml). To this was added dropwise a solution of the isocyanide derivative (**3.19a-b**) (1 eq.) in dry $CH_2Cl_2$ (3 ml) at rt. over 10 min and the resulting mixture was stirred for 24 h. After solvent removal the residues were purified by column chromatography in hexane/ ethyl acetate (9:1).

**Method B**

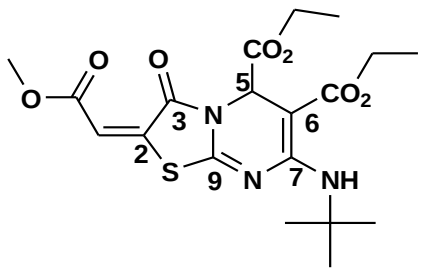
In a round bottomed flask equipped with a magnetic stirrer bar, isocyanide derivative (**3.19a-b**) (1 eq.) in dry $CH_2Cl_2$ (2.5 ml) was slowly added to a solution of DMAD or DEtAD (1eq.) in dry dichloromethane (5 ml) at 0ºC under inert atmosphere, and stirred for 0.5 h. A solution of compound **3.5a-b, 3.8, 3.11, 3.13** or **3.15** in dry $Cl_2CH_2$ (2.5ml) was slowly added and then the mixture was stirred for 24h at rt. After solvent removal crude material was purified by column chromatography using hexane/ ethyl acetate (9:1) as eluent unless stated otherwise.

*8.3.2.1 (E)-Dimethyl 7-(tert-butylamino)-2-(2-methoxy-2-oxoethylidene)-3-oxo-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine-5,6-dicarboxylate (**3.20a**)*



**Method B:** (*E*)-Methyl 2-(2-amino-4-oxothiazol-5(4*H*)-ylidene)acetate (**3.5a**) (0.10 g, 0.54 mmol), DMAD (**3.4a**) (0.08 g, 0.54 mmol) and *tert*-butyl isocyanide (**3.19a**) (0.04 g, 0.54 mmol) were reacted to give (*E*)-dimethyl 7-(*tert*-butylamino)-2-(2-methoxy-2-oxoethylidene)-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidine-5,6-dicarboxylate (**3.120a**); **Physical characteristics**: red solid; Yield: 0.18 g, 82%; **Mp**: 220-222 ºC, **$R_f$** : 0.9; **$^1$H NMR** (400 MHz, $CDCl_3$) δ 9.05 (1H, s, NH), 7.03 (1H, s, CH=C-S), 5.78 (1H, s, H-5), 3.88 (3H, s, $OCH_3$), 3.74 (3H, s, $OCH_3$), 3.70 (3H, s, $OCH_3$), 1.44 (9H, s, $C(CH_3)_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 169.8 (C=O), 168.5 (C=O), 166.2 (C=O), 163.94 (C=O), 159.5 (C-7), 156.2 (C-9), 139.9 (C-2), 119.0 (*C*H=C-S), 71.0 (C-6), 53.23 ($OCH_3$) , 53.17 ($OCH_3$), 53.0 ($OCH_3$), 52.3 (N-*C*$(CH_3)_3$), 50.9 (C-5), 30.9 (C(*C*$H_3)_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3061, 2951, 1736, 1688, 1653, 1629, 1611, 1580, 1436, 1381; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{17}H_{22}N_3O_7S^+$ 412.1173, found 412.1207.

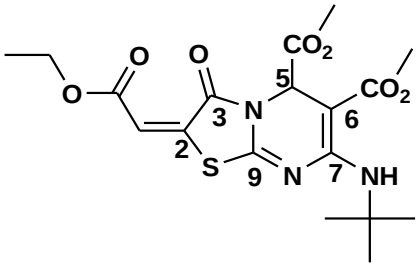
*8.3.2.2. (E)-Diethyl 7-(tert-butylamino)-2-(2-methoxy-2-oxoethylidene)-3-oxo-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine-5,6-dicarboxylate (**3.20b**)*



**Method B:** (*E*)-Methyl 2-(2-amino-4-oxothiazol-5(4*H*)-ylidene)acetate (**3.5a**) (0.10 g, 0.54 mmol), DEtAD (**3.4b**) (0.09 g, 0.54 mmol) and *tert*-butyl isocyanide (**3.19a**) (0.04 g, 0.54 mmol) were reacted to give (*E*)-diethyl 7-(*tert*-butylamino)-2-(2-methoxy-2-oxoethylidene)-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidine-5,6-dicarboxylate (**3.20b**); **Physical**

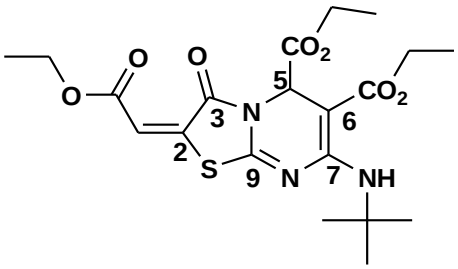
**characteristics**: red solid; Yield: 0.193 g, 80%; **Mp**: 165-167 ºC, **$R_f$** : 0.9; **$^1$H NMR** (400 MHz, $CDCl_3$) δ 9.07 (1H, s, NH), 7.04 (1H, s, CH=C-S), 5.78 (1H, s, H-5), 4.30-4.06 (4H, m, 2 × $OCH_2$), 3.89 (3H, s, $OCH_3$), 1.44 (9H, s, $C(CH_3)_3$), 1.31 (3H, t, *J*= 7.2 Hz, $CH_3CH_2O$), 1.23 (3H, t, *J*= 7.2 Hz, $CH_3CH_2O$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 169.5 (C=O), 168.2 (C=O), 166.2 (C=O), 164.1 (C=O), 159.4 (C-7), 156.1 (C-9), 140.1 (C-2), 118.9 (*C*H=C-S), 71.3 (C-6), 62.3 and 59.5 (2 × $OCH_2$), 53.1 (N-$C(CH_3)_3$), 53.0 ($CH_3$-O), 52.5 (C-5) 30.9 ($C(CH_3)_3$), 14.8 and 14.2 (2 × $CH_3CH_2O$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 2980, 1723, 1701, 1646, 1608, 1429, 1369, 1325, 1268; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{19}H_{26}N_3O_7S^+$ 440.1486, found 440.1528.

*8.3.2.3 (E)-Dimethyl 7-(tert-butylamino)-2-(2-ethoxy-2-oxoethylidene)-3-oxo-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine-5,6-dicarboxylate (**3.20c**)*



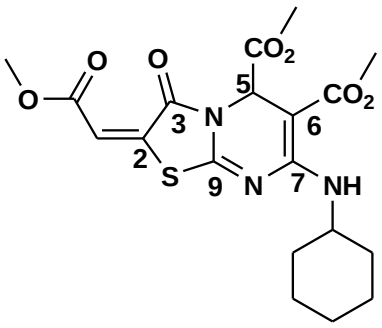

**Method B:** (*E*)-Ethyl 2-(2-amino-4-oxothiazol-5(4H)-ylidene)acetate (**3.5b**) (0.25 g, 1.25 mmol), DMAD (**3.4a**) (0.18 g, 1.25 mmol) and *tert*-butyl isocyanide (**3.19a**) (0.10 g, 1.25 mmol) were reacted to give (*E*)-dimethyl 7-(*tert*-butylamino)-2-(2-ethoxy-2-oxoethylidene)-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidine-5,6-dicarboxylate (**3.20c**); **Physical characteristics**: red solid; Yield: 0.47 g, 87%; **Mp**: 172-174 ºC, **$R_f$** : 0.8; **$^1$H NMR** (400 MHz, $CDCl_3$) δ 9.05 (1H, s, NH), 7.02 (1H, s, CH=C-S), 5.78 (1H, s, H-5), 4.33 (2H, q, *J* = 7.2 Hz, O-$CH_2$), 3.74 (3H, s, $OCH_3$), 3.69 (3H, s, $OCH_3$), 1.43 (9H, s, $C(CH_3)_3$), 1.35 (3H, t, *J* = 7.2 Hz, $CH_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 169.8 (C=O), 168.5 (C=O), 165.7 (C=O), 164.0 (C=O), 159.6 (C-7), 156.2 (C-9), 139.5 (C-2), 119.6 (*C*H=C-S), 71.0 (C-6), 62.3 (O-$CH_2$), 53.2 ($OCH_3$), 53.2 ($OCH_3$), 52.3 (N-$C(CH_3)_3$), 50.9 (C-5), 30.9 ($C(CH_3)_3$), 14.3 ($CH_3CH_2$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3055, 2971, 1724, 1689, 1653, 1609, 1578, 1443, 1372; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{18}H_{24}N_3O_7S^+$426.1329, found 426.1345.

*8.3.2.4 (E)-Diethyl 7-(tert-butylamino)-2-(2-ethoxy-2-oxoethylidene)-3-oxo-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine-5,6-dicarboxylate (**3.20d**)*



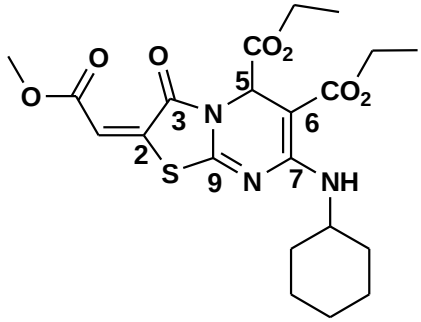
**Method B:** (*E*)-Ethyl 2-(2-amino-4-oxothiazol-5(4*H*)-ylidene)acetate (**3.5b**) (0.25 g, 1.25 mmol), DEtAD (**3.4b**) (0.21 g, 1.25 mmol) and *tert*-butyl isocyanide (**3.19a**) (0.10 g, 1.25 mmol) were reacted to give **(***E*)-diethyl 7-(*tert*-butylamino)-2-(2-ethoxy-2-oxoethylidene)-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidine-5,6-dicarboxylate (**3.20d**); **Physical characteristics**: red solid; Yield: 0.48 g, 86%; **Mp**: 155-157 ºC, **$R_f$** : 0.9, hexane/ ethyl acetate (9:1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 9.06 (1H, s, NH), 7.02 (1H, s, CH=C-S), 5.78 (1H, s, H-5), 4.33 (2H, q, *J* = 7.2 Hz, O-C*H*$_2$CH$_3$), 4.25-4.09 (4H, m, 2 × OC*H*$_2$CH$_3$), 1.43 (9H, s, C(C*H*$_3$)$_3$), 1.35 (3H, t, *J* = 7.2 Hz, C*H*$_3$CH$_2$O), 1.31 (3H, t, *J*= 7.2 Hz, *CH*$_3$CH$_2$O), 1.23 (3H, t, *J* = 7.2 Hz, C*H*$_3$CH$_2$O), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 169.5 (C=O), 168.2 (C=O), 165.7 (C=O), 164.1 (C=O), 159.5 (C-7), 156.2 (C-9), 139.7 (C-2), 119.4 (*C*H=C-S), 71.3 (C-6), 62.3 (O*C*H$_2$CH$_3$), 62.2 (O*C*H$_2$CH$_3$), 59.51 (O*C*H$_2$CH$_3$), 53.1 (N-*C*(CH$_3$)$_3$), 52.5 (C-5) 30.9 (C(*C*H$_3$)$_3$), 14.8 (*C*H$_3$CH$_2$O), 14.3 (*C*H$_3$CH$_2$O), 14.1 (*C*H$_3$CH$_2$O); **FT-IR $\nu_{max}$/cm$^{-1}$**: 2981, 2805, 1732, 1691, 1614, 1583, 1382, 1311; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{20}H_{28}N_3O_7S^+$ 454.1642, found 454.1642.

*8.3.2.5 (E)-Dimethyl 7-(cyclohexylamino)-2-(2-methoxy-2-oxoethylidene)-3-oxo-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine-5,6-dicarboxylate (**3.20e**)*



**Method B:** (*E*)-Methyl 2-(2-amino-4-oxothiazol-5(4*H*)-ylidene)acetate (**3.5a**) (0.10 g, 0.54 mmol), DMAD (**3.4a**) (0.08 g, 0.5 mmol) and cycohexyl isocyanide (**3.19b**) (0.06 g, 0.54 mmol) were reacted to give **(***E*)-dimethyl 7-(cyclohexylamino)-2-(2-methoxy-2-oxoethylidene)-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidine-5,6-dicarboxylate (**3.20e**)**; Physical characteristics: orange solid**; Yield: 0.19 g, 79%; **Mp**: 174-176 ºC, **R**$_{\mathbf{f}}$: 0.9, hexane/ ethyl acetate(90% : 10%); **$^{1}$H NMR** (400 MHz, $CDCl_3$) δ 8.87 (1H, s, NH), 7.03 (1H, s, CH=C-S), 5.77 (1H, s, H-5), 4.06 (1H, s, C*H*-NH), 3.88 (3H, s, $OCH_3$), 3.75 (3H, s, $OCH_3$), 3.69 (3H, s, $OCH_3$), 1.95 -1.76 (5H, m, equatorial cyclohexyl $CH_2$ protons), 1.73 – 1.39 (5H, m, axial cyclohexyl $CH_2$ protons ), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 169.9 (C=O), 168.4 (C=O), 166.2 (C=O), 163.9 (C=O), 160.9 (C-7), 155.0 (C-9), 139.9 (C-2), 119.1 (*C*H=C-S), 70.3 (C-6), 53.2 ($OCH_3$), 53.0 ($OCH_3$), 52.5 ($OCH_3$), 50.9 (C-5), 49.9 (CH-NH), 34.3 (cyclohexyl $CH_2$), 33.7 (cyclohexyl $CH_2$), 25.6 (cyclohexyl $CH_2$), 24.8 (cyclohexyl $CH_2$), 24.7 (cyclohexyl $CH_2$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 2931, 2852, 1730, 1704, 1654, 1604, 1556, 1440, 1422, 1375; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{19}H_{24}N_3O_7S^+$ 438.1329, found 438.1359.

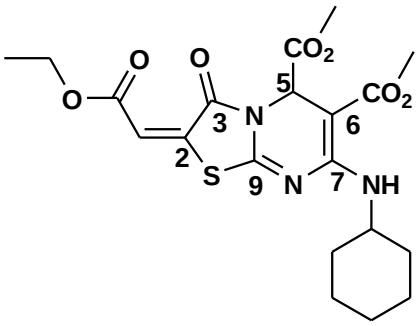
*8.3.2.6 (E)-Diethyl 7-(cyclohexylamino)-2-(2-methoxy-2-oxoethylidene)-3-oxo-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine-5,6-dicarboxylate (**3.20f**)*




**Method B:** (*E*)-Methyl 2-(2-amino-4-oxothiazol-5(4*H*)-ylidene)acetate (**3.5a**) (0.10 g, 0.54 mmol), DEtAD (**3.4b**) (0.09 g, 0.54 mmol) and cyclohexyl isocyanide (**3.19b**) (0.06 g, 0.54 mmol) were reacted to give (*E*)-diethyl 7-(cyclohexylamino)-2-(2-methoxy-2-oxoethylidene)-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidine-5,6-dicarboxylate (**3.20f**); **Physical characteristics**: red solid; Yield: 0.198 g, 79%; **Mp**: 138-140 ºC, **R**$_{\mathbf{f}}$ : 0.9; **$^{1}$H NMR** (400 MHz, $CDCl_3$) δ 8.89 (1H, s, NH), 7.03 (1H, s, CH=C-S), 5.77 (1H, s, H-5), 4.09-4.04 (4H, m, 2 × O*C*$H_2$$CH_3$), 4.03 (1H, s, C*H*-NH), 3.88 (3H, s, $OCH_3$), 1.96 -1.59 (10H, m, 5 × cyclohexyl $CH_2$), 1.36 (3H, t, *J* = 7.2 Hz, C*H*$_3$$CH_2$), 1.29 (3H, t, *J* = 7.2 Hz, C*H*$_3$$CH_2$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 169.5 (C=O), 168.1 (C=O), 166.2 and 164.0 (C=O), 160.7 (C-7), 154.9 (C-9), 140.0 (C-2), 118.9 (*C*H=C-S), 70.5 (C-6), 62.2 (O*C*$H_2$$CH_3$), 59.4 (O*C*$H_2$$CH_3$), 53.0 ($CH_3$-O),

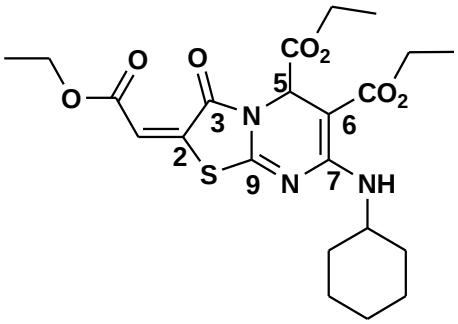
52.6 (C-5), 49.8 (*C*H-NH), 34.3 (cyclohexyl $CH_2$), 33.7 (cyclohexyl $CH_2$), 25.6 (cyclohexyl $CH_2$), 24.8 (cyclohexyl $CH_2$), 24.7 (cyclohexyl $CH_2$), 14.78 ($CH_3$), 14.13 ($CH_3$); **FT-IR** $\nu_{max}$**/cm$^{-1}$** 2930, 2853, 1729, 1699, 1643, 1622, 1606, 1572, 1434, 1370; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{21}H_{28}N_3O_7S^+$ 466.1642, found 466.1683.

*8.3.2.7 (E)-Dimethyl 7-(cyclohexylamino)-2-(2-ethoxy-2-oxoethylidene)-3-oxo-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine-5,6-dicarboxylate (**3.20g**)*



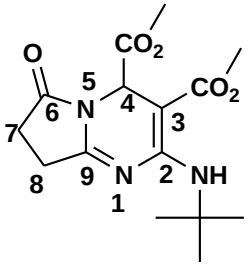

**Method B:** (*E*)-Ethyl 2-(2-amino-4-oxothiazol-5(4*H*)-ylidene)acetate (**3.5b**) (0.25 g, 1.25 mmol), DMAD (**3.4a**) (0.18 g, 1.25 mmol) and cyclohexyl isocyanide (**3.19b**) (0.14 g, 1.25 mmol) were reacted to give (*E*)-dimethyl 7-(cyclohexylamino)-2-(2-ethoxy-2-oxoethylidene)-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidine-5,6-dicarboxylate (**3.20 g**); **Physical characteristics:** orange solid; Yield: 0.47 g, 84%; **Mp**: 159-161 ºC, **$R_f$**: 0.8; **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.87 (1H, s, NH), 7.02 (1H, s, CH=C-S), 5.77 (1H, s, H-5), 4.33 (2H, q, *J* = 7.2 Hz, $OCH_2$), 4.06 (1H, s, C*H*-NH), 3.75 (3H, s, $OCH_3$), 3.69 (3H, s, $OCH_3$), 1.96 -1.60 (6H, m, from cyclohexyl $CH_2$ and $CH_2CH_3$), 1.37-1.25 (7H, m, cyclohexyl $CH_2$ and $CH_2CH_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 169.9 (C=O), 168.4 (C=O), 165.7 (C=O), 163.9 (C=O), 161.6 (C-7), 155.1 (C-9), 139.5 (C-2), 119.7 (*C*H=C-S), 70.3 (C-6), 62.3 ($CH_2$), 53.2 ($OCH_3$), 52.44 ($OCH_3$), 50.9 (C-5), 49.8 (CH-NH), 34.3 (cyclohexyl $CH_2$), 33.7 (cyclohexyl $CH_2$), 25.6 (cyclohexyl $CH_2$), 24.74 (cyclohexyl $CH_2$), 24.66 (cyclohexyl $CH_2$), 14.3 ($CH_3$); **FT-IR** $\nu_{max}$**/cm$^{-1}$**: 2928, 2861, 1728, 1697, 1648, 1603, 1572, 1443; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{20}H_{26}N_3O_7S^+$ 452.1486, found 452.1484.

*8.3.2.8 (E)-Diethyl 7-(cyclohexylamino)-2-(2-ethoxy-2-oxoethylidene)-3-oxo-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine-5,6-dicarboxylate (**3.20h**)*



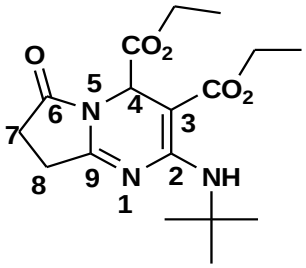

**Method B:** (*E*)-Ethyl 2-(2-amino-4-oxothiazol-5(4*H*)-ylidene)acetate (**3.5b**) (0.25 g, 1.25 mmol), DEtAD (**3.4b**) (0.21 g, 1.25 mmol) and cyclohexyl isocyanide (**3.19b**) (0.27 g, 1.25 mmol) were reacted to give (*E*)-diethyl 7-(cyclohexylamino)-2-(2-ethoxy-2-oxoethylidene)-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidine-5,6-dicarboxylate (**3.20h**); **Physical characteristics:** orange solid; Yield: 0.47 g, 80%; **Mp**: 132-134 ºC, $\mathbf{R_f}$ : 0.9; **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.88 (1H, s, NH), 7.03 (1H, s, CH=C-S), 5.77 (1H, s, H-5), 4.33 (2H, q, *J* = 7.2 Hz, C*H*$_3$CH$_2$), 4.25 (1H, s, C*H*-NH), 4.23-4.06 (4H, m, 2 × OC*H*$_2$CH$_3$), 1.96 -1.68 (5H, m, 5 × cyclohexyl $CH_2$ and $CH_3$ from $CH_2CH_3$), 1.59 -1.56 (8H, m, 5 X cyclohexyl $CH_2$), 1.38-1.34 (6H, m, 2 × $CH_3$ of $CH_2CH_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 169.5 (C=O), 168.1 (C=O), 165.7 (C=O), 164.1 (C=O), 160.8 (C-7), 155.0 (C-9), 139.7 (C-2), 119.5 (*C*H=C-S), 70.6 (C-6), 62.2 (O*C*H$_2$CH$_3$), 62.2 (O*C*H$_2$CH$_3$), 59.5 (O*C*H$_2$CH$_3$), 52.7 (C-5), 49.8 (CH-NH), 34.4 (cyclohexyl $CH_2$), 33.8 (cyclohexyl $CH_2$), 25.7 (cyclohexyl $CH_2$), 24.8 (cyclohexyl $CH_2$ ), 24.7 (cyclohexyl $CH_2$), 14.8 (*C*H$_3$CH$_2$O), 14.3 (*C*H$_3$CH$_2$O), 14.2 (*C*H$_3$CH$_2$O); **FT-IR $\nu_{max}$/cm$^{-1}$** 2931, 2855, 1730, 1698, 1648, 1610, 1573, 1434, 1369; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{22}H_{30}N_3O_7S^+$ 480.1799, found 480.1830.

*8.3.2.9 Dimethyl 2-(tert-butylamino)-6-oxo-4,6,7,8-tetrahydropyrrolo[1,2-a]pyrimidine-3,4-dicarboxylate (**3.21a**)*



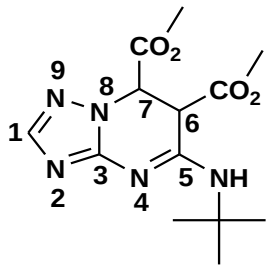
**Method B**: Pyrrolidine-2,5-diimine (**3.8**) (0.19 g, 1.9 mmol), DMAD (**3.4a**) (0.28 g, 1.9 mmol) and *tert*-butyl isocyanide (**3.19a**) (0.16 g, 1.9 mmol) were reacted to give dimethyl 2-(*tert*-butylamino)-6-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]pyrimidine-3,4-dicarboxylate (**3.21a**); **Physical characteristics**: yellow solid; Yield: 0.48 g, 80%; **Mp**:172-174 ºC **$R_f$**: 0.9; **$^1$H NMR** (400 MHz, $CDCl_3$) δ 9.08 (1H, s, NH), 5.59 (1H ,s, H-4), 3.70 (3H, s, $OCH_3$), 3.67 (3H, s, $OCH_3$), 3.01-2.68 (4H, m, $CH_2CH_2$), 1.43 (9H, s, $C(CH_3)_3$; **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 175.7 (C=O), 171.0 (C=O), 168.8 (C=O), 163.9 (C-2), 158.0 (C-9), 70.4 (C-3), 52.9 ($OCH_3$), 52.8 ($OCH_3$), 50.7 (N-*C*$(CH_3)_3$), 50.3 (C-4), 30.8 (C(*C*$H_3)_3$), 27.5 and 26.8 (C-7 and C-8); **FT-IR $\nu_{max}$/cm$^{-1}$**: 2959, 1747, 1636, 1581, 1440, 1421, 1274, 1244, 1177, 1115; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{15}H_{24}N_3O_6^+$ 342.1660 found 342.1683.

*8.3.2.10 Diethyl 2-(tert-butylamino)-6-oxo-4,6,7,8-tetrahydropyrrolo[1,2-a]pyrimidine-3,4-dicarboxylate (**3.21b**)*



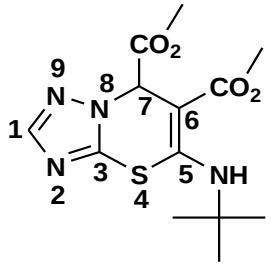

**Method B:** Pyrrolidine-2,5-diimine (**3.8**) (0.10 g, 1.03 mmol), DEtAD (**3.4b**) (0.18 g, 1.03 mmol) and *tert*-butyl isocyanide (**3.19a**) (0.09 g, 1.03 mmol) were reacted to give diethyl 2-(*tert*-butylamino)-6-oxo-4,6,7,8-tetrahydropyrrolo[1,2-a]pyrimidine-3,4-dicarboxylate (**3.21b**); **Physical characteristics**: yellow solid; Yield: 0.27 g, 75%; **Mp**:129-131 ºC, **$R_f$**: 0.9, hexane/ ethyl acetate (9 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 9.10 (1H, s, NH), 5.60 (1H, s, H-4), 4.24-4.04 (4H, m, 2 × OC*H*$_2$CH$_3$) 3.07-2.63 (4H, m, C*H*$_2$-C*H*$_2$), 1.44 (9H, s, $C(CH_3)_3$), 1.29 (3H, t, *J* = 7.2 Hz, C*H*$_3$CH$_2$O), 1.23 (3H, t, *J* = 7.2 Hz, C*H*$_3$CH$_2$O); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 175.9 (C=O), 170.6 (C=O), 168.5 (C=O), 163.7 (C-2), 157.93 (C-9), 70.6 (C-3), 61.8 (O*C*$H_2CH_3$), 59.2 (O*C*$H_2CH_3$), 52.8 (N-*C*$(CH_3)_3$), 50.4 (C-4), 30.9 (C(*C*$H_3)_3$), 27.5 and 26.8 (C-7 and C-8), 14.8 (*C*$H_3CH_2$), 14.2 (*C*$H_3CH_2$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 2988, 1758, 1633, 1589, 1428, 1365, 1330, 1296, 1276, 1241; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for 370.1973 $C_{17}H_{28}N_3O_6^+$ found 370.1995.

*8.3.2.11 Dimethyl 5-(tert-butylamino)-6,7-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine-6,7-dicarboxylate (**3.22a**)*



**Method B:** 1*H*-1, 2, 4-Triazol-5-amine (**3.11**) (0.11 g, 1.22 mmol), DMAD (**3.4a**) (0.18 g, 1.22 mmol) and *tert*-butyl isocyanide (**3.19a**) (0.10 g, 1.22 mmol) were reacted to give dimethyl 5-(*tert*-butylamino)-6,7-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidine-6,7-dicarboxylate (**3.22a**); **Physical characteristics**: brown solid; Yield: 0.12g, 30%; **Mp:** 176-178 ºC, **$R_f$:** 0.7, hexane-ethyl acetate (9 : 1); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 7.89 (1H, s, NH) 7.63 (1H, s, H-1), 5.53 (1H, s, H-7), 4.27 (1H, s, H-6), 3.68 (3H, s, $OCH_3$), 3.66 (3H, s, $OCH_3$), 1.38 (9H, s, $C(CH_3)_3$); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 168.0 (C=O), 167.4 (C=O), 156.5 (C-3), 155.3 (C-5), 150.4 (C-1), 56.2 (C-6), 53.4 ($OCH_3$), 53.3 ($OCH_3$), 52.4 (N-*C*$(CH_3)_3$), 45.4 (C-7), 27.92 ($C(CH_3)_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 2957, 2890, 1744, 1518, 1590, 1450, 1364; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{13}H_{20}N_5O_4^+$ 310.1510, found 310.1528.

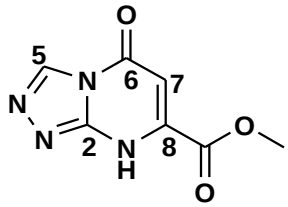
*8.3.2.12 Dimethyl 5-(tert-butylamino)-7H-[1,2,4]triazolo[5,1-b][1,3]thiazine-6,7-dicarboxylate ( **3.23a**)*



**Method B:** 1*H*-1,2,4-Triazole-5-thiol (**3.13**) (0.11 g, 1.14 mmol), DMAD (**3.4a**) (0.16 g, 1.14 mmol) and *tert*-butyl isocyanide (**3.19a**) (0.09g, 1.14 mmol) were reacted to give dimethyl 5-(*tert*-butylamino)-7*H*-[1,2,4]triazolo[5,1-*b*][1,3]thiazine-6,7-dicarboxylate (**3.23a**); **Physical characteristics**: white solid; Yield: 0.09 g, 24%; **Mp:** 162-164 ºC**, $R_f$:** 0.7, hexane/ ethyl acetate(9 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 9.88 (1H, s, NH), 7.99 (1H, s, H-1)), 6.45 (1H, s, H-7), 3.79 (3H, s, $OCH_3$), 3.72 (3H, s, $OCH_3$), 1.50 (9H, s, $C(CH_3)_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 168.5 (C=O), 167.4 (C=O), 155.9 (C-3), 152.8 (C-1), 144.8 (C-5) 86.1 (C-6), 57.6

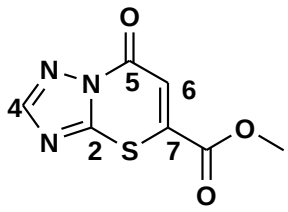
(C-7), 54.9 ($OCH_3$), 53.06 ($OCH_3$), 51.7 (N-*C*($CH_3$)$_3$), 30.7 (C(*C*$H_3$)$_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 2924, 2894, 1748, 1657, 1585, 1489, 1437; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{13}H_{19}N_4O_4S^+$ 327.1122, found 327.1125.

*8.3.2.13 Methyl 5-oxo-5,8-dihydro-[1,2,4]triazolo[4,3-a]pyrimidine-7-carboxylate (**3.22b**)*



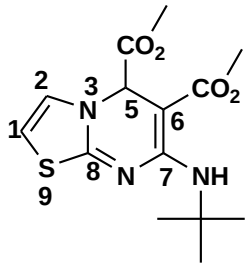

1*H*-1, 2, 4-Triazol-5-amine (**3.11**) (0.11 g, 1.22 mmol), DMAD (**3.4a**) (0.18 g, 1.22 mmol) and *tert*-butyl isocyanide (**3.19a**) (0.10g, 1.22 mmol) were reacted to give dimethyl 5-(*tert*-butylamino)-6,7-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidine-6,7-dicarboxylate (**3.22a**). **Method A. Physical characteristics**: yellow solid; Yield: 0.06 g, 25%; **Mp**: 160-162 ºC (Lit[104]. 218-222 ºC); **R$_f$** 0.5; **$^1$H NMR** (400 MHz, DMSO-$d_6$) 9.04 (1H, s, H-5), 7.44 (1H, s, NH), 6.67 (1H, s, H-7), 3.95 (3H, s, $OCH_3$) **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 161.1 (C=O), 160.2 (C=O), 149.0 ( C-2), 135.3 (C-5), 133.6 (C-8) 113.6 (C-7), 53.8 ($OCH_3$); **FTIR $\nu_{max}$/cm$^{-1}$**: 2970, 1732, 1691, 1628, 1426; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_7H_7N_4O_3^+$ 195.0513, found 195.0526.

*8.3.2.14 Methyl 7-oxo-7H-[1,2,4]triazolo[5,1-b][1,3]thiazine-5-carboxylate (**3.23b**)*




1*H*-1,2,4-Triazole-5-thiol (**3.13**) (0.11 g, 1.14 mmol), DMAD (**3.4a**) (0.16 g, 1.14 mmol) and *tert*-butyl isocyanide (**3.19a**) (0.09g, 1.14 mmol) were reacted to give dimethyl 5-(*tert*-butylamino)-7*H*-[1,2,4]triazolo[5,1-*b*][1,3]thiazine-6,7-dicarboxylate (**3.23b**). **Method A. Physical characteristics**: white solid ; Yield:0.06 g; 20% ; **Mp:** 185-187 ºC (Lit[105]. 184-185ºC); **R$_f$** 0.7; **$^1$H NMR** (400 MHz, DMSO-$d_6$) 8.69 (1H, s, H-4), 7.54 (1H, s, H-6), 3.98 (3H, s, O-$CH_3$) **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 161.5 (C=O), 155.3 (C=O), 153.7 (C-4), 151.5 (C-2), 139.3 (C-7) 122.4 (C-6), 54.7 ($OCH_3$); **FTIR $\nu_{max}$/cm$^{-1}$**: 2964, 1706, 1589, 1427, 1491; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_7H_6N_3O_3S^+$ 212.0124, found 212.0134.

*8.3.2.15 Dimethyl 7-(tert-butylamino)-5H-thiazolo[3,2-a]pyrimidine-5,6-dicarboxylate (**3.24**)*

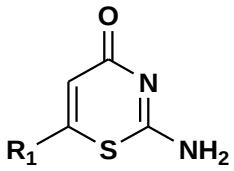



**Method B**: Thiazol-2-amine (**3.15**) (0.15 g, 1.51 mmol), DMAD (**3.4a**) (0.21 g, 1.51 mmol) and *tert*-butyl isocyanide (**3.19a**) (0.23 g, 1.51 mmol) were reacted to give dimethyl 7-(*tert*-butylamino)-5*H*-thiazolo[3,2-*a*]pyrimidine-5,6-dicarboxylate (**3.24**), **Physical characteristics**: yellow solid; Yield: 0.13. g, 27%; **Mp**: 179-181 ºC, $\mathbf{R_f}$: 0.6, hexane-ethyl acetate (8:2); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.30 (1H, d, *J* = 5.2 Hz, N-C*H*=CH), 5.93 (1H, d, *J* = 5.2 Hz, N-CH=C*H*), 4.84 (1H, s, NH), 4.23 (1H, s, H-5), 3.74 (3H, s, $OCH_3$), 3.72 (3H, s, $OCH_3$), 1.27 (9H, s, $C(CH_3)_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 170.4 (C=O), 168.6 (C=O), 162.3 (C-8), 141.0 (C-7), 123.0 (C-1), 101.3 (C-2), 70.3 (C-6), 61.2 (C–5), 53.4 and 52.9 (2 × $OCH_3$), 42.3 (N-*C*$(CH_3)_3$), 31.02 (C (*C*$H_3)_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 2964, 2880, 1732, 1724, 1682, 1624, 1460, 1440, **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{14}H_{20}N_3O_4S^+$ 326.1169, found 326.1134.

## 8.4 Synthetic methods: Chapter 4

*8.4.1 General method for the synthesis of 2-amino-4H-1,3-thiazin-4-one derivatives (**4.3a-e**)*

**Method A**




**4.3**

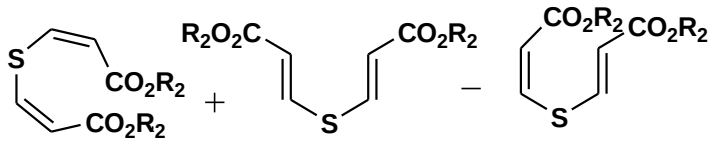
| 4.3 | $R_1$ | $R_2$ |
|---|---|---|
| 4.3b: | $CH_3$ | $CH_2CH_3$ |
| 4.3c: | $CH_2CH_3$ | $CH_2CH_3$ |
| 4.3d: | $CH_2CH_2CH_3$ | $CH_3$ |
| 4.3e: | Ph | $CH_2CH_3$ |

In a round bottomed flask equipped with a magnetic stirrer, mixtures of thiourea (**4.1**) (1eq.) and alkyl propiolate derivative (**4.2**) (1.5 eq.) in isopropanol (30 ml) were refluxed for 0.5 h-2 h, while monitoring by TLC. Reactions were then allowed to cool to room temperature, forming precipitates which were then filtered and washed with water and ether to obtain solid products **4.3b-e**.

**Method B**

2-Amino-4*H*-1,3-thiazin-4-one (**4.3a**) was prepared by introducing methyl propiolate to a mixture of thiourea and methanol at 0-5 ºC, as reported by Choudhary and Peddinti[108]. The mixture was left in a refrigerator for 8h to form a crude solid which was filtered, and washed with water to obtain the product (**4.3a**).

**Method C**




**4.3f1** *cis-cis* **4.3f2** *trans-trans* **4.3f3** *cis-trans*

*cis,cis-*, *cis,trans-*, *trans,trans*-Dimethyl 3,3'-thiodiacrylate **4.3f** was prepared by introducing methyl propiolate (**4.2a**) to a mixture of thiourea (**4.1**)and water or methanol at room

temperature, as reported by Choudhary and Peddinti.[108] The mixture was allowed to stir for 2h to form a crude solid which was filtered, and washed with water to obtain the product **4.1a2**.

*8.4.1.1 2-Amino-4H-1,3-thiazin-4-one (**4.3a**)*

Thiourea (**4.1**) (0.50 g, 5.24 mmol) and methyl propiolate (**4.2a**) (0.66 g, 3.93 mmol) were reacted to give 2-amino-4*H*-1,3-thiazin-4-one (**4.3a**) ; **Physical characteristics**: white solid; Yield: 0.42 g, 75%; **Mp**: 180-182 ºC (lit.[202] 179-181 ºC); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 8.20 (2H, br s, $NH_2$), 7.67 (1H, d, *J* = 10 Hz, Ar-H), 6.33 (1H, d, *J* = 10 Hz, Ar-H);**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 168.6 (C=O), 164.8 ( Ar-C), 134.5 (Ar-CH), 118.4 (Ar-CH).

*8.4.1.2 cis,cis-, cis,trans-, trans,trans-dimethyl 3,3'-thiodiacrylate **4.3f***

Thiourea (**4.1**) (1.00 g, 10.47 mmol) and methyl propiolate (**4.2a**) (1.32 g, 15.70 mmol) were reacted to give **4.3f**; **Physical characteristics:** white solid; Yield: 0.2 g, 95%; **Mp**: 138-140 ºC (lit.[108] 137-139 ºC); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 8.09 (1H, d, *J* = 15.6 Hz) 7.95 (0.95H, d, *J* = 10.4 Hz), 7.76 (2.66H, d, *J* = 10.4 Hz), 6.26 (0.92H, d, *J* = 15.6 Hz,), 6.11 (0.49H, s), 6.09 (1.28H s), 6.08 (0.45H, s) 3.68 (8.6H, s, $CH_3$), 3.67 (s, $CH_3$) ;**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 165.9, 165.8, 164.6, 148.9, 146.7, 145.2, 117.4, 114.7, 114.7, 51.5, 51.5, 51.4.

*8.4.1.3 2-Amino-6-methyl-4H-1,3-thiazin-4-one (**4.3b**)*

Thiourea (**4.1**) (3.00 g, 39.41 mmol) and ethyl 2-butynoate (**4.2b**) (6.63 g, 59.17 mmol) were reacted to give 2-amino-6-methyl-4*H*-1,3-thiazin-4-one (**4.3b**); **Physical characteristics**: white solid; Yield: 4.5 g, 83%; **Mp**: 209-2011 ºC (lit. [202]209-211 ºC); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 8.24 (2H, br s, $NH_2$), 7.68 (1H, s, Ar-H), 2.17 (3H, s, Ar-$CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 170.4 (C=O), 164.8 (Ar-C), 145.5 (Ar-C), 116.1 (Ar-CH), 21.7 ($CH_3$)

*8.4.1.4 2-Amino-6-ethyl-4H-1,3-thiazin-4-one (**4.3c**)*

Thiourea (**4.1**) (3.00 g, 39.41 mmol) and ethyl 2-pentynoate (**4.2c**) (7.46 g, 59.17 mmol) were reacted to give 2-amino-6-ethyl-4*H*-1,3-thiazin-4-one (**4.3c**); **Physical characteristics**: white solid; Yield: 5.31 g, 87%; **Mp**: 210-212 ºC; **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 8.24 (2H, br s, $NH_2$), 6.21 (1H, s, Ar-H), 2.47 (2H, q, *J* = 7.2 Hz, Ar-$CH_2$), 1.15 (3H, t, *J* = 7.2 Hz, $CH_2C\mathit{H}_3$);

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 170.5 (C=O), 164.7 ( Ar-C), 151.4 (Ar-C), 114.8 (Ar-CH), 28.7 ($CH_2$), 12.7 ($CH_3$); **FT-IR *ν*max/cm$^{-1}$**: 3248, 3045, 2979, 1617, 1489, 1459; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_6H_9N_2OS^+$ 157.0430 found 157.0433.

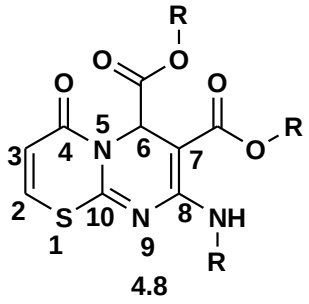
*8.4.1.5 2-Amino-6-propyl-4H-1,3-thiazin-4-one (**4.3d**)*

Thiourea (**4.1**) (3.00 g, 39.41 mmol) and methyl 2-hexynoate (**4.2d**) (7.46 g, 59.17 mmol) were reacted to give 2-amino-6-propyl-4*H*-1,3-thiazin-4-one (**4.3d**); **Physical characteristics**: yellow solid crystals; Yield: 5.31 g, 87%; **Mp**: 192-194 ºC; **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 8.24 (2H, br s, $NH_2$), 6.21 (1H, s, Ar-H), 2.45 (2H, t, *J* = 7.2 Hz, Ar-$CH_2$), 1.17 (2H, sextet, *J* = 7.2 Hz, C*H*$_2$CH$_3$), 0.90 (3H, t, *J* = 7.2 Hz, $CH_2$C*H*$_3$); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 170.4 (C=O), 164.8 (Ar-C), 149.7 (Ar-C), 115.7 (Ar-CH), 37.2 ($CH_2$-Ar), 21.2 ($CH_2$), 13.0 ($CH_3$); **FT-IR *ν*max/cm$^{-1}$**: 3264, 3049, 2964, 1615, 1489, 1416; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_7H_{11}N_2OS^+$ 171.0587 found 171.0586.

*8.4.1.6 2-Amino-6-phenyl-4H-1,3-thiazin-4-one (**4.3e**)*

Thiourea (**4.1**) (3.00 g, 39.41 mmol) and ethyl phenylpropiolate (4.**2e**) (10.29 g, 59.17 mmol) were reacted to give 2-amino-6-phenyl-4*H*-1,3-thiazin-4-one **(4.3e)**; **Physical characteristics**: white solid; Yield: 6.71 g, 84%; **Mp**: found 107-109 ºC, (lit.[202] 208-210 ºC); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 8.43 (2H, br s, $NH_2$), 7.65-7.60 (2H, m, Ar-H), 7.56-7.50 (3H, m, Ar-H), 6.71 (1H, d, *J* = 7.2, Ar-H), **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 170.4 (C=O), 164.2 (Ar-C), 145.9 (Ar-C), 134.8 (Ar-C), 130.8 (Ar-CH), 129.3 (Ar-CH), 126.1 (Ar-CH), 115.2 (Ar-CH).

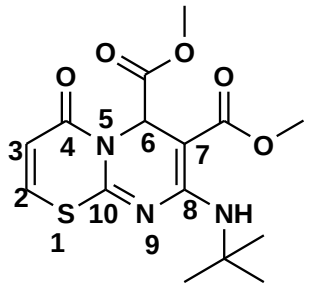
*8.4.2 General synthesis of 4-Oxo-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate derivatives (**4.8a-u**)*



**4.8**

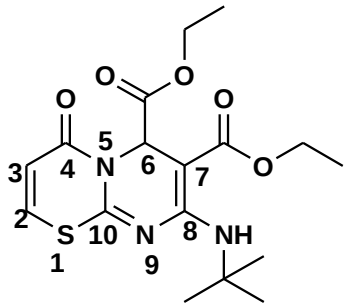
In a round bottomed flask equipped with a magnetic stirrer, isocyanide derivative (**4.4a-c**) in dry $CH_2Cl_2$ (2.5 ml) was slowly added to DMAD (**4.5a)** or DEtAD (**4.5b**) in dry $CH_2$ $Cl_2$ (5 ml) at 0 ºC under inert argon and the solution was allowed to stir for 0.5 h. 2-Amino-4*H*-1,3-thiazin-4-one derivatives **4.3a-e** in dry $CH_2Cl_2$ (2.5 ml) was slowly added to the solution and stirred at room temperature under argon for 6-24 h. The solvent was removed under vacuum and the residue purified by silica gel column chromatography using hexane-ethyl acetate as eluent to afford the products.

*8.4.2.1 Dimethyl 8-(tert-butylamino)-4-oxo-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8a**)*



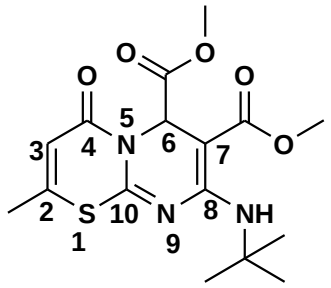

2-Amino-4*H*-1,3-thiazin-4-one (**4.3a**) (0.25 g, 1.95 mmol), *tert*-butyl isocyanide (**4.4a**) (0.16 g, 1.95 mmol) and dimethyl acetylenedicarboxylate (**4.5a**) (0.28 g, 1.95 mmol) were reacted to give dimethyl 8-(*tert*-butylamino)-4-oxo-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate (**4.8a**); **Physical characteristics**: yellow solid; Yield: 0.55 g, 81%; **Mp**: 145-147 ºC, $\mathbf{R_f}$ : 0.5, hexane/ethyl acetate (9 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.73 (1H, br s, NH), 7.36 (1H, d, *J* = 10.8 Hz, Ar-CH ), 6.51 (1H, d, *J* = 10.4 Hz, Ar-CH), 6.35 (1H, s, CH), 3.75 (3H, s, OC$H_3$), 3.71 (3H, s, OC$H_3$), 1.42 (9H, s, C(C$H_3$)$_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 170.9 (C=O), 168.4 (C=O), 160.7 (C=O), 157.5 (C-10), 154.9 (C-8), 136.8 (C-3), 118.2 (Ar-CH), 72.3 (C-6), 52.9 (C-7), 52.7 (O$CH_3$), 51.1 (O$CH_3$), 50.8(*C*(*C*H$_3$)$_3$), 30.9 (C(*C*H$_3$)$_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 2982, 2913, 1728, 1690, 1648, 1600, 1533, 1472, 1431; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{15}H_{20}N_3O_5S^+$ 354.1118 found 354.1113.

*8.4.2.2 Diethyl 8-(tert-butylamino)-4-oxo-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8b**)*



2-Amino-4*H*-1,3-thiazin-4-one (**4.3a**) (0.25 g, 1.95 mmol), *tert*-butyl isocyanide (**4.4a**) (0.16 g, 1.95 mmol) and diethyl acetylenedicarboxylate (**4.5b**) (0.33 g, 1.95 mmol) were reacted to give diethyl 8-(*tert*-butylamino)-4-oxo-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate (**4.8b**); **Physical characteristics**: yellow solid; Yield: 0.63 g, 85%; **Mp**: 143-145 ºC, $\mathbf{R_f}$ : 0.6, hexane/ethyl acetate (9 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.75 (1H, br s, NH), 7.34 (1H, d, *J* = 10.8 Hz, Ar-CH), 6.51 (1H, d, *J* = 10.4 Hz, Ar-CH), 6.34 (1H, s, CH), 4.31-4.11 (4H, m, 2 × $CH_2$), 1.43 (9H, s, $C(CH_3)_3$), 1.33 (3H, t, *J* = 7.2 Hz, $CH_3$), 1.26 (3H, t, *J* = 7.2 Hz, $CH_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 170.6 (C=O), 168.1 (C=O), 160.8 (C=O), 157.3 (C-10), 154.8 (C-8), 136.7 (C-3), 118.2 (Ar-CH), 72.5 (C-6), 61.9 (O-$CH_2$), 59.3 (O-$CH_2$), 52.6 (C-7),) 51.3($C(CH_3)_3$, 30.9 ($C(CH_3)_3$, 14.9($CH_3$), 14.2 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3067, 2983, 2864, 1728, 1690, 1647, 1599, 1534, 1431; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{17}H_{24}N_3O_5S^+$ 382.1431 found 382.1439.

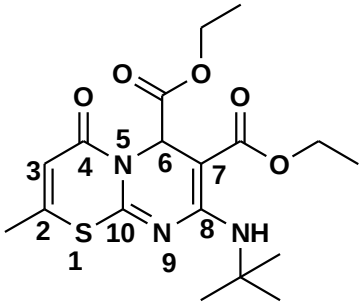
*8.4.2.3 Dimethyl 8-(tert-butylamino)-2-methyl-4-oxo-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8c**)*



2-Amino-6-methyl-4*H*-1,3-thiazin-4-one (**4.3b**) (0.25 g, 1.76 mmol), *tert*-butyl isocyanide (**4.4a**) (0.15 g, 1.76 mmol) and dimethyl acetylenedicarboxylate (**4.5a**) (0.25 g, 1.76 mmol) were reacted to give dimethyl 8-(*tert*-butylamino)-2-methyl-4-oxo-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate (**4.8c**); **Physical characteristics**: yellow solid; Yield: 0.53 g,

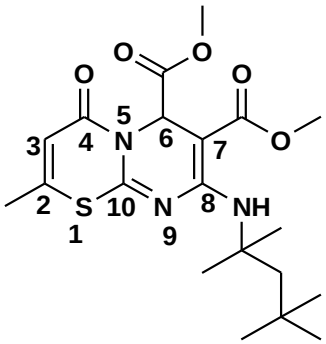
82%; **Mp**: 149-151 ºC, **$R_f$** : 0.5, hexane/ethyl acetate (9 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.63 (1H, br s, NH), 6.24 and 6.23 (2H, 2 × s, Ar-CH and H-6), 3.66 (3H, s, O-$CH_3$), 3.61 (3H, s, O-$CH_3$), 2.17 (Ar-$CH_3$), 1.33 (9H, s, C(C$H_3$)$_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 171.1 (C=O), 168.5 (C=O), 161.2 (C=O), 157.8 (C-10), 155.3 (C-8), 149.7 (C-2), 116.1 (C-3), 72.1 (C-6), 52.9 (C-7), 52.6 ($OCH_3$), 51.0 ($OCH_3$), 50.8 (*C*($CH_3$)$_3$), 30.8 (C(*C*$H_3$)$_3$) 22.1 (Ar-$CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 2992, 2963, 2902, 1734, 1648, 1682, 1653, 1602, 1525; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{16}H_{22}N_3O_5S^+$ 368.1275 found 368.1279.

*8.4.2.4 Diethyl 8-(tert-butylamino)-2-methyl-4-oxo-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8d**)*



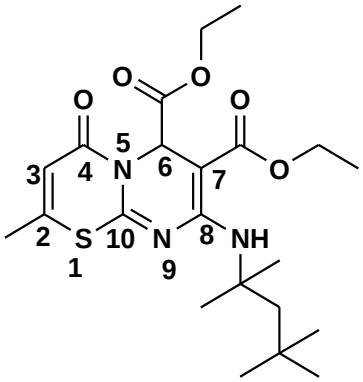

2-Amino-6-methyl-4*H*-1,3-thiazin-4-one **(4.3b)** (0.25 g, 1.76 mmol), *tert*-butyl isocyanide (**4.4a**) (0.15 g, 1.76 mmol) and diethyl acetylenedicarboxylate (**4.5b**) (0.30 g, 1.76 mmol) were reacted to give diethyl 8-(*tert*-butylamino)-2-methyl-4-oxo-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate (**4.8d**); **Physical characteristics**: yellow solid; Yield: 0.57 g, 83%; **Mp**: 135-137 ºC, **$R_f$** : 0.6, hexane/ethyl acetate (9 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.72 (1H, br s, NH), 6.28 (2H, 2 × s, Ar-CH and H-6), 4.27-4.06 (4H, m, 2 × $CH_2$), 2.22 (3H, Ar-$CH_3$), 1.39 (9H, s, C(C$H_3$)$_3$), 1.29 (3H, t, *J* = 7.2 Hz, C$H_3$), 1.22 (3H, t, *J* = 7.2 Hz, $CH_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 170.7 (C=O), 168.2 (C=O), 161.4 (C=O), 157.7 (C-10), 155.2 (C-8), 149.7 (C-2), 116.1 (C-3), 72.4 (C-6), 61.8 (O-$CH_2$), 59.2 (O-$CH_2$), 52.6 (C-7), 51.3 (N-*C*($CH_3$)$_3$), 30.9 (C(*C*$H_3$)$_3$, 22.1 (Ar-$CH_3$), 14.9 ($CH_3$), 14.2 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3285, 2975, 2890, 1735, 1690, 1643, 1600, 1535, 14377; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{18}H_{26}N_3O_5S^+$ 396.1588 found 396.1597.

*8.4.2.5 Dimethyl 2-methyl-4-oxo-8-((2,4,4-trimethylpentan-2-yl)amino)-4,6-dihydropyrimido [2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8e**)*



2-Amino-6-methyl-4*H*-1,3-thiazin-4-one (**4.3b**) (0.25 g, 1.76 mmol), 1,1,3,3-tetramethylbutyl isocyanide (**4.4b**) (0.25 g, 1.76 mmol) and dimethyl acetylenedicarboxylate (**4.5a**) (0.25 g, 1.76 mmol) were reacted to give dimethyl 2-methyl-4-oxo-8-((2,4,4-trimethylpentan-2-yl)amino)-4,6-dihydropyrimido [2,1-*b*][1,3]thiazine-6,7-dicarboxylate (**4.8e**); **Physical characteristics**: yellow solid; Yield: 0.56 g, 76%; **Mp**: 114-116 ºC, $\mathbf{R_f}$ : 0.6, hexane/ethyl acetate (9 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.74 (1H, br s, NH), 6.34 and 6.33 (2H, 2 × s, Ar-CH and H-6), 3.76 (3H, s, $OCH_3$), 3.69 (3H, s, $OCH_3$), 2.27 (3H, Ar-$CH_3$), 1.83 ($CH_2$), 1.48 and 1.46 (6H, 2 × s, $2CH_3$), 0.97 (9H, s, C($CH_3$)$_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 171.1 (C=O), 168.5 (C=O), 161.3 (C=O), 157.6 (C-10), 155.4 (C-8), 149.8, (C-2), 116.1 (C-3), 72.0 (C-6), 56.2 (*C*($CH_3$)$_3$), 53.3 ($CH_2$), 52.9 (C-7), 51.1 ($OCH_3$), 50.8 ($OCH_3$), 31.7 (*C*($CH_2$)$_2$), 31.8 (C(*C*$H_3$)$_3$), 31.6 ($CH_3$), 31.5 ($CH_3$), 22.1 (Ar-$CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3285, 2951, 2899, 1739, 1687, 1649, 1599, 1535, 1442; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{20}H_{30}N_3O_5S^+$ 424.1901 found 424.1906.

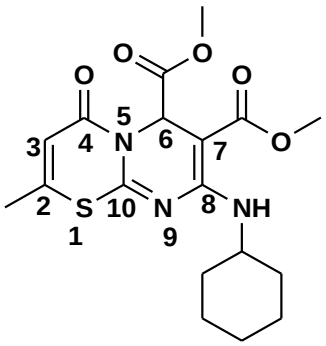
*8.4.2.6 Diethyl 2-methyl-4-oxo-8-((2,4,4-trimethylpentan-2-yl)amino)-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8f**)*



2-Amino-6-methyl-4*H*-1,3-thiazin-4-one (**4.3b**) (0.25 g, 1.76 mmol), 1,1,3,3-tetramethyl butyl isocyanide (**4.4b**) (0.25 g, 1.76 mmol) and diethyl acetylenedicarboxylate (**4.5b**) (0.30 g, 1.76

mmol) were reacted to give diethyl 2-methyl-4-oxo-8-((2,4,4-trimethylpentan-2-yl)amino)-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate (**4.8f**); **Physical characteristics**: yellow solid; Yield: 0.61 g, 77%; **Mp**: 112-114 ºC, **$R_f$** : 0.7, hexane/ethyl acetate (9: 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.73 (1H, br s, N*H*), 6.29 (2H, 2 × s, Ar-CH, H-6), 4.26-4.01 (4H, m, 2 × $CH_2O$) 2.23 (3H, s, Ar-$CH_3$), 1.80 (2H, q, *J* = 14.4 Hz, C*H*$_2$), 1.44 and 1.42 (6H, 2 × s, 2 × $CH_3$), 1.31 (3H, t, *J* = 6.8 Hz, $CH_3$), 1.21 (3H, t, *J* = 7.2 Hz, $CH_3$), 0.94 (9H, s, C(C*H*$_3$)$_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 170.7 (C=O), 168.2 (C=O), 161.4 (C=O), 157.4 (C-10), 155.3 (C-8), 149.6 (C-2), 116.1 (C-3), 72.3 (C-6), 61.8 (O-$CH_2$), 59.1 (O-$CH_2$), 56.2 (*C*-$(CH_3)_3$), 53.1 ($CH_2$), 51.3 (C-7), 31.6 (C-(*C*$H_3$)$_3$), 22.1 (Ar-$CH_3$), 14.9 (*C*$H_3CH_2$), 14.2 (*C*$H_3CH_2$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3285, 2975, 2890, 1735, 1690, 1643, 1600, 1535, 1477; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{22}H_{34}N_3O_5S^+$ 452.2214 found 452.2223.

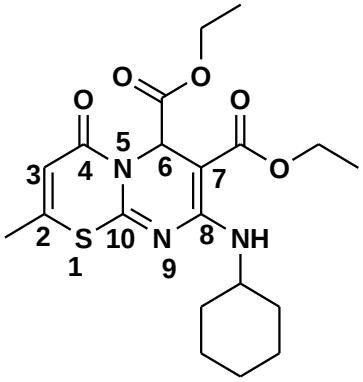
*8.4.2.7 Dimethyl8-(cyclohexylamino)-2-methyl-4-oxo-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8g**)*




2-Amino-6-methyl-4*H*-1,3-thiazin-4-one (**4.3b**) (0.25 g, 1.76 mmol), cyclohexyl isocyanide (**4.4c**) (0.19 g, 1.76 mmol) and dimethyl acetylenedicarboxylate (**4.5a**) (0.25 g, 1.76 mmol) were reacted to give Dimethyl 8-(cyclohexylamino)-2-methyl-4-oxo-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate (**4.8g**); **Physical characteristics**: yellow solid; Yield: 0.69 g, 84 %; **Mp**: 148-150 ºC, **$R_f$** : 0.4, hexane/ethyl acetate (9 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.52 (1H, br s, NH), 6.29 (2H, 2 × s, Ar-CH, H-6), 3.90 (1H, s N-CH), 3.72 (3H, s, $OCH_3$), 3.67 (3H, s, $OCH_3$), 2.23 ($CH_3$, s, Ar-$CH_3$), 1.93-1.70 (5H, m, cyclohexyl) 1.67-1.32 (5H, m, cyclohexyl); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 171.1 (C=O), 168.3 (C=O), 161.2 (C=O), 159.1 (C-10), 154.2 (C-8), 149.6 (C-2), 116.0 (C-3), 71.5 (C-6), 52.9 (O-$CH_3$), 51.2 (C-7), 50.7 (O-$CH_3$), 49.6 (CH-NH), 34.3 ($CH_2$, cyclohexyl), 33.7 ($CH_2$, cyclohexyl), 25.7 ($CH_2$, cyclohexyl), 24.8 ($CH_2$, cyclohexyl), 24.7 ($CH_2$, cyclohexyl), 22.1 (Ar-

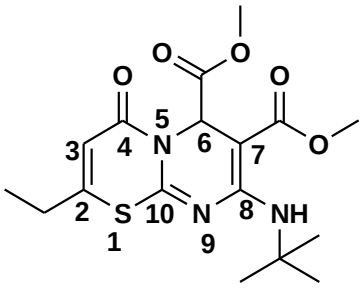
$CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3285, 2946, 2858, 1762, 1730, 1692, 1653, 1618, 1590, 1525, 1445; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{18}H_{24}N_3O_5S^+$ 394.1431 found 394.1433.

*8.4.2.8 Diethyl 8-(cyclohexylamino)-2-methyl-4-oxo-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8h**)*



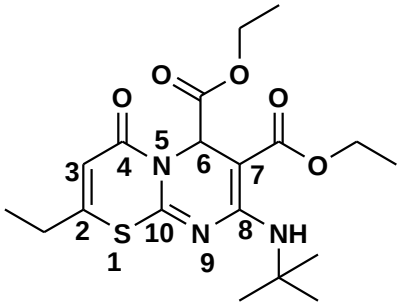

2-Amino-6-methyl-4*H*-1,3-thiazin-4-one (**4.3b**) (0.25 g, 1.76 mmol), cyclohexyl isocyanide (**4.4c**) (0.19 g, 1.76 mmol) and diethyl acetylenedicarboxylate (**4.5b**) (0.30 g, 1.76 mmol) were reacted to give diethyl 8-(cyclohexylamino)-2-methyl-4-oxo-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate (**4.8h**); **Physical characteristics**: yellow solid; Yield: 0.63 g, 85%; **Mp**: 170-172 ºC, **$R_f$** : 0.50, hexane/ethyl acetate (9 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.55 (1H, br s, NH), 6.28 (2H, 2 × s, Ar-CH, H-6), 4.27-4.09 (4H, m, 2 × $CH_2$), 3.92 (1H, s, C*H*NH), 2.23 (3H, s, Ar-$CH_3$), 1.93-1.69 (4H, m, cyclohexyl), 1.66-1.55 (4H, m, cyclohexyl), 1.41-1.33 (2H, m, cyclohexyl) 1.34 (3H, s, *J* = 7.2 Hz, $CH_3$), 1.28 (3H, s, *J* = 6.8 Hz, $CH_3$) ; **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 170.7 (C=O), 168.2 (C=O), 161.3 (C=O), 158.9 (C-10), 154.1 (C-8), 149.6 (C-2), 115.9 (C-3), 71.8 (C-6), 61.7 (O-$CH_2$), 59.1 (O-$CH_2$), 51.4 (C-7), 49.6 (CH), 34.3($CH_2$, cyclohexyl), 33.7 ($CH_2$, cyclohexyl), 25.7 ($CH_2$, cyclohexyl), 24.8 ($CH_2$, cyclohexyl), 24.7 ($CH_2$, cyclohexyl), 22.1 (Ar-$CH_3$), 14.9 ($CH_2CH_3$), 14.1 ($CH_2CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 2992, 2945, 2867, 1735, 1689, 1648, 1588, 1540, 1440; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{20}H_{28}N_3O_5S^+$ 422.1744 found 422.1712.

*8.4.2.9 Dimethyl 8-(tert-butylamino)-2-ethyl-4-oxo-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8i**)*



2-Amino-6-ethyl-4*H*-1,3-thiazin-4-one (**4.3c**) (0.25 g, 1.60 mmol), *tert*-butyl isocyanide (**4.4a**) (0.13 g, 1.60 mmol) and dimethyl acetylenedicarboxylate (**4.5a**) (0.22 g, 1.60 mmol) were reacted to give dimethyl 8-(*tert*-butylamino)-2-ethyl-4-oxo-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8i**); **Physical characteristics**: yellow solid; Yield: 0.51 g, 84%; **Mp**: 135-137 ºC, $\mathbf{R_f}$ : 0.48, hexane/ethyl acetate (9 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.70 (1H, br s, NH), 6.31 (2H, 2 × s, Ar-CH, H-6), 3.73 (3H, s, OC$H_3$), 3.68 (3H, s, OC$H_3$), 2.50 (2H, q, *J* = 7.2 Hz, Ar-$CH_2$), 1.40 (9H, s, C(C$H_3$)$_3$), 1.28 (3H, t, *J* = 7.6 Hz , $CH_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 171.2 (C=O), 168.5 (C=O), 161.5 (C=O), 157.9 ( C-10), 155.8 (C-8), 155.3 (C-2), 114.4 (C-3), 72.1 (C-6), 52.9 ($OCH_3$), 52.6 (*C*-($CH_3$)$_3$), 51.2 (C-7), 50.8 (O*C*$H_3$), 30.8 (C(*C*$H_3$)$_3$), 29.4 (*C*$H_2$CH$_3$), 12.5 ($CH_2$*C*$H_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3273, 2967, 2876, 1739, 1674, 1644, 1616, 1591, 1531, 1438; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{17}H_{24}N_3O_5S^+$ 382.1430 found 382.1427.

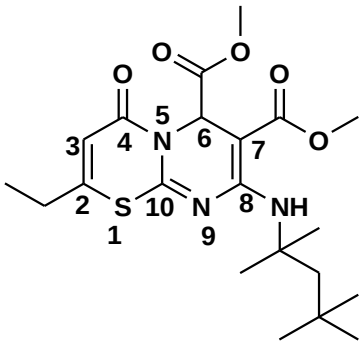
*8.4.2.10 Diethyl 8-(tert-butylamino)-2-ethyl-4-oxo-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8j**)*



2-Amino-6-ethyl-4*H*-1,3-thiazin-4-one (**4.3c**) (0.25 g, 1.60 mmol), *tert*-butyl isocyanide (**4.4a**) (0.13 g, 1.60 mmol) and diethyl acetylenedicarboxylate (**4.5b**) (0.27 g, 1.60 mmol) were reacted to give diethyl 8-(*tert*-butylamino)-2-ethyl-4-oxo-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate (**4.8j**); **Physical characteristics**: yellow solid; Yield: 0.52 g, 79%; **Mp**: 94-96 ºC, $\mathbf{R_f}$ : 0.6, hexane/ethyl acetate (9 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.72

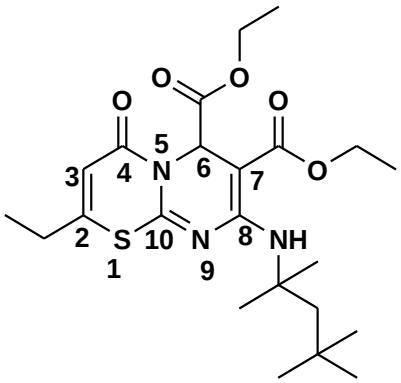
(1H, br s, NH), 6.30 and 6.28 (2H, 2 × s, Ar-CH, H-6), 4.27-4.09 (4H, m, 2 × $CH_2$), 2.50 (2H, q, *J* = 7.6 Hz, Ar-$CH_2$), 1.40 (9H, s, C(C*H*$_3$)$_3$), 1.29-1.26 (6H, m, 2 × $CH_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 170.7 (C=O), 168.2 (C=O), 161.6 (C=O), 157.8 (C-10), 155.7 (C-8), 155.2 (C-2), 114.4 (C-3), 72.31 (C-6), 61.8 (O-$CH_2$), 59.2 (O-$CH_2$), 52.5 (*C*($CH_3$)$_3$), 51.4 (C-7), 30.9 (C(*C*$H_3$)$_3$, 29.3 (Ar-$CH_2$), 14.9 ($CH_3$), 14.2 ($CH_3$), 12.5 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3285, 2975, 2890, 1735, 1690, 1643, 1600, 1535, 14377; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{19}H_{28}N_3O_5S^+$ 410.1744 found 410.1749.

*8.4.2.11 Dimethyl 8-((2,4-dimethylpentan-2-yl)amino)-2-ethyl-4-oxo-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8k**)*



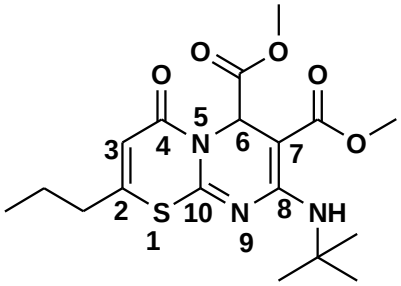

2-Amino-6-ethyl-4*H*-1,3-thiazin-4-one **(4.3c)** (0.25 g, 1.60 mmol), 1,1,3,3-tetramethylbutyl isocyanide (**4.4b**) (0.22 g, 1.76 mmol) and dimethyl acetylenedicarboxylate (**4.5a**) (0.23 g, 1.60 mmol) were reacted to give dimethyl 8-((2,4-dimethylpentan-2-yl)amino)-2-ethyl-4-oxo-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate (**4.8k**); **Physical characteristics**: yellow paste; Yield: 0.57 g, 81%, **R$_f$** : 0.5, hexane/ethyl acetate (9 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.64 (1H, br s, NH), 6.25 and 6.24 (2H, 2 × s, Ar-CH, H-6), 3.66 (3H, s, $OCH_3$), 3.59 (3H, s, $OCH_3$), 2.44 (2H, q, *J* = 7.6 Hz , Ar-$CH_2$), 1.73 ($CH_2$), 1.38 and 1.36 (6H, 2 × s, 2 × $CH_3$), 1.21 (3H, t, *J* = 7.2 Hz, $CH_3$C*H*$_2$), 0.87 (9H, s, C(C*H*$_3$)$_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 171.1 (C=O), 168.4 (C=O), 161.4 (C=O), 157.7 (C-10), 155.8 (C-8), 155.4 (C-2), 114.3 (C-3), 71.9 (C-6), 56.2 (*C*($CH_3$)$_3$), 53.3 ($CH_2$), 52.8 ($OCH_3$), 51.1 (C-7), 50.7 ($OCH_3$), 31.8 (C(*C*$H_3$)$_3$), 31.7, 31.6 (2 × $CH_3$) 29.3 (Ar-$CH_2$), 12.4 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3285, 2950, 2902, 1780, 1738, 1687, 1649, 1598, 1532; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{21}H_{32}N_3O_5S^+$ 438.2057 found 438.2021.

*8.4.2.12 Diethyl 8-((2,4-dimethylpentan-2-yl)amino)-2-ethyl-4-oxo-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8l**)*



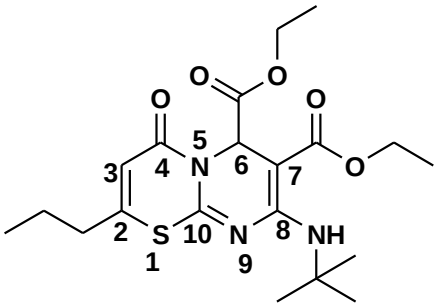
2-Amino-6-ethyl-4*H*-1,3-thiazin-4-one (**4.3c**) (0.25 g, 1.60 mmol), 1,1,3,3-tetramethylbutyl isocyanide (**4.4b**) (0.22 g, 1.60 mmol) and diethyl acetylenedicarboxylate (**4.5b**) (0.27 g, 1.60 mmol) were reacted to give Diethyl 8-((2,4-dimethylpentan-2-yl)amino)-2-ethyl-4-oxo-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate (**4.8l**); **Physical characteristics**: yellow solid; Yield: 0.61 g, 81%; **Mp**: 98-100 ºC, $\mathbf{R_f}$ : 0.6, hexane/ethyl acetate (9 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.73 (1H, br s, NH), 6.30 and 6.29 (2H, 2 × s, Ar-CH, H-6), 4.26-4.08 (4H, m, 2 × $CH_2O$), 2.50 (2H, q, *J* = 7.2 Hz, Ar-$CH_2$), 1.80 (2H, q, *J* = 14.8 Hz, $CH_2$), 1.45 and 1.42 (6H, 2 × s, $(CH_3)_2$-C), 1.31-1.25 (6H, m, 2 × $OCH_2CH_3$), 1.21 (3H, s, *J* = 7.2 Hz, $ArCH_2CH_3$), 0.94 (9H, s, $C(CH_3)_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 170.7 (C=O), 168.2 (C=O), 161.6 (C=O), 157.6 (C-10), 155.7 (C-8), 155.3 (C-2), 114.4 (C-3), 72.2 (C-6), 61.8 (O-$CH_2$), 59.1 (O-$CH_2$), 56.1 ($C(CH_3)_3$), 53.3 (Ar-$CH_2$), 51.4 (C-7), 31.7 ($C(CH_3)_2$), 29.3 (C-$(CH_3)_3$), 29.3 ($CH_2$), 14.9 ($CH_3$), 14.2 ($CH_3$), 12.5 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3270, 2973, 2913, 1737, 1688, 1646, 1600, 1535, 1432; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{23}H_{36}N_3O_5S^+$ 466.2370 found 466.2333.

*8.4.2.13 Dimethyl 8-(tert-butylamino)-4-oxo-2-propyl-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8m**)*



2-Amino-6-propyl-4*H*-1,3-thiazin-4-one (**4.3d**) (0.25 g, 1.47 mmol), *tert*-butyl isocyanide (**4.4a**) (0.12 g, 1.47 mmol) and dimethyl acetylenedicarboxylate (**4.5a**) (0.21 g, 1.47 mmol) were reacted to give dimethyl 8-(*tert*-butylamino)-4-oxo-2-propyl-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate (**4.8m**); **Physical characteristics**: yellow paste; Yield: 0.48 g, 84%, $\mathbf{R_f}$ : 0.5, hexane/ethyl acetate (9 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.72 (1H, br s, NH), 6.32 (2H, s, Ar-CH, H-6), 3.74 (3H, s, $OCH_3$), 3.69 (3H, s, $OCH_3$), 2.46 (2H, t, *J* = 7.6 Hz, Ar-$CH_2$), 1.71 (2H, sextet, *J* = 7.6 Hz, $CH_2$), 1.42 (9H, s, C($CH_3$)$_3$), 1.03 (3H, t, *J* = 7.2 Hz ,$CH_3CH_2$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 171.1 (C=O), 168.4 (C=O), 161.3 (C=O), 157.9 (C-10), 155.3 (C-8), 154.4 (C-2), 115.1 (C-3), 72.0 (C-6), 52.9 ($OCH_3$), 52.6 ($C(CH_3)_3$), 51.1 (C-7), 50.8 ($OCH_3$), 37.9 ($C(CH_3)_3$), 30.8 (Ar-$CH_2$), 21.6 ($CH_3CH_2$) 13.4 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3282, 2960, 2916, 1739, 1685, 1647, 1597, 1567, 1531, 1441; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{18}H_{26}N_3O_5S^+$ 396.1588 found 396.1545.

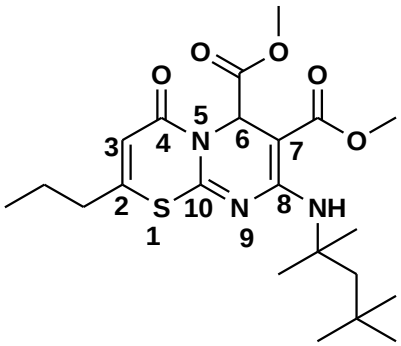
*8.4.2.14 Diethyl 8-(tert-butylamino)-4-oxo-2-propyl-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8n**)*




2-Amino-6-propyl-4*H*-1,3-thiazin-4-one (**4.3d**) (0.25 g, 1.47 mmol), *tert*-butyl isocyanide (**4.4a**) (0.12 g, 1.47 mmol) and diethyl acetylenedicarboxylate (**4.5b**) (0.25 g, 1.60 mmol) were reacted to form diethyl 8-(*tert*-butylamino)-4-oxo-2-propyl-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate (**4.8n**); **Physical characteristics**: yellow paste; Yield: 0.53 g, 85 %, $\mathbf{R_f}$ : 0.56, hexane/ethyl acetate (9: 1) ; **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.74 (1H, br s, NH), 6.30 (2H, s, Ar-CH, H-6), 4.29-4.13 (4H, m, 2 × $CH_2$), 2.46 (2H, t, *J* = 7.6 Hz, Ar-$CH_2$), 1.71 (2H, sextet, *J* = 7.2 Hz, $CH_2$), 1.42 (9H, s, C($CH_3$)$_3$), 1.34-1.32 (6H, m, 2 × $CH_3$), 1.32 (3H, t, *J* = 7.2 Hz, $CH_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 170.7 (C=O), 168.2 (C=O), 161.5 (C=O), 157.8 (C-10), 155.2 (C-8), 154.3 (C-2), 115.2 (C-3), 72.3 (C-6), 61.7 (O-$CH_2$), 59.1 (O-$CH_2$), 52.5 ($C(CH_3)_3$), 51.3 (C-7), 38.0 (Ar-$CH_2$), 30.8 ($C(CH_3)_3$), 21.6 ($CH_3CH_2$), 14.9, 14.1 and 13.4 (3 × $CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3285, 2975, 2910, 2870, 1737, 1687, 1646, 1600, 1535,

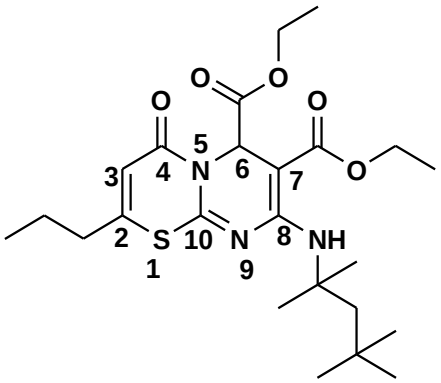
1432; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{20}H_{30}N_3O_5S^+$ 424.1901 found 424.1873.

*8.4.2.15 Dimethyl 4-oxo-2-propyl-8-((2,4,4-trimethylpentan-2-yl)amino)-4,6-dihydropyrimido [2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8o**)*



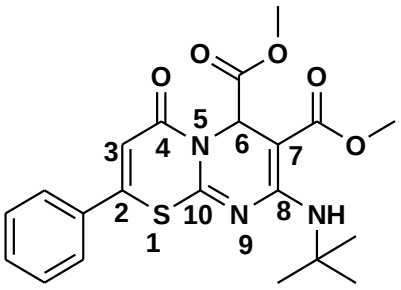

2-Amino-6-propyl-4*H*-1,3-thiazin-4-one (**4.3d**) (0.25 g, 1.47 mmol), 1,1,3,3-tetramethylbutyl isocyanide (**4.4b**) (0.21 g, 1.47 mmol) and dimethyl acetylenedicarboxylate (**4.5a**) (0.21 g, 1.47 mmol) were reacted to give dimethyl 4-oxo-2-propyl-8-((2,4,4-trimethylpentan-2-yl)amino)-4,6-dihydropyrimido [2,1-*b*][1,3]thiazine-6,7-dicarboxylate (**4.8o**); **Physical characteristics**: yellow paste; Yield: 0.52 g, 79%, $\mathbf{R_f}$ : 0.5, hexane/ethyl acetate (9: 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.64 (1H, br s, NH), 6.24 (2H, 2 × s, Ar-CH, H-6), 3.65 (3H, s, $OCH_3$), 3.59 (3H, s, $OCH_3$), 2.38 (2H, t, $J$ = 7.6 Hz , Ar-$CH_2$), 1.73 (2H, s, C-$CH_2$), 1.64 (2H, sextet, $J$ = 7.2 Hz, $CH_3CH_2$), 1.38 and 1.36 (6H, 2 × s, 2 × $CH_3$), 0.94 (3H, $J$ = 7.2 Hz, $CH_3$), 0.87 (9H, s, $C(CH_3)_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 171.1 (C=O), 168.4 (C=O), 161.3 (C=O), 157.8 (C-10), 155.4 (C-8), 154.4 (C-2), 115.1 (C-3), 71.9 (C-6), 56.2 ($C(CH_3)_3$), 53.3 (Ar-$CH_2$), 52.8 ($OCH_3$), 51.1 (C-7), 50.7 ($OCH_3$), 37.9 (C-$CH_2$), 31.8 ($C(CH_3)_3$), 31.7, 31.6 (2 × $CH_3$) 21.6 ($CH_3CH_2$), 13.4 ($CH_2CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3279, 2951, 2873, 1739, 1686, 1648, 1598, 1598, 1532, 1442; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{22}H_{34}N_3O_5S^+$ 452.2214 found 452.2190.

*8.4.2.16 Diethyl 4-oxo-2-propyl-8-((2,4,4-trimethylpentan-2-yl)amino)-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8p**)*



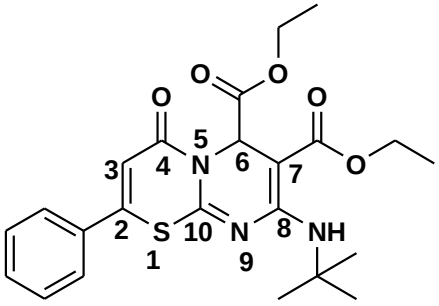

2-Amino-6-propyl-4*H*-1,3-thiazin-4-one (**4.3d**) (0.25 g, 1.47 mmol), 1,1,3,3-tetramethylbutyl isocyanide (**4.4b**) (0.21 g, 1.47 mmol) and diethyl acetylenedicarboxylate (**4.5b**) (0.25 g, 1.60 mmol) were reacted to give diethyl 4-oxo-2-propyl-8-((2,4,4-trimethylpentan-2-yl)amino)-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate (**4.8p**); **Physical characteristics**: yellow paste; Yield: 0.55 g, 79%, $\mathbf{R_f}$ : 0.6, hexane/ethyl acetate (9 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.75 (1H, br s, NH), 6.30 (2H,2 × s, Ar-CH, H-6), 4.29-4.09 (4H, m, 2 × C*H*$_2$CH$_3$), 2.46 (2H, t, *J* = 7.2 Hz, Ar-$CH_2$), 1.85-1.69 (2 × 2H, m, 2 × $CH_2$), 1.46 and 1.44 (6H, 2 × s, $(CH_3)_2$-C), 1.33 and 1.21 (6H, 2 × s, 2 x $CH_3$), 1.31 (3H, t, *J* = 6.8 Hz, $CH_3$), 1.22 (3H, t, *J* = 7.2 Hz, $CH_3$), 1.03 (3H, t, *J* = 7.2 Hz, $CH_3$), 0.96 (9H, s, C$(CH_3)_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 170.7 (C=O), 168.1(C=O), 161.5 (C=O), 157.6 (C-10), 155.3 (C-8), 154.3 (C-2), 115.1 (C-3), 72.1 (C-6), 61.7 (O-$CH_2$), 59.1 (O-$CH_2$), 56.1 (*C*$(CH_3)_3$), 53.2 (O-$CH_2$), 51.3 (C-7), 37.9 (Ar-$CH_2$), 31.6 (C(*C*$H_3)_3$), 21.6 ($CH_3CH_2$), 14.9 ($CH_3$), 14.1 ($CH_3$), 13.4 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3285, 2957, 2910, 2870, 1737, 1687, 1646, 1600, 1535, 1432; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{24}H_{38}N_3O_5S^+$ 480.2527 found 480.2526.

*8.4.2.17 Dimethyl 8-(tert-butylamino)-4-oxo-2-phenyl-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (4.8q)*



2-Amino-6-phenyl-4*H*-1,3-thiazin-4-one (**4.3e**) (0.25 g, 1.22 mmol), *tert*-butyl isocyanide (**4.4a**) (0.10 g, 1.22 mmol) and dimethyl acetylenedicarboxylate (**4.5a**) (0.17 g, 1.22 mmol) were reacted to give Dimethyl 8-(*tert*-butylamino)-4-oxo-2-phenyl-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate (**4.8q**); **Physical characteristics**: yellow solid; Yield: 0.40 g, 77%; **Mp**: 99-101 ºC, **$R_f$** : 0.58, hexane/ethyl acetate (9 : 1) ; **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.74 (1H, br s, NH), 7.59-7.48 (5H, m, Ar-H) 6.69 (1H, s, Ar-CH), 6.36 (1H, s, H-6), 3.75 (3H, s, $OCH_3$), 3.71 (3H, s, $OCH_3$), 1.43 (9H, s, $C(CH_3)_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 171.1 (C=O), 168.5 (C=O), 161.8 (C=O), 157.7 (C-10), 155.1 (C-8), 150.9 (C-2), 133.9 (Ar-C), 132.0 (Ar-C) , 129.6 (Ar-CH), 126.6 (Ar-CH), 126.5 (Ar-CH), 113.9 (C-3), 72.1 (C-6), 53.0 (C-7), 52.7 ($OCH_3$), 51.3 ($OCH_3$), 50.9 ($C(CH_3)_3$), 30.9 ($C(CH_3)_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 2977, 2841, 1735, 1683, 1582, 1448, 1370; **HRMS (ESI-TOF)** m/z: [M+H]+ Calculated for $C_{21}H_{24}N_3O_5S^+$ 430.1431 found 430.1445.

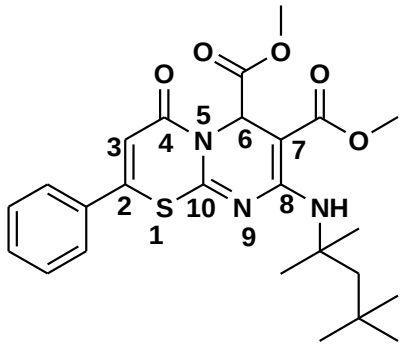
*8.4.2.18 Diethyl 8-(tert-butylamino)-4-oxo-2-phenyl-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8r**)*




2-Amino-6-phenyl-4*H*-1,3-thiazin-4-one (**4.3e**) (0.25 g, 1.22 mmol), *tert*-butyl isocyanide (**4.4a**) (0.10 g, 1.22 mmol) diethyl acetylenedicarboxylate (**4.5b**) (0.21 g, 1.22 mmol) were reacted to give diethyl 8-(*tert*-butylamino)-4-oxo-2-phenyl-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate (**4.8r**); **Physical characteristics**: yellow solid; Yield: 0.45 g, 80%; **Mp**: 145-147 ºC, **$R_f$** : 0.5, hexane/ethyl acetate (9 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.76 (1H, br s, NH), 7.59-7.47 (5H, m, Ar-H), 6.68 (1H, s, Ar-CH), 6.34 (1H, s, H-6), 4.29-4.10 (4H, m, 2 × $CH_2$), 1.43 (9H, s, $C(CH_3)_3$), 1.33-1.22 (6H, m, 2 × $CH_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 170.7 (C=O), 168.2 (C=O), 161.8 (C=O), 157.5 (C-10), 155.2 (C-8), 150.9 (C-2), 133.9 (Ar-C), 131.9 (Ar-C), 129.6 (Ar-C), 126.4 (ArCH), 113.9 (C-3), 72.4 (C-6), 61.9 (O-$CH_2$), 59.2 (O-$CH_2$), 52.6 (C-7),) 51.5 ($C(CH_3)_3$), 30.9 ($C(CH_3)_3$), 14.9 ($CH_3$), 14.2 ($CH_3$); **FT-**

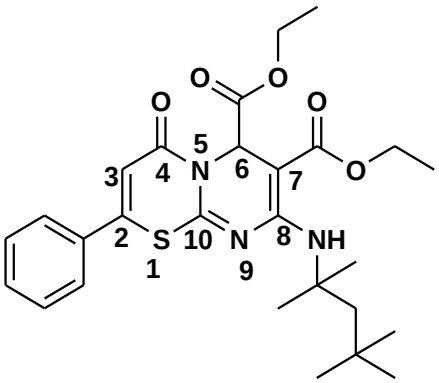
**IR $\nu_{max}$/cm$^{-1}$**: 3279, 2976, 2873, 1734, 1676, 1645, 1606, 1535, 1462; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{23}H_{28}N_3O_5S^+$ 458.1744 found 458.1744.

*8.4.2.19 Dimethyl 4-oxo-2-phenyl-8-((2,4,4-trimethylpentan-2-yl)amino)-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8s**)*



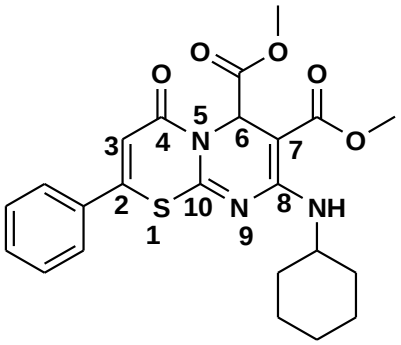

2-Amino-6-phenyl-4*H*-1,3-thiazin-4-one (**4.3e**) (0.25 g, 1.22 mmol), 1,1,3,3-tetramethylbutyl isocyanide (**4.4b**) (0.17 g, 1.22 mmol) and dimethyl acetylenedicarboxylate (**4.5b**) (3.(0.17 g, 1.22 mmol) were reacted to give dimethyl 4-oxo-2-phenyl-8-((2,4,4-trimethylpentan-2-yl)amino)-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate (**4.8s**); **Physical characteristics**: yellow solid; Yield: 0.45 g, 76%; **Mp**: 162-164 ºC, **$R_f$** : 0.4, hexane-ethyl acetate (9 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.77 (1H, br s, NH), 7.62-7.49 (5H, m, Ar-H), 6.71 (1H, s, Ar-CH), 6.39 (1H, s, H-6), 3.77 (3H, s, $OCH_3$), 3.71 (3H, s, $OCH_3$) 1.85 ($CH_2$), 1.50 and 1.48 (6H, 2 × s, 2 × $CH_3$), 0.98 (9H, s, C(C$H_3$)$_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 171.0 (C=O), 168.4 (C=O), 161.6 (C=O), 157.4 (C-10), 155.4 (C-8), 150.9 (C-2), 133.8(Ar-C) , 131.9 (Ar-CH) , 129.5 (Ar-CH), 126.4 (Ar-CH), 113.9 (C-3) , 71.2 (C-6), 56.2 (*C*($CH_3$)$_3$), 53.3 ($CH_2$), 52.9 (C-7), 51.3 and 50.7 (2 × O*C*$H_3$), 31.8 (*C*($CH_3$)$_2$), 31.7 (C(*C*$H_3$)$_3$), 31.6 ($CH_3O$), 31.5 ($CH_3O$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 2953, 2878, 1732, 1681, 1651, 1613, 1542, 1450; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{25}H_{32}N_3O_5S^+$ 486.2057 found 486.2044.

*8.4.2.20 Diethyl 4-oxo-2-phenyl-8-((2,4,4-trimethylpentan-2-yl)amino)-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8t**)*



2-Amino-6-phenyl-4*H*-1,3-thiazin-4-one (**4.3e**) (0.25 g, 1.22 mmol), 1,1,3,3-tetramethylbutyl isocyanide (**4.4b**) (0.17 g, 1.22 mmol) and dimethyl acetylenedicarboxylate (**4.5b**) (0.21 g, 1.22 mmol) were reacted to give Diethyl 4-oxo-2-phenyl-8-((2,4,4-trimethylpentan-2-yl)amino)-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate (**4.8t**); **Physical characteristics**: yellow solid; Yield: 0.52 g, 83 %; Mp: 88-90 ºC, $R_f$ : 0.5, hexane/ethyl acetate (9 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.00 (1H, br s, NH), 7.63-7.50 (5H, m, Ar-H), 6.72 (1H, s, Ar-CH), 6.37 (1H, s, H-6), .4.34-4.11 (4H, m, 2 × O-$CH_2$), 1.87 (2H, q, *J* = 14.8 Hz, $CH_2$), 1.50 and 1.48 (6H, 2 × s, 2 x $CH_3$), 1.34 (3H, t, *J* = 7.2 Hz, $CH_2CH_3$), 1.26 (3H, t, *J* = 6.8 Hz, $CH_2CH_3$), 0.98 (9H, s, $C(CH_3)_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 170.7 (C=O), 168.2 (C=O), 161.8 (C=O), 157.31 (C-10), 155.3 (C-8), 150.9 (C-2), 133.9 (Ar-C), 131.9 (Ar-CH), 129.6 (Ar-CH), 126.4 (Ar-CH), 113.9 (C-3), 72.2 (C-6), 61.8 (O-$CH_2$), 59.2 (O-$CH_2$), 56.2 ($C(CH_3)_3$), 53.3 (O-$CH_2$), 51.5 (C-6), 31.8 ($C(CH_3)_3$), 31.8 ($CH_3$), 31.7 ($CH_3$), 14.9 ($CH_3$), 14.2 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 2963, 2925, 2864, 1735, 1671, 1641, 1611, 1535, 1441; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{27}H_{36}N_3O_5S^+$ 514.2370 found 514.2350.

*8.4.2.21 Dimethyl 8-(cyclohexylamino)-4-oxo-2-phenyl-4,6-dihydropyrimido[2,1-b][1,3]thiazine-6,7-dicarboxylate (**4.8u**)*

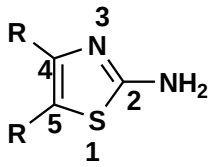


2-Amino-6-phenyl-*4H*-1,3-thiazin-4-one (**4.3e**) (0.25 g, 1.22 mmol), cyclohexyl isocyanide (**4.4c**) (0.13 g, 1.22 mmol) and dimethyl acetylenedicarboxylate (**4.5a**) (0.17 g, 1.22 mmol)

were reacted to give dimethyl 8-(cyclohexylamino)-4-oxo-2-phenyl-4,6-dihydropyrimido[2,1-*b*][1,3]thiazine-6,7-dicarboxylate (**4.8u**); **Physical characteristics**: yellow solid; Yield: 0.44 g, 79 %; Mp: 168-170 ºC, $R_f$ : 0.4, hexane/ethyl acetate (9 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.59 (1H, br s, NH), 7.63-7.51 (5H, m, Ar-H), 6.71 (1H, s, Ar-CH), 6.39 (1H, s, CH-6), 3.99 (1H, s, N-CH), 3.78 (3H, s, $OCH_3$), 3.73 (3H, s, $OCH_3$), 1.99-1.59 (6H, m, cyclohexyl), 1.45-1.24 (4H, m, cyclohexyl); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 171.1 (C=O), 168.4 (C=O), 161.7 (C=O), 159.0 (C-10), 154.2 (C-8), 150.9 (C-2), 133.9 (Ar-C), 132.0 (Ar-CH), 129.6 (Ar-CH), 126.4 (Ar-CH), 113.8 (C-3), 71.4 (C-6), 52.9 (O-$CH_3$), 51.5 (C-7), 50.8 (O-$CH_3$), 49.7 (*C*HNH), 34.3 ($CH_2$, cyclohexyl), 33.7 ($CH_2$, cyclohexyl), 25.7 ($CH_2$, cyclohexyl), 24.8 ($CH_2$, cyclohexyl), 24.7 ($CH_2$, cyclohexyl); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3282, 2918, 2846, 1740, 1677, 1651, 1604, 1533, 1444; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{23}H_{26}N_3O_5S^+$ 456.1588 found 456.1592.

## 8.5 Synthetic methods: Chapter 5

*8.5.1 Preparation of 2-Amino thiazole derivatives (**5.5a-c**)*




A solution of thiourea (**5.12**) and an appropriate α-haloketone (**5.13a-c**) in ethanol (25 ml), was refluxed for 12 h in a round-bottomed flask equipped with a magnetic stirrer. The reaction was monitored by TLC (hexane/ethyl acetate). The reaction mixture was filtered, washed with ethanol and dissolved in water (25 ml) and the solution was adjusted to pH 11 by adding sodium carbonate and extracted with dichloromethane (2 × 30 ml). The extracts were dried over sodium sulphate and evaporated under reduced pressure to obtain the product.

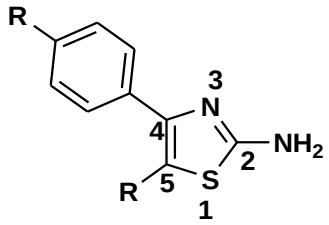
*8.5.1.1 4-Methylthiazol-2-amine (**5.7b**)*

Thiourea (**5.12**) (5.00 g, 66 mmol) and chloroacetone (**5.13b**) (7.00 g, 66 mmol) were reacted to give 4-methylthiazol-2-amine (**5.7b**); **Physical characteristics**: yellow oil; Yield: 6.8 g, 91%; **Mp**: 45-47 ºC (lit.[212] 46 ºC); **$^{1}$H NMR (**400 MHz, $CDCl_3$) δ 6.00 (1H, s, H-5), 5.53 (2H, s, $NH_2$), 2.16 (3H, s, $CH_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 168.1 (Ar-C), 148.3 (Ar-CH), 102.2 (Ar-CH), 17.1 ($CH_3$).

*8.5.1.2.3 4,5-Dimethylthiazol-2-amine (**5.7c**)*

Thiourea (**5.12**) (5.00 g, 66 mmol) and 3-chlorobutane-2-one (**5.13c**) (7.00 g, 66 mmol) were reacted to give 4,5-dimethylthiazol-2-amine (**5.7c**); **Physical characteristics**: Brown solid crystals; Yield: 6.9 g, 81%; **Mp**: 81-83 ºC (lit.[210] 80-81 ºC); **$^{1}$H NMR** (400 MHz, $CDCl_3$) δ 5.34 (2H, s, $NH_2$), 2.11 (3H, s, $CH_3$), 2.04 (3H, s, $CH_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 164.4 (Ar-C), 142.6 (Ar-C), 114.8 (Ar-C), 14.3 ($CH_3$), 11.0 ($CH_3$).

*8.5.2 Preparation of 4-phenylthiazol-2-amines derivatives (5.7d-h)*




To a solution of THF (30 ml) and phenacyl halide (**5.13d-h**) in a round-bottomed flask was added thiourea (**5.12**) and the mixture was stirred at room temperature for 0.5 h. The resulted precipitate was filtered, washed with THF and water and dried to obtain a solid product which was recrystallized from ethanol.

*8.5.2.1 4 4-Phenylthiazol-2-amine (**5.7d**)*

2-Bromoacetophenone (**5.13d**) (5.10 g, 26 mmol) and thiourea (**5.12**) (1.90 g, 26 mmol) were reacted to give 4-phenylthiazol-2-amine (**5.7d**); **Physical characteristics**: White solid crystals; Yield: 4.2 g, 92%; **Mp**: 151-153 ºC (lit.[214] 150-151 ºC); **$^{1}$H NMR** (400 MHz, DMSO-*d6*) δ 7.42-7.13 (2H, m, Ar-H), 7.51-7.42 (3H, m, Ar-H), 7.24 (1H, s, Ar-H), 4.03 (2H, br s, $NH_2$) , **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 170.2 (Ar-CH), 139.3 (Ar-CH), 129.4(Ar-CH), 129.0 (Ar-C), 128.9 (Ar-CH), 125.8 (Ar-CH), 102.9 (Ar-CH).

*8.5.2.2 4-(4-Methylphenyl)thiazol-2-amine (**5.7e**)*

2-Bromo-4′-methylacetophenone (**5.13e**) (5.00 g, 23 mmol) and thiourea (**5.12**) (2.01 g, 23 mmol) were reacted to give 4-(4-methylphenyl)thiazol-2-amine (**5.7e**); **Physical characteristics**: White solid; Yield: 3.71 g, 85%; **Mp**: 129-131 ºC (lit.[215] 130-132 ºC); **$^{1}$H NMR** (400 MHz, DMSO-*d6*) δ 7.61 (2H, d, *J* = 8.0 Hz, ArH), 7.29 (2H, d, *J* = 8.4 Hz, ArH), 7.19 (1H, s, Ar-H), 4.14 (2H, br s, $NH_2$), 2.33 (3H, s, $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 170.3 (Ar-C), 139.3 (Ar-C), 139.0 (Ar-C), 129.7 (Ar-CH), 125.9 (Ar-C), 125.7 (Ar-CH), 102.0 (Ar-CH), 20.9 ($CH_3$).

*8.5.2.3 4-(4-Fluorophenyl)thiazol-2-amine (**5.7f**)*

2-Chloro-4′-fluoroacetophenone (**5.13f**) (3.00 g, 17 mmol) and thiourea (**5.12**) (1.30 g, 17 mmol) were reacted to give 4-(4-fluorophenyl)thiazol-2-amine (**5.7f**); **Physical characteristics**: White solid crystals; Yield: 2.6 g, 79%; **Mp**: 99-101 ºC (lit.[214] 102-103 ºC);

**$^1$H NMR** (400 MHz, DMSO-*d6*) δ 7.86-7.83 (2H, m, ArH), 7.35-7.31 (2H, m, ArH), 7.21 (1H, s, ArH), 4.03 (2H, br s, $NH_2$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 170.3 (Ar-C), 162.4 (d, $J_{CF}$ = 246.5 Hz, Ar-C), 138.4 (Ar-C), 128.0 (d, $J_{CF}$ = 8 Hz, Ar-CH), 125.6 (d, $J_{CF}$ = 4.0 Hz, Ar-C), 116.0 (d, $J_{CF}$ = 22.1 Hz, Ar-CH), 102.4 (Ar-CH).

*8.5.2.4 4-(4-Chlorophenyl)thiazol-2-amine (**5.7h**)*

2-Bromo-4′-chloroacetophenone (**5.13h**) (5.11 g, 22 mmol) and thiourea (**5.12**) (2.01 g, 23 mmol) were reacted to give 4-(4-chlorophenyl)thiazol-2-amine (**5.7h**); **Physical characteristics**: White solid ; Yield: 4.01 g, 87%; **Mp**:202-204 ºC (lit.[215] 203-205 ºC);**$^1$H NMR** (400 MHz, DMSO-*d6*) δ 7.76 (2H, d, *J* = 8.8 Hz, ArH), 7.55 (2H, d, *J* = 8.4 Hz, ArH), 7.28 (1H, s, Ar-H), 3.96 (2H, br s, $NH_2$);**$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 170.0 (Ar-C), 133.8 (Ar-C), 129.1 (Ar-CH), 128.4 (Ar-C), 127.6 (Ar-CH), 103.6 (Ar-CH).

*8.5.2.5 4-(4-Methoxyphenyl)thiazol-2-amine (**5.7g**)*

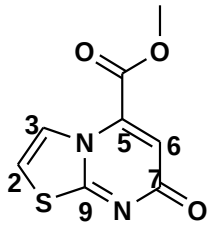
2-Bromo-4′-methoxyacetophenone (**5.13g**) (5.12 g, 22 mmol) and thiourea (**5.12**) (2.02 g, 23 mmol) were reacted to give 4-(4-methoxyphenyl)thiazol-2-amine (**5.7g**); **Physical characteristics**: White solid ; Yield: 3.94 g, 86%; **Mp**: 202-204 ºC (lit. [215] 200-203 ºC) ; **$^1$H NMR** (400 MHz, DMSO-*d6*) δ 7.66 (2H, d, *J* = 8.4 Hz, ArH), 7.09 (1H, s, Ar-CH), 7.04 (2H, d, *J* = 8.8 Hz, ArH), 3.97 (2H, br s, $NH_2$), 3.79 (3H, s, $OCH_3$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 170.3 (Ar-C), 160.2 (Ar-C), 138.9 (Ar-C), 127.4 (Ar-CH), 121.3 (Ar-CH), 114.5 (Ar-C), 100.8 (Ar-CH).

*8.5.3a Synthesis of methyl 7-oxo-7H-thiazolo[3,2-a]pyrimidine-5-carboxylate derivatives (**5.17a-n**). Method B (3-CR)*

In a round-bottomed flask equipped with a magnetic stirrer bar, thiourea (**5.12**) was added to a mixture of phenacyl α-halide/ α-haloketone (**5.13**), absolute ethanol (15 ml) and tetrahydrofuran (THF) (15 ml) and the reaction mixture was allowed to stir at room temperature for at least 15 minutes. After this time dimethyl acetylenedicarboxylate (DMAD) or diethyl acetylenedicarboxylate (DEtAD) (**5.16**) was slowly added over at least 10 minutes and the reaction mixture was heated at 80 ºC for 6 h-12 h while being monitored by TLC. After completion the reaction was cooled and the solvents were removed under reduced pressure to

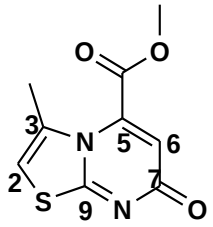
obtain a residue which was washed with cold ethanol and filtered to give solid products. Product **5.17l** was extracted by ethyl acetate.

*8.5.3.1 Methyl 7-oxo-7H-thiazolo[3,2-a]pyrimidine-5-carboxylate (**5.17a**)*



Thiourea (**5.12**) (0.31 g, 4.1 mmol), chloroacetaldehyde (**5.13a**) (0.50 g, 4.1 mmol) and DMAD (**5.16a**) (0.58g, 4.1mmol) were reacted to give methyl 7-oxo-7*H*-thiazolo[3,2-*a*]pyrimidine-5-carboxylate (**5.17a**); **Physical properties**: yellow solid; Yield: 0.75 g, 87%; **Mp**: 267-269 ºC, (Lit.[217] 268-273 ºC); **$R_f$** 0.5, hexane/ethyl acetate (2: 3); **$^1$H NMR** (400 MHz, DMSO-$d_6$) 8.30 (1H, d, *J* = 4.8 Hz, H-2 ), 7.36 (1H, d, *J* = 5.2 Hz, H-3 ), 6.75 (1H, s, H-6), 3.92 (3H, s, O-$CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 166.8 (C=O) , 165.9 (C=O), 160.9 (C-9), 136.5 (C-5), 124.6 (C-2), 114.3 (C-6), 109.9 (C-3), 53.7 (O-$CH_3$); **FTIR *ν*max/cm$^{-1}$**: 3076, 2958, 1727, 1621, 1571, 1479; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calc. for $C_8H_7N_2O_3S^+$ 211.0172 , found 211.0162.

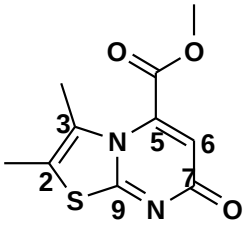
*8.5.3.2 Methyl 3-methyl-7-oxo-7H-thiazolo[3,2-a]pyrimidine-5-carboxylate (**5.17b**)*



Thiourea (**5.12**) (0.41 g, 5.4 mmol), chloroacetone (**5.13b**) (0.5 g, 5.4 mmol) and DMAD (**5.14a**) (0.77 g, 5.4 mmol) were reacted to give methyl 3-methyl-7-oxo-7*H*-thiazolo[3,2-*a*]pyrimidine-5-carboxylate (**5.17b**); **Physical properties**: yellow solid; Yield: 0.94 g, 78%; **Mp**: 156-159 ºC (lit.[218] 155-157 ºC); **$R_f$** 0.4, hexane/ethyl acetate (2 : 3); **$^1$H NMR** (400 MHz, DMSO-*d6*) 7.09 (1H, s, H-2), 6.53 (1H, s, H-6), 3.95 (3H, s, O-$CH_3$), 2.21 (3H, s, $OCH_3$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 166.7(C=O), 164.9 (C=O), 161.4 (C-9), 138.9 (C-5), 132.8 (C-

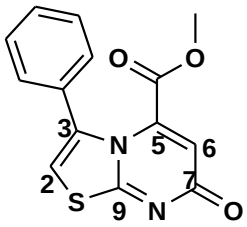
3), 111.9 (C-6), 107.1 (C-2), 54.0 (O-$CH_3$), 15.12 ($CH_3$); **FTIR *ν*max/cm$^{-1}$**: 3063, 1737, 1637, 1558, 1495, 1415; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calc. for $C_9H_9N_2O_3S^+$ 225.0328, found 225.0323.

*8.5.3.3 Methyl 2,3-dimethyl-7-oxo-7H-thiazolo[3,2-a]pyrimidine-5-carboxylate (**5.17c**)*




Thiourea (**5.12**) (0.36 g, 4.6 mmol), 3-chloro-2-butanone (**5.13c**) (0.50 g, 4.6 mmol) and DMAD (**5.17a**) ( 0.65 g, 4.6 mmol) were reacted to give methyl 2,3-dimethyl-7-oxo-7*H*-thiazolo[3,2-*a*]pyrimidine-5-carboxylate (**5.17c**); **Physical properties** brown solid; Yield: 0.89 g, 82%; **Mp**: 165-167 ºC; **R$_f$** 0.5, hexane/ethyl acetate (2 : 3); **$^1$H NMR** (400 MHz, DMSO-*d6*), 6.50 (1H, s, H-6), 3.95 (3H, s, O-$CH_3$), 2.28 (3H, s, $CH_3$), 2.10 (3H, s, $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 164.9 (C=O), 164.7 (C=O), 161.5 (C-9), 138.9 (C-5), 127.9 (C-2), 116.7 (C-3), 112.1 (C-6), 54.0 (O-$CH_3$), 12.6 ($CH_3$), 11.8 ($CH_3$); **FTIR *ν*max/cm$^{-1}$**: 2993, 1734, 1638, 1608, 1491, 1413; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calc. for $C_{10}H_{11}N_2O_3S^+$ 239.0485, found 239.0486.

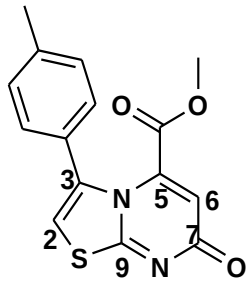
*8.5.3.4 Methyl 7-oxo-3-phenyl-7H-thiazolo[3,2-a]pyrimidine-5-carboxylate (**5.17d**)*




Thiourea (**5.12**) (0.19 g, 2.6 mmol), 2-bromoacetophenone (**5.13d**) (0.51 g, 2.6 mmol) and DMAD (**5.16a**) (0.37 g, 2.6 mmol) were reacted to give methyl 7-oxo-3-phenyl-7*H*-thiazolo[3,2-*a*]pyrimidine-5-carboxylate (**5.17d**); **Physical properties**:yellow solid; Yield: 0.55 g, 74%; **Mp**: 181-183 ºC; **R$_f$** 0.4, hexane/ethyl acetate (2 : 3); **$^1$H NMR** (400 MHz, DMSO-*d6*) 7.52-7.34 (5H, m, ArH), 7.34 (1H, s, H-2), 6.48 (1H, s, H-6), 3.13 (3H, s, O-$CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 166.8 (C=O), 165.34 (C=O), 160.6 (C-9), 138.8 (C-5), 136.5 (Ar-C), 130.4 (Ar-C), 129.6 (Ar-CH) , 128.9 (Ar-CH), 127.2 (ArCH), 113.1 (Ar-CH),

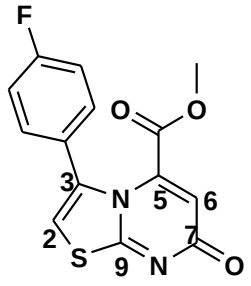
108.7 ( Ar-CH), 52.8 (O-$CH_3$); **FTIR *ν*max/cm$^{-1}$**: 3067, 1737, 1647, 1587, 1566, 1485; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calc. for $C_{14}H_{11}N_2O_3S^+$ 287.0485, found 287.0498.

*8.5.3.5 Methyl 7-oxo-3-(p-tolyl)-7H-thiazolo[3,2-a]pyrimidine-5-carboxylate (**5.17e**)*




Thiourea (**5.12**) (0.19 g, 2.4 mmol), 2-bromo-4-methylacetophenone (**5.17e**) (0.52 g, 2.4 mmol) and DMAD (**5.16a**) (0.34 g, 2.4 mmol) were reacted to form methyl 7-oxo-3-(*p*-tolyl)-7*H*-thiazolo[3,2-*a*]pyrimidine-5-carboxylate (**5.17e**); **Physical properties**: orange solid; Yield: 0.51 g, 71%; **Mp**: 176-178 ºC; **R$_f$** 0.4, hexane/ethyl acetate (2 : 3) ; **$^1$H NMR** (400 MHz, DMSO-*d$_6$*) 7.35-7.33 (2H, m, ArH), 7.30-7.27 (3H, m, ArH), 6.47 (1H, s, H-6), 3.15 (3H, s, O-$CH_3$), 2.35 (3H, s, $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 166.8 (C=O), 165.3 (C=O), 160.6 (C-9), 139.4 (C-5), 138.9 (Ar-C), 136.6 (Ar-C) , 129.4 (Ar-CH), 127.5 (Ar-C), 127.2 (ArCH), 113.01 (C-6), 108.14 (C-2), 52.8 (O-$CH_3$); **FTIR *ν*max/cm$^{-1}$**: 3041, 1742, 1639, 1587, 1509, 1485, 1416; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calc. for $C_{15}H_{13}N_2O_3S^+$ 301.0641 , found 301.0648.

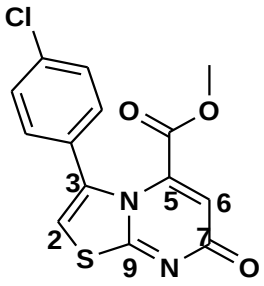
*8.5.3.6 Methyl 3-(4-fluorophenyl)-7-oxo-7H-thiazolo[3,2-a]pyrimidine-5-carboxylate (**5.17f**)*




Thiourea (**5.12**) (0.19 g, 2.3 mmol), 2-bromo-4-fluoroacetophenone (**5.13f**) (0.51 g, 2.3 mmol) and DMAD (**5.16a**) (0.33 g, 2.3 mmol) were reacted to give methyl 3-(4-fluorophenyl)-7-oxo-7*H*-thiazolo[3,2-a]pyrimidine-5-carboxylate (**5.17f**); **Physical properties**: yellow solid; Yield: 0.49 g, 70% ; **Mp**: 220-222 ºC; **R$_f$** 0.5, hexane/ethyl acetate (2 : 3); **$^1$H NMR** (400 MHz,

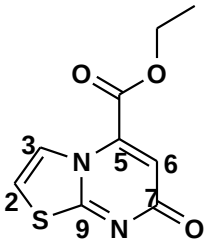
DMSO-*d6*) 7.56-7.53 (2H, m, ArH), 7.37-7.34 (3H, m, ArH), 6.49 (1H, s, H-6), 3.23 (3H, s, O-$CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 166.7 (C=O), 165.3 (C=O), 162.6 (d, $J_{CF}$ = 246 Hz, Ar-C), 161.3 (ArC), 160.6 (ArC), 138.7 (Ar-C) ,135.4 (ArC), 129.9 (d, $J_{CF}$ = 8.1 Hz, Ar-CH), 126.9 (d, $J_{CF}$ = 3.0 Hz, Ar-C), 115.9 (d, $J_{CF}$ = 22.1 Hz, Ar-CH), 113.1 (C-6), 109.0 (C-2), 52.9 (O-$CH_3$); **FTIR *ν*max/cm$^{-1}$**: 3058, 1742, 1640, 1581, 1489; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calc. for $C_{14}H_{10}FN_2O_3S^+$ 305.0391, found 305.0391.

*8.5.3.7 Methyl 3-(4-chlorophenyl)-7-oxo-7H-thiazolo[3,2-a]pyrimidine-5-carboxylate (**5.17g**)*




Thiourea (**5.12**) (0.16 g, 2.1 mmol), 2-bromo-4-chloroacetophenone (**5.13g**) (0.50 g, 2.1 mmol) and DMAD (**5.16a**) (0.30 g, 2.1 mmol)were reacted to give methyl 3-(4-chlorophenyl)-7-oxo-7*H*-thiazolo[3,2-*a*]pyrimidine-5-carboxylate (**5.17g**); **Physical properties**: yellow solid; Yield: 0.48 g, 71%; **Mp**: 200-202 ºC; **R$_f$** 0.8, hexane/ethyl acetate (2 : 3); **$^1$H NMR** (400 MHz, DMSO-*d6*) 7.58 (2H, d, *J*= 8.4 Hz, ArH), 7.50 (2H, d, *J*= 8.4 Hz, ArH), 7.39 (1H, s, H-2), 6.50 (1H, s, H-6), 3.23 (3H, s, O-$CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 166.8 (C=O), 165.3 (C=O), 160.6 (C-9), 138.6 (Ar-C), 135.2 (Ar-C), 134.3 (Ar-C), 129.3 (Ar-C), 129.1 (Ar-CH), 129.0 (Ar-CH) , 128.7 (Ar-CH), 127.4 (Ar-CH), 113.2 (C-6), 109.5 (C-2), 52.90 (O-$CH_3$); **FTIR *ν*max/cm$^{-1}$**: 3049, 1734, 1633, 1583, 1494, 1481; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calc. for $C_{14}H_{10}ClN_2O_3S^+$ 321.0095, found 321.0087.

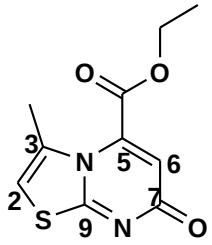
*8.5.3.8 Ethyl 7-oxo-7H-thiazolo[3,2-a]pyrimidine-5-carboxylate (**5.17h**)*




Thiourea (**5.12**) (0.31 g, 4.1 mmol), chloroacetaldehyde (**5.13a**) (0.50 g, 4.1 mmol) and DEtAD (**5.16b**) (0.69 g, 4.1 mmol).were reacted to give ethyl 7-oxo-7*H*-thiazolo[3,2-a]pyrimidine-5-

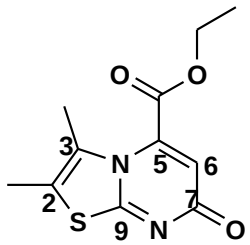
carboxylate (**5.17h**); **Physical properties**: yellow solid; Yield: 0.84 g, 91%; **Mp**: 180-182 ºC; **$R_f$** 0.4, hexane/ethyl acetate (2 : 3); **$^1$H NMR** (400 MHz, DMSO-*d6*) 8.30 (1H, d, *J* = 4.8 Hz, H-2), 7.36 (1H, d, *J* = 4.8 Hz, H-3), 6.76 (1H, s, H-6), 4.38 (2H, q, *J* = 6.8 Hz, O-$CH_2$), 1.34 (3H, t, *J* = 7.2 Hz, $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 166.8 (C=O), 166.0 (C=O), 160.3 (C-9), 136.6 (C-5), 124.6 (C-2), 114.3 (C-6), 110.0 (C-3), 62.9 (O-$CH_2$), 13.7 ($CH_3$); **FTIR *ν*max/cm$^{-1}$**: 3176, 3077, 2978, 1720, 1621, 1557, 1471; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calc. for $C_9H_9N_2O_3S^+$ 225.0328, found 225.0339.

*8.5.3.9 Ethyl 3-methyl-7-oxo-7H-thiazolo[3,2-a]pyrimidine-5-carboxylate (**5.17i**)*




Thiourea (**5.12**) (0.41 g, 5.4 mmol), chloroacetone (**5.13b**) (0.5 g, 5.4 mmol) and DEtAD (**5.16b**) (0.92 g, 5.4 mmol) were reacted to form ethyl 3-methyl-7-oxo-7*H*-thiazolo[3,2-*a*]pyrimidine-5-carboxylate (**5.17i**); **Physical properties**: brown solid; Yield: 1.1 g, 86%; **Mp**: 150-152 ºC; **$R_f$** 0.5, hexane/ethyl acetate (2 : 3); **$^1$H NMR** (400 MHz, DMSO-*d6*) 7.10 (1H, s, H-2), 6.53 (1H, s, H-6), 4.38 (2H, q, *J* = 6.8 Hz, O-$CH_2$), 2.23 (3H, s, $CH_3$) 1.34 (3H, t, *J* = 6.8 Hz, $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 166.8 (C=O), 165.0 (C=O), 160.9 (C-9), 139.2 (Ar-C), 132.9 (Ar-C), 111.9 (C-6), 107.1 (C-2), 63.5 ($CH_2$), 15.3 ($CH_3$) 13.6 ($CH_3$); **FTIR *ν*max/cm$^{-1}$**: 2973, 1731, 1634, 1493; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calc. for $C_{10}H_{11}N_2O_3S^+$ 239.0485, found 239.0497.

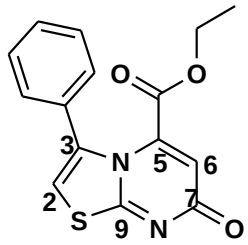
*8.5.3.10 Ethyl 2,3-dimethyl-7-oxo-7H-thiazolo[3,2-a]pyrimidine-5-carboxylate (**5.17j**)*




Thiourea (**5.12**) (0.36 g, 4.6 mmol), 3-chloro-2-butanone (**5.13c**) (0.50 g, 4.6 mmol) and DEtAD (**5.16b**) (0.78 g, 4.6 mmol) were reacted to give ethyl 2,3-dimethyl-7-oxo-7*H*-

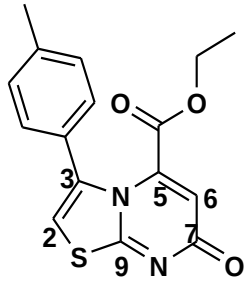
thiazolo[3,2-*a*]pyrimidine-5-carboxylate (**5.17j**); **Physical properties**: brown solid; Yield: 0.94 g, 81%; **Mp**: 153-155 ºC; **$R_f$** 0.5, hexane/ethyl acetate (2 : 3); **$^1$H NMR** (400 MHz, DMSO-*d6*), 6.5 (1H, s, H-6), 4.40 (2H, q, *J* = 5.2 Hz, $CH_2$), 2.28 (3H, s, $CH_3$), 2.12 (3H, s, $CH_3$), 1.33 (3H, t, *J* = 7.6 Hz, $CH_2CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 165.0 (C=O), 164.7 (C=O), 161.0 (C-9), 139.2 (Ar-C), 127.9 (Ar-C), 116.7 (Ar-C), 112.1 (C-6), 63.5 (O-$CH_2$), 13.6, 12.7, (2 × $CH_3$), 11.8 ($CH_2CH_3$) ; **FTIR *ν*max/cm$^{-1}$**: 2988, 1732, 1641, 1489, 1452; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calc. for $C_{11}H_{13}N_2O_3S^+$ 253.0641, found 253.0652.

*8.5.3.11 Ethyl 7-oxo-3-phenyl-7H-thiazolo[3,2-a]pyrimidine-5-carboxylate (**5.17k**)*



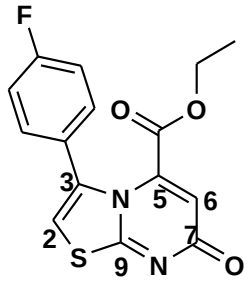

Thiourea (**5.12**) (0.19 g, 2.6 mmol), 2-bromoacetophenone (**5.13d**) (0.51 g, 2.6 mmol) and DEtAD (**5.16b**) ( 0.44 g, 2.6 mmol) were reacted to give ethyl 7-oxo-3-phenyl-7*H*-thiazolo[3,2-*a*]pyrimidine-5-carboxylate (**5.17k**); **Physical properties**: yellow solid; Yield: 0.63 g, 80%; **Mp**: 157-159 ºC; **$R_f$** 0.8, hexane/ethyl acetate (2 : 3); **$^1$H NMR** (400 MHz, DMSO-*d6*) 7.58-7.42 (5H, m, ArH), 7.45 (1H, s, H-2), 6.49 (1H, s, H-6), 3.49 (2H, q, *J* = 7.2 Hz, O-$CH_2$), 0.99 (3H, t, *J* = 7.2 Hz, $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 166.9 (C=O), 165.4 (C=O), 160.2 (C-9), 139.0 (Ar-C), 136.6 (Ar-C), 130.5 (Ar-C), 129.6 (Ar-CH) , 128.9 (Ar-CH), 127.2 (Ar-CH) , 125.8 (Ar-CH), 113.1 (Ar-CH), 108.8 (Ar-CH), 102.6 (Ar-CH), 62.6 (O-$CH_2$), 13.3 ($CH_3$); **FTIR *ν*max/cm$^{-1}$**: 3056, 1734, 1649, 1487; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calc. for $C_{15}H_{13}N_2O_3S^+$ 301.0641, found 301.0662.

*8.5.3.1 2 Ethyl 7-oxo-3-(p-tolyl)-7H-thiazolo[3,2-a]pyrimidine-5-carboxylate (**5.17l**)*



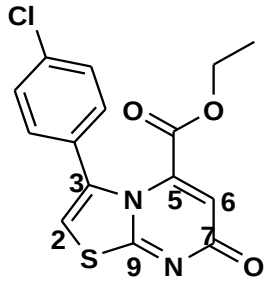
Thiourea (**5.12**) (0.19 g, 2.4 mmol), 2-bromo-4-methylacetophenone (**5.13e**) (0.52 g, 2.4 mmol) and DEtAD (**5.16b**) (0.41 g, 2.4 mmol) were reacted to form ethyl 7-oxo-3-(*p*-tolyl)-7*H*-thiazolo[3,2-*a*]pyrimidine-5-carboxylate (**5.17l**); **Physical properties**: yellow solid; Yield: 0.65 g, 79%; **Mp**: 155-157 ºC; **$R_f$** 0.9, hexane/ethyl acetate (2 : 3); **$^1$H NMR** (400 MHz, $CDCl_3$) 7.24-7.13 (4H, m, ArH), 6.74 (1H, s, H-2), 6.50 (1H, s, H-6), 3.56 (2H, q, *J* = 7.2 Hz O-$CH_2$), 2.33 (3H, s, $CH_3$) 1.02 (3H, t, *J* = 7.2 Hz, $CH_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 167.2 (C=O), 166.4 (C=O), 160.3 (C-9), 140.4 (Ar-C), 139.5 (Ar-C), 137.4 (Ar-C), 129.9 (Ar-CH), 127.5 (Ar-C) , 126.9 (Ar-CH), 113.9 (C-6), 107.9 (C-2), 63.0 ($CH_2$-O), 21.4 ($CH_3$), 13.5 ($CH_3$); **FTIR *ν*max/cm$^{-1}$**: 2924, 1731, 1643, 1587, 1489; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calc. for $C_{16}H_{15}N_2O_3S^+$ 315.0798, found 315.0824.

*8.5.3.13 Ethyl 3-(4-fluorophenyl)-7-oxo-7H-thiazolo[3,2-a]pyrimidine-5-carboxylate (**5.17m**)*




Thiourea (**5.12**) (0.19 g, 2.3 mmol), 2-bromo-4-fluoroacetophenone (**5.13f**) (0.51 g, 2.3 mmol) and DEtAD (**5.16b**) (0.39 g, 2.3 mmol) were reacted to form ethyl 3-(4-fluorophenyl)-7-oxo-7*H*-thiazolo[3,2-*a*]pyrimidine-5-carboxylate (**5.17m**); **Physical properties**: yellow solid, Yield 0.63 g, 86%; **Mp**: 180-182 ºC; **$R_f$** 0.5, hexane/ethyl acetate (2 : 3); **$^1$H NMR** (400 MHz, DMSO-*d6*) 7.58-7.52 (2H, m, ArH), 7.37-7.27 (3H, m, ArH), 6.49 (1H, s, H-6), 3.61 (2H, q, *J* = 7.2 Hz, O-$CH_2$) 1.04 (3H, t, *J* = 7.2 Hz, $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 166.8 (C=O), 165.4 (C=O), 162.6 (d, $J_{CF}$ = 246 Hz, Ar-C), 161.4 (Ar-C) , 160.2 (Ar-C), 138.9 (Ar-C), 135.4 (Ar-C), 129.9 (d, $J_{CF}$ = 9.1 Hz, Ar-CH), 128.0 (d, $J_{CF}$ = 8.1 Hz, Ar-CH), 126.9 (d, $J_{CF}$ = 4.0 Hz, Ar-CH), 115.9 (d, $J_{CF}$ = 22.2 Hz, Ar-CH) 113.1 (C-6) , 109.1 C-2), 62.7 ($CH_2$-O), 13.3 (O-$CH_3$); **FTIR *ν*max/cm$^{-1}$**: 3061, 1733, 1639, 1489; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calc. for $C_{15}H_{12}FN_2O_3S^+$ 319.0547, found 319.0565.

*8.5.3.14 Ethyl 3-(4-chlorophenyl)-7-oxo-7H-thiazolo[3,2-a]pyrimidine-5-carboxylate (**5.17n**)*



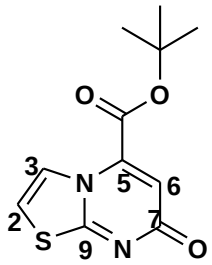

Thiourea (**5.12**) (0.16 g, 2.1 mmol), 2-bromo-4-chloroacetophenone (**5.13g**) (0.50 g, 2.1 mmol) and DEtAD (**5.16b**) ( 0.36 g, 2.1 mmol) were reacted to give ethyl 3-(4-chlorophenyl)-7-oxo-7*H*-thiazolo[3,2-*a*]pyrimidine-5-carboxylate (**5.17n**); **Physical properties**: yellow solid, Yield: 0.59 g, 84% ; **Mp**: 198-200 ºC; $\mathbf{R_f}$ 0.9, hexane/ethyl acetate (2 : 3); **$^1$H NMR** (400 MHz, DMSO-*d6*) 7.58 (2H, d, *J* = 8.4 Hz, Ar-H), 7.51 (2H, d, *J* = 8.4 Hz, Ar-H), 7.39 (1H, s, H-2), 6.51 (1H, s, H-6), 3.63 (2H, q, *J* = 7.2 Hz, O-$CH_2$), 1.03 (3H, t, *J* = 6.8, Hz, $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 166.8 (C=O), 165.3 (C=O), 160.1 (C-9), 138.7 (Ar-C), 135.3 (Ar-CH), 134.3 (Ar-C), 129.4 (Ar-C), 129.1 (Ar-CH), 128.9 (Ar-CH), 113.2 (C-6), 109.38 (C-2), 62.7 ($CH_2$-O), 13.2 ($CH_3$); **FTIR *ν*max/cm$^{-1}$**: 3057, 2986, 1733, 1640, 1580, 1501, 1482; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calc. for $C_{15}H_{12}ClN_2O_3S^+$ 335.0252, found 335.0267.

*8.5.4 Synthesis of Methyl 7-oxo-7H-thiazolo[3,2-a]pyrimidine-5-carboxylate derivatives (**5.5.1a-b**, **5.6a-b**, **5.17a-o** and **5.18a-b**) Method A (2-CR)*

**Method A**

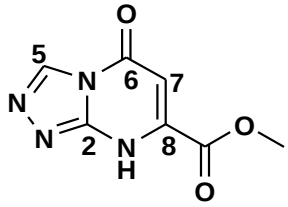
In a round bottomed flask equipped with magnetic stirrer bar, DMAD / DEtAD (**5.16**) was added to the mixture of 2-Aminothiazole derivative (**5.7a-h**) / 2-Imino-1,3-oxazolidine (**5.8**) / 1*H*-1,2,4-triazol-5-amine (**5.3**) /1*H*-1,2,4-triazole-5-thiol (**5.4**) and with ethanol. The reaction mixture was allowed to reflux for 12-15h.monitored by TLC. After completion the reaction was cooled and the solvents was removed under reduced pressure to obtain a residue which was washed with cold ethanol and filtered to give solid products.

*8.5.4.1 N-(tert-butyl)- 7-oxo-5H-thiazolo[3,2-a]pyrimidine-5-carboxylate (**5.17o**)*



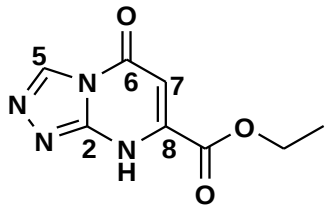

Thiazol-2-amine (**5.7a**) (0.20 g, 1.99 mmol), di-*tert*-butyl acetylenedicarboxylate (DTAD) (**5.16c**) (0.45 g, 1.99 mmol) were reacted to give *N*-(*tert*-butyl)-7-oxo-5H-thiazolo[3,2-*a*]pyrimidine-5-carboxylate (**5.17o**); **Physical characteristics**: yellow solid; Yield: 0.36 g, 72%; **Mp**:246-248 ºC; **$R_f$** 0.5, hexane/ethyl acetate (2 : 3) ; **$^1$H NMR** (400 MHz, DMSO-*d6*) 8.29 (1H, d, *J* = 4.4 Hz, H-2), 7.34 (1H, d, *J* = 5.2 Hz, H-3), 6.73 (1H, s, H-6), 1.57 (9H, s, $((CH_3)_3)$; **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 166.8 (C=O), 166.2 (C=O), 159.3 (C-9), 137.4 (C-5), 124.5 (C-2), 114.2 (C-6), 109.9 (C-3), 85.0 ($C(CH_3)_3$), 27.4 ($(CH_3)_3$); **FTIR *ν*max/cm$^{-1}$**: 2923, 1729, 1639; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{11}H_{13}N_2O_3S^+$ 253.0641, found 253.0642.

*8.5.4.2 Methyl 5-oxo-1,5-dihydro-[1,2,4]triazolo[4,3-a]pyrimidine-7-carboxylate (**5.5a**)*



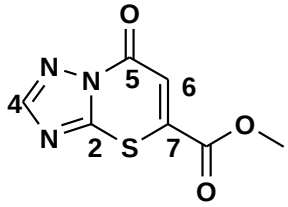

1*H*-1,2,4-Triazol-5-amine (**5.3**) (0.50 g, 6.0 mmol) and DMAD (**5.16a**) (0.85 g, 6.0 mmol) were reacted to give methyl 5-oxo-1,5-dihydro-[1,2,4]triazolo[4,3-a]pyrimidine-7-carboxylate (**5.5a**); **Physical characteristics**: yellow solid ; Yield: 1.1 g, 83%; **Mp**: 160-162 ºC (lit.[104] 218-222 ºC); **$R_f$** 0.5, ethyl acetate (100%); **$^1$H NMR** (400 MHz, DMSO-*d6*) 9.04 (1H, s, H-5), 7.44 (1H, s, NH), 6.67 (1H, s, H-7), 3.95 (3H, s, O-$CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 161.1 (C=O), 160.2 (C=O), 149.0 ( C-2), 135.3 (CH-5), 133.6 (C-8) 113.6 (CH-7), 53.8 (O-$CH_3$); **FTIR *ν*max/cm$^{-1}$**: 2970, 1732, 1691, 1628, 1426; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_7H_7N_4O_3^+$ 195.0513, found 195.0526.

*8.5.4.3 Ethyl 5-oxo-5,8-dihydro-[1,2,4]triazolo[4,3-a]pyrimidine-7-carboxylate (**5.5b**)*



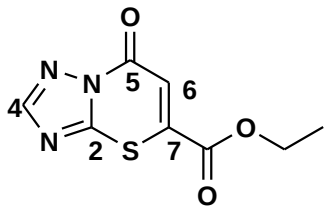
1*H*-1,2,4-triazol-5-amine (**5.3**) (0.50 g, 6.0 mmol) and DEtAD (**5.16b**) (1.02 g, 6.0 mmol) were reacted to give ethyl 5-oxo-5,8-dihydro-[1,2,4]triazolo[4,3-*a*]pyrimidine-7-carboxylate (**5.5b**); **Physical properties**: Brown solid: Yield: 1.1 g, 88%; **Mp**: 185-187 ºC; **$R_f$** 0.5, ethyl acetate (100%) ; **$^1$H NMR** (400 MHz, DMSO-*d6*) 8.91 (1H, s, H-5), 6.66 (1H, s, H-7), 4.50 (2H, q, *J* = 6.8 Hz, O-$CH_2$), 1.40 (3H, t, *J* = 7.2 Hz, $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 160.5 (C=O), 158.9 (C=O), 151.9 (C-5), 134.1 (Ar-C), 132.9 (Ar-C) 112.9 (C-7), 62.5 ($CH_2$), 12.9 ($CH_3$); **FTIR *ν*max/cm$^{-1}$**: 3150, 3068, 2909, 2731, 1722, 1680, 1610, 1579; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calc. for $C_8H_9N_4O_3^+$ 209.0669, found 209.0677.

*8.5.4.4 Methyl 5-oxo-5H-[1,2,4]triazolo[3,4-b][1,3]thiazine-7-carboxylate (**5.6a**)*



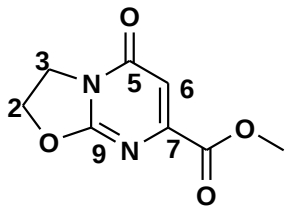
1*H*-1,2,4-Triazole-5-thiol (**5.4**) (0.50 g, 5.0 mmol) and DMAD (**5.16a**) (0.7 g, 5.0 mmol) were reacted to give methyl 5-oxo-5*H*-[1,2,4]triazolo[3,4-*b*][1,3]thiazine-7-carboxylate (**5.6a**); **Physical characteristics**: white solid ; Yield: 0.86 g, 89%; **Mp:** 185-187 ºC ( lit.[105] 184-185 ºC); **$R_f$** 0.7, hexane/ethyl acetate (1 : 9) ; **$^1$H NMR** (400 MHz, DMSO-*d6*) 8.69 (1H, s, H-4), 7.54 (1H, s, H-6), 3.98 (3H, s, O-$CH_3$) **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 161.5 (C=O), 155.3 (C=O), 153.7 (CH-4), 151.5 (C-2), 139.3 (C-7) 122.4 (CH-6), 54.7 (O-$CH_3$); **FTIR *ν*max/cm$^{-1}$**: 2964, 1706, 1589, 1427, 1491; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_7H_6N_3O_3S^+$ 212.0124, found 212.0134.

*8.5.4.5 7-Oxo-7H-[1,2,4]triazolo[5,1-b][1,3]thiazine-5-carboxylate (**5.6b**)*



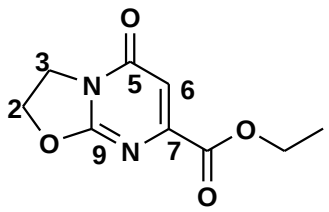

1*H*-1,2,4-Triazole-5-thiol (**5.4**) (0.50 g, 5.0 mmol) and DEtAD (**5.16b**) (0.85 g, 5.0 mmol). were reacted to give 7-oxo-7*H*-[1,2,4]triazolo[5,1-*b*][1,3]thiazine-5-carboxylate (**5.6b**); **Physical properties**: White solid; Yield: 0.92 g, 82%; **Mp**: 110-112 ºC; **$R_f$** 0.9 hexane/ethyl acetate (2 : 8); **$^1$H NMR** (400 MHz, DMSO-*d6*) 8.68 (1H, s, H-4), 7.54 (1H, s, H-6), 4.48 (2H, q, *J* = 7.2 Hz, O-$CH_2$), 1.38 (3H, t, *J* = 6.8 Hz, $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 160.9 (C=O), 155.3 (C=O), 153.7 (C-4), 151.56 (C-2), 139.6 (C-7) 122.2 (C-6), 64.1 ($CH_2$), 13.8 ($CH_3$); **FTIR *ν*max/cm$^{-1}$**: 3123, 3053, 2987, 1698, 1582, 1491; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calc. for $C_8H_8N_3O_3S^+$ 226.0281, found 226.0288.

*8.5.4.6 Methyl 5-oxo-3,5-dihydro-2H-oxazolo[3,2-a]pyrimidine-7-carboxylate (**5.18a**)*




2-Imino-1,3-oxazolidine **(5.8**) (0.51 g, 5.8 mmol) and DMAD (**5.16a**) (0.83 g, 5.8 mmol) were reacted to give methyl 5-oxo-3,5-dihydro-2*H*-oxazolo[3,2-*a*]pyrimidine-7-carboxylate (**5.18a**); **Physical properties**: White solid; Yield: 0.95 g, 82%; **Mp**: 251-253 ºC; **$R_f$** 0.5, ethyl acetate (100%); **$^1$H NMR** (400 MHz, DMSO-*d6*) 6.37 (1H, s, H-6) 4.67 (2H, t, *J* = 8.4 Hz, $CH_2$), 4.49 (2H, t, *J* = 8.4 Hz, $CH_2$), 3.87 (3H, s, O-$CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 170.9, (C=O) 162.1 (C=O), 160.8 (C-9), 138.1 (C-7), 110.9 (C-6), 66.6 ($CH_2$), 53.5 (O-$CH_3$), 47.03 ($CH_2$); **FTIR *ν*max/cm$^{-1}$**: 2965, 1732, 1624, 1535, 1476, 1421; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calc. for $C_8H_9N_2O_4^+$ 197.0557, found 197.0549.

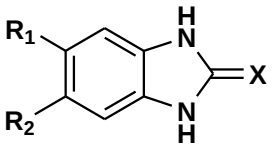
*8.5.4.7 Ethyl 5-oxo-3,5-dihydro-2H-oxazolo[3,2-a]pyrimidine-7-carboxylate (**5.18b**)*




2-Imino-1,3-oxazolidine (**5.8**) (0.51 g, 5.8 mmol) and DEtAD (**5.16b**) (0.98 g, 5.8 mmol) were reacted to give ethyl 5-oxo-3,5-dihydro-2*H*-oxazolo[3,2-*a*]pyrimidine-7-carbo×ylate (**5.18b**); **Physical properties**: White solid: Yield 0.98 g, 80%; **Mp**: 297-299 ºC; **$R_f$** 0.5, ethyl acetate (100%); **$^1$H NMR** (400 MHz, DMSO-*d6*) 5.64 (1H, s, H-6), 4.27 (2H, q, *J* = 7.2 Hz, O-$CH_2$), 4.04 (2H, t, *J* = 6.0 Hz, $CH_2$), 3.62 (2H, t, *J* = 5.6 Hz, $CH_2$) , 1.27 (3H, t, *J* = 7.2 Hz, $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-*d6*) δ 170.4 (C=O) 163.2 (C=O), 161.6 (C-9), 155.7 (C-7), 89.5 (C-6), 61.1 ($CH_2$), 58.5 ($CH_2$), 48.1 ($CH_2$), 13.8 ($CH_3$); **FTIR *ν*max/cm$^{-1}$**: 3125, 2958, 1700, 1698, 1662, 1642, 1637, 1381; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calc. for $C_9H_{11}N_2O_4^+$ 211.0713, found 211.0708.

## 8.6 Synthetic methods: Chapter 6

### *8.6.1 General procedure for the synthesis of 1H-Benzo[d]imidazol-2(3H)-one derivatives (**6.3a-k**)*



**6.3**

| 6.3: $R_1$ | $R_2$ | X |
|---|---|---|
| 6.3a: H | H | O |
| 6.3b: $CH_3$ | H | O |
| 6.3c: $CO_2CH_3$ | H | O |
| 6.3d: F | H | O |
| 6.3e: Cl | H | O |
| 6.3f: Br | H | O |
| 6.3g: H | H | S |
| 6.3h: H | H | N |
| 6.3i: $CO_2CH_3$ | H | S |
| 6.3j: $CH_3$ | $CH_3$ | O |
| 6.3k: Cl | Cl | O |

The synthesis was done following the earlier method report by Kadhim A. J.[227] In a round bottomed flask equipped with a magnetic stirrer bar, either urea (**6.2a**), thiourea (**6.2b**) or guanidine hydrochloride (**6.2c**) were reacted with a phenylenediamine derivative(**6.1**) and concentrated hydrochloric acid (HCl) in ethanol 30 ml by refluxing for 8h with TLC monitoring. The reaction was then allowed to cool to room temperature; forming a precipitate which was filtered and washed with isopropanol to obtain solid product.

#### *8.6.1.1 1H-Benzo[d]imidazol-2(3H)-one (**6.3a**)*

Urea (**6.2a**) (2.81 g, 46 mmol), *o*-phenylenediamine (**6.1a**) ( 5.01g, 46 mmol) and HCl (1.68 g, 46 mmol) were reacted to form 1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3a**); **Physical characteristics**: yellow solid; Yield: 5.21 g, 84%; **Mp**: 274-276 ºC (lit.[227] 275-279 ºC); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 6.62 (2H, d, *J* = 3.2 Hz, Ar-H), 6.53 (2H, s, Ar-H), 5.48 (2H, br s, N-H); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 159.7 (C=O), 132.9 (Ar-C), 119.3(Ar-CH), 116.8 (Ar-CH).

*8.6.1.2 Methyl-1H-benzo[d]imidazol-2(3H)-one (**6.3b**)*

Urea (**6.2a**) (2.46 g, 40.9 mmol), 3,4-diaminotoluene (**6.1b**) ( 5.02g, 40.9 mmol) and HCl (1.49 g, 40.9 mmol) were reacted to form 5-methyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3b**); **Physical characteristics**: brown solid; Yield: 5.2 g, 86%; **Mp**: 291-293 ºC (lit.[229] 292 ºC); **$^{1}$H NMR** (400 MHz, DMSO-$d_6$) δ 6.49 (1H, d, *J* = 7.2 Hz, Ar-H), 6.39 (1H, s, Ar-H), 6.25 (1H, d, *J* = 6.4 Hz, Ar-H), 5.48 (2H, br s, N-H), 2.07 (3H, s, $CH_3$) ; **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 159.8 (C=O), 135.2 (Ar-CH), 130.4 (Ar-C), 127.4 (Ar-C) , 118.2 (Ar-CH), 116.1 (Ar-C), 116.1 (Ar-CH), 20.5 ($CH_3$).

*8.6.1.3 Methyl 2-oxo-2,3-dihydro-1H-benzo[d]imidazole-5-carboylate (**6.3c**)*

Urea (**6.2a**) (1.81 g, 30.09 mmol), methyl 3,4-diaminobenzoate (**6.1c**) (5.01g, 30.09 mmol) and HCl (1.09g, 30.09 mmol) were reacted to form methyl 2-oxo-2,3-dihydro-1*H*-benzo[d]imidazole-5-carboxylate (**6.3c**); **Physical characteristics**: brown solid; Yield: 4.61 g, 80%; **Mp**: 311-313 ºC (lit.[230] 312-313 ºC); **$^{1}$H NMR** (400 MHz, DMSO-$d_6$) δ 7.57 (1H, s, Ar-H), 7.45 (1H, d, *J*= 8.4 Hz, Ar-H), 6.74 (1H, d, *J*= 8.4 Hz, Ar-H), 5.54 (2H, br s, N-H), 3.75 (3H, s, O-$CH_3$) ; **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 165.9 (C=O), 159.7 (C=O), 143.9 (Ar-C), 126.3 (Ar-CH), 123.2 (Ar-C), 121.7 (Ar-C), 117.3 (Ar-CH), 114.8 (Ar-CH), 51.5 (O-$CH_3$).

*8.6.1.4 5-Fluoro-1H-benzo[d]imidazol-2(3H)-one (**6.3d**)*

Urea (**6.2a**) (0.95 g, 15.86 mmol), 4-fluoro-1,2 phenylenediamine (**6.1d**) (2.01 g, 15.86 mmol) and HCl (0.59g, 15.86 mmol) were reacted to form 5-fluoro-1*H*-benzo*[d]*imidazol-2(3*H*)-one (**6.3d**); **Physical characteristics**: brown solid; Yield: 1.95 g, 81%; **Mp**: 299-301 ºC (lit.[231] 300 ºC); **$^{1}$H NMR** (400 MHz, DMSO-$d_6$) δ 6.71 (1H, t, *J* = 8 Hz, Ar-H), 6.42 (1H, d, *J* = 10.8 Hz, Ar-H), 6.27-6.22 (1H, m, Ar-H), 5.45 (2H, br s, N-H); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 159.7 (C=O), 157.9 (d, $J_{CF}$ = 233.4 Hz, Ar-C), 139.2 (d, $J_{CF}$ = 11.1 Hz, Ar-C), 124.6, 118.5 (d, $J_{CF}$ = 10.1 Hz, Ar-CH), 102.4 (d, $J_{CF}$ = 22.1 Hz, Ar-CH), 101.5 (d, $J_{CF}$ = 25.2 Hz, Ar-CH).

*8.6.1.5 5-Chloro-1H-benzo[d]imidazol-2(3H)-one (**6.3e**)*

Urea (**6.2a**) (0.84 g, 14 mmol), 4-chloro-1,2 phenylenediamine (**6.1e**) (2.02 g, 14 mmol) and HCl (0.51 g, 14 mmol) were reacted to form 5-chloro-1*H*-benzo[*d*]imidazol-2(3*H*)-one **(6.3e)**;

**Physical characteristics**: brown solid; Yield: 1.8 g, 75%; **Mp**: 323-325 ºC (lit.[229] 324-326 ºC); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 6.75-6.69 (2H, m, Ar-H), 6.53-6.50 (1H, m, Ar-H), 5.49 (2H, br s, N-H); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 159.7 (C=O), 137.5 (Ar-C), 128.6 (Ar-C), 124.4 (Ar-C), 118.8 (Ar-CH), 117.1 (Ar-CH), 114.9 (Ar-CH).

*8.6.1.6 5-Bromo-1H-benzo[d]imidazol-2(3H)-one (**6.3f**)*

Urea (**6.2a**) (0.66 g, 10.9 mmol), 4-bromo-1,2 phenylenediamine (**6.1f**) ( 2.01 g, 10.9 mmol) and HCl ( 0.39 g, 10.9 mmol) were reacted to form 5-bromo-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3f**); **Physical characteristics**: brown solid; Yield: 1.92 g, 83%; **Mp**: 335-337 ºC (lit.[232] 336-337 ºC); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 6.82 (1H, Ar-H), 6.65 (2H, d, $J$ = 9.6 Hz, Ar-H), 5.55 (2H, br s, N-H), **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 159.9 (C=O), 137.4 (Ar-C), 129.7 (Ar-C), 120.2 (Ar-CH), 119.0 (Ar-CH), 117.8 (Ar-CH), 111.8 (Ar-C).

*8.6.1.7 1H-Benzo[d]imidazole-2(3H)-thione (**6.3g**)*

Thiourea (**6.2c**) (3.51 g, 46.2 mmol), *o*-phenylenediamine (**6.1a**) (5.02 g, 46.2 mmol) and HCl (1.68 g, 46.2 mmol) were reacted to form 1*H*-benzo[*d*]imidazole-2(3*H*)-thione (**6.3g**); **Physical characteristics**: brown solid; Yield: 5.6 g, 81%; **Mp**: 115-117 ºC (lit.[227] 114-118-279 ºC); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 6.69 (2H, t, $J$ = 5.6 Hz, Ar-H), 6.58-6.56 (2H, m, Ar-H), 6.16 (2H, br s, N-H); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 183.9 (C=O), 132.7 (Ar-C), 119.6 (Ar-CH), 117.0 (Ar-CH).

*8.6.1.8 1H-Benzo[d]imidazol-2(3H)-imine (**6.3h**)*

Guanidine hydrochloride (**6.2b**) (4.39 g, 46 mmol), *o*-phenylenediamine (**6.1a**) ( 5.01 g, 46 mmol) and HCl ( 1.68 g, 46 mmol) were reacted to form 1*H*-benzo[*d*]imidazol-2(3*H*)-imine (**6.3h**); **Physical characteristics**: brown solid; Yield: 4.61 g, 75%; **Mp**:181-183 ºC (lit[228] 182-184 ºC); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 7.09 (2H, br s, N-H); 6.59-6.57 (2H, m, Ar-H), 6.45 (2H, t, $J$ = 3.6 Hz, Ar-H), 5.51 (H, br s, N-H); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 158.2 (C=O), 133.9 (Ar-C), 118.3 (Ar-CH), 115.6 (Ar-CH).

*8.6.1.9 Methyl 2-thioxo-2,3-dihydro-1H-benzo[d]imidazole-5-carboxylate (**6.3i**)*

Thiourea (**6.2c**) (2.29 g, 30.09 mmol), methyl 3,4-diaminobenzoate (**6.1c**) (5.01g, 30.09 mmol) and HCl (1.09g, 30.09 mmol) were reacted to form methyl 2-thioxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-5-carboxylate (**6.3i**); **Physical characteristics**: brown crystalline solid; Yield: 5.11 g, 82%; **Mp**: 285-287 ºC (lit.[233] 1284-287 ºC); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 7.59 (1H, s, Ar-H), 7.49 (1H, d, *J* = 8.4 Hz, Ar-H), 7.11 (2H, br s, N-H), 6.75 (1H, d, *J* = 8.4 Hz, Ar-H), 3.75 (3H,s, O-$CH_3$) ; **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 183.9 (C=S), 165.9 (C=O), 144.1 (Ar-C), 126.7 (Ar-C), 122.6 (Ar-C), 122.1 (Ar-CH), 117.3 (Ar-CH), 114.9 (Ar-CH), 51.5 (O-$CH_3$).

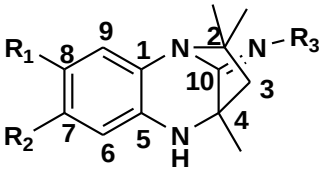
*8.6.1.10 5,6-Dimethyl-1H-benzo[d]imidazol-2(3H)-one (**6.3j**)*

Urea (**6.2a**) (0.88 g, 14.7 mmol), 4,5-dimethyl-1,2-phenylenediamine (**6.1.g**) (2.01 g, 14.7 mmol) and HCl (0.53 g, 14.7 mmol) were reacted to form 5,6-dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3j**); **Physical characteristics**: brown solid; Yield: 1.81 g, 76%; **Mp**: 394-396 ºC, (lit.[234] 395 ºC); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 6.46 (2H, s, Ar-H), 5.49 (2H, br s, N-H); 2.01 (6H, s, 2 × $CH_3$); $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 159.9 (C=O), 130.8 (Ar-C), 126.4 (Ar-C) 118.4 (Ar-CH), 18.69 (2 × $CH_3$).

*8.6.1.11 5,6-Dichloro-1H-benzo[d]imidazol-2(3H)-one (**6.3k**)*

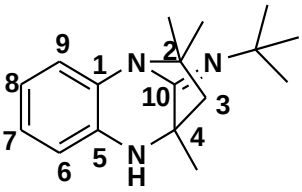
Urea (**6.2a**) (0.68 g, 11.3 mmol), 4,5-dichloro-*o*-phenylenediamine (**6.1h**) (5.01g, 11.3 mmol) and HCl (1.68 g, 11.3 mmol) were reacted to form 5,6-dichloro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3k**); **Physical characteristics**: black solid; Yield: 1.6 g, 70%; **Mp**: 397-399 ºC (lit.[233] 398 ºC); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 6.85 (2H, s, Ar-H), 5.49 (2H, br s, N-H); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 159.9 (C=O), 133.4 (Ar-C), 119.8 (Ar-C), 117.0 (Ar-CH).

*8.6.2 General procedure for the synthesis of 2-methyl-N-(2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[b][1,4]diazepin-10-ylidene)propan-2-amine derivatives (**6.8a-h**)*



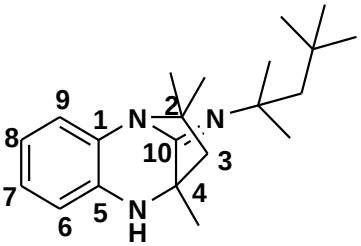
In a round bottomed flask equipped with a magnetic stirrer bar, a mixture of 1H-benzo[*d*]imidazol-2(3*H*)-one derivatives (**6.3a-l**), isocyanide derivative (**6.4**) and 2 equivalents of acetone/ acetone–D or 3-pentanone (**6.9**) was stirred at room temperature (rt) for 24 h, while being monitored by TLC. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using hexane-ethyl acetate as eluent to obtain the product.

*8.6.2.1 2-Methyl-N-(2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[b][1,4]diazepin-10-ylidene)propan-2-amine (**6.8a**)*



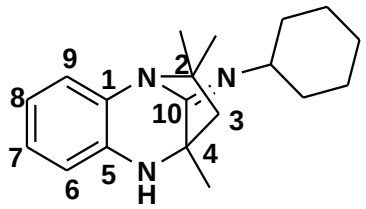

1*H*-Benzo[*d*]imidazol-2(3*H*)-imine (**6.3a**) (0.25 g, 1.87 mmol), *tert*-butyl isocyanide (**6.4a**) (0.17 g, 1.87 mmol) and acetone (**6.9a**) (0.22 g, 3.74 mmol) were reacted to give 2-methyl-*N*-(2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[*b*][1,4]diazepin-10-ylidene)propan-2-amine (**6.8a**); **Physical characteristics**: yellow crystalline solid; Yield: 0.43 g, 84%; **Mp**: 125-127 ºC, **R$_f$** : 0.8, hexane/ethyl acetate (8 : 2); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 6.94 (1H, t, *J* = 7.6 Hz, Ar-CH), 6.83 (1H, d, *J* = 7.6 Hz, Ar-CH), 6.67 (1H, t, *J* = 7.2 Hz, Ar-CH), 6.53 (1H, d, *J* = 8.0 Hz, Ar-CH), 3.90 (1H, br s, NH), 2.02 (1H, d, *J* = 13.2 Hz, $CH_2$), 1.78 (1H, d, *J* = 13.2 Hz, $CH_2$), 1.39 (3H, s, $CH_3$), 1.28 (9H, s, ($CH_3$)$_3$), 1.16 (3H, s, $CH_3$), 1.08 (3H, s, $CH_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 167.2 (C-10), 140.7 (Ar-C), 137.3 (Ar-C), 129.2 (Ar-CH), 126.7 (Ar-CH), 118.9 (Ar-CH), 116.5 (Ar-CH), 65.4, 61.1, 55.7, 53.4 (C-3), 32.5 ($CH_3$), 30.9 (($CH_3$)$_3$), 26.6 ($CH_3$), 21.3 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3257, 2922, 1696, 1596, 1469; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{17}H_{26}N_3^+$ 272.2121 found 272.2119.

*8.6.2.2 2,4,4-Trimethyl-N-(2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[b][1,4]diazepin-10-ylidene)pentan-2-amine (**6.8b**)*



1*H*-Benzo[d]imidazol-2(3*H*)-imine (**6.3a**) (0.25 g, 1.87 mmol ), 1,1,3,3-tetramethylbutyl isocyanide (**6.4b**) (0.26 g, 1.87 mmol) and acetone (**6.9a**) (0.22 g, 3.74 mmol) were reacted to give 2,4,4-trimethyl-*N*-(2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[*b*][1,4]diazepin-10-ylidene)pentan-2-amine (**6.8b**); **Physical characteristics**: brown paste; Yield: 0.49 g, 80%, $\mathbf{R_f}$ : 0.9, hexane/ethyl acetate (8 : 2); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 6.94 (1H, t, *J* = 7.6 Hz, Ar-CH), 6.82 (1H, d, *J* = 8 Hz, Ar-CH), 6.66 (1H, t, *J* = 7.6 Hz, Ar-CH), 6.52 (1H, d, *J* = 8.0 Hz, Ar-CH), 3.87 (1H, br s, NH), 2.01 (1H, d, *J* = 13.2 Hz, $CH_2$), 1.78 (1H, d, *J* = 13.2 Hz, $CH_2$),1.61 (1H, d, *J* = 14.0 Hz, $CH_2$), 1.48-1.46 (1H, m, $CH_2$), 1.46 (3H, s, $CH_3$), 1.38 (3H, s, $CH_3$), 1.23 (3H, s, $CH_3$), 1.16 (3H, s, $CH_3$), 1.08 (3H, s, $CH_3$), 0.87 (9H, s, ($CH_3$)$_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 164.9 (C-10), 140.9 (Ar-C), 137.2 (Ar-C), 129.3 (Ar-CH), 126.7 (Ar-CH), 118.9 (Ar-CH), 116.4 (Ar-CH), 65.3, 61.3, 59.6, 57.4 ($CH_2$), 53.4 (C-3), 32.7 ($CH_3$), 31.8 (($CH_3$)$_3$), 30.9 ($CH_3$), 30.2 ($CH_3$), 26.7 ($CH_3$), 21.4 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 2955, 1708, 1601, 1482; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{21}H_{34}N_3^+$ 328.2747 found 328.2731.

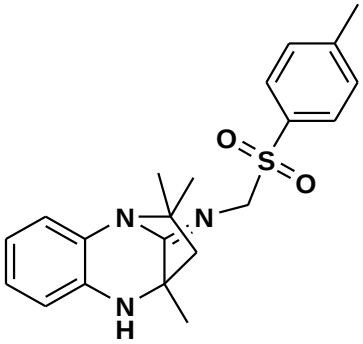
*8.6.2.3 N-(2,2,4-Trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[b][1,4]diazepin-10-ylidene)cyclohexanamine (**6.8c**)*




1*H*-benzo[*d*]imidazol-2(3*H*)-imine (**6.3a**) (0.25 g, 1.87 mmol), cyclohexyl isocyanide (**6.4c**) (0.21 g, 1.87 mmol) and acetone (**6.9a**) (0.22 g, 3.74 mmol) were reacted to give *N*-(2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[*b*][1,4]diazepin-10-ylidene)cyclohexanamine **(6.8c)**; **Physical characteristics**: yellow crystalline solid; Yield: 0.47 g, 84%, **Mp**: 120-122 ºC, $\mathbf{R_f}$ : 0.5, hexane/ethyl acetate (8: 2); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 6.95 (1H, t, *J* = 7.6 Hz, Ar-CH), 6.87 (1H, d, *J* = 7.6 Hz, Ar-CH), 6.66 (1H, t, *J* = 7.6 Hz, Ar-CH), 6.52 (1H, d, *J* = 8.0 Hz, Ar-CH), 4.03 (1H, s, CH), 3.76 (1H, br s, NH), 2.06 (1H, d, *J* = 13.2 Hz, $CH_2$), 1.74-1.60 (4H, m, cyclohexyl), 1.45 (3H, s, $CH_3$),1.36-1.1.33 (6H, m, cyclohexyl), 1.25 (3H, s, $CH_3$), 1.21 (3H, s, $CH_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 168.7 (C-10), 140.8 (Ar-C), 136.8 (Ar-C), 128.9 (Ar-CH), 126.9 (Ar-CH), 118.9 (Ar-CH), 116.1 (Ar-CH), 64.8, 60.1, 58.7 (CH), 54.0 (C-3), 33.9 ($CH_2$), 33.6 ($CH_2$), 32.1 ($CH_3$), 26.2 ($CH_3$), 25.88 ($CH_2$), 25.1 ($CH_2$), 24.9 ($CH_2$), 20.6

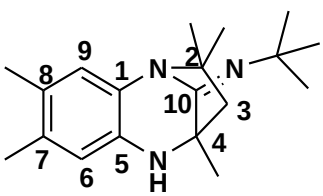
($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3235, 2923, 2851, 1696, 1598, 1470; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{19}H_{28}N_3^+$ 298.2278 found 298.2278.

*8.6.2.4 1-Tosyl-N-(2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[b][1,4]diazepin-10-ylidene)methanamine (**6.8d**)*



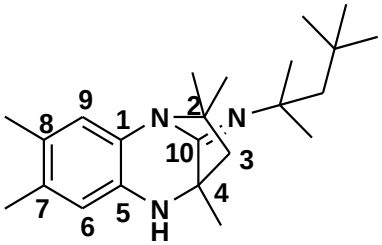

1*H*-benzo[*d*]imidazol-2(3*H*)-imine (**6.3a**) (0.25 g, 1.87 mmol), toluenesulfonylmethyl isocyanide (**6.4d**) (0.36 g, 1.87 mmol) and acetone (**6.9a**) (0.22 g, 3.74 mmol) were reacted to give 1-tosyl-*N*-(2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[*b*][1,4]diazepin-10-ylidene)methanamine (**6.8d**); **Physical characteristics**: yellow solid; Yield: 0.5 g, 71%, **Mp**: 95-97 ºC, **$R_f$** : 0.2, hexane/ethyl acetate (8 : 2) ; **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.67 (2H, d, *J* = 8 Hz, Ar-H), 7.11 (2H, d, *J* = 7.6 Hz, Ar-H), 6.84 (1H, t, *J* = 7.2 Hz, Ar-CH), 6.64 (1H, d, *J* = 7.6 Hz, Ar-CH), 6.53 (1H, t, *J* = 7.6 Hz, Ar-CH), 6.33 (1H, d, *J* = 7.6 Hz, Ar-CH), 5.31 (1H, d, *J* = 13.6 Hz, S-$CH_2$), 4.69 (1H, d, *J* = 13.2 Hz, S-$CH_2$), 3.77 (1H, br s, NH), 2.29 (Ar-$CH_3$), 1.97 (1H, d, *J* = 13.2 Hz, $CH_2$), 1.68 (1H, d, *J* = 13.2 Hz, $CH_2$), 1.34 (3H, s, $CH_3$), 0.91 (3H, s, $CH_3$), 0.82 (3H, s, $CH_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 177.3 (C-10), 143.4 (Ar-C), 139.3(Ar-C), 133.7 (Ar-C), 133.6 (Ar-C), 128.3, (Ar-CH) 128.0 (Ar-CH), 127.7 (Ar-CH), 126.4 (Ar-CH), 118.0 (Ar-CH), 115.0 (Ar-CH), 71.0 (S-$CH_2$), 64.7, 59.5, 52.3 (C-3), 30.4 ($CH_3$), 24.9 ($CH_3$), 20.7 ($CH_3$), 18.9 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3348, 2961, 2925, 2855, 1704, 1598, 1480; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{21}H_{26}N_3O_2S^+$ 384.1740 found 384.1742.

8.6.2.5 2-Methyl-N-(2,2,4,7,8-pentamethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[b][1,4]diazepin-10-ylidene)propan-2-amine (**6.8e***)*



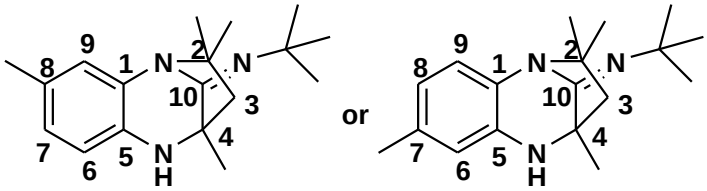
5,6-Dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3j**) (0.25 g, 1.54 mmol), *tert*-butyl isocyanide (**6.4a**) (0.17 g, 1.54 mmol) and acetone (**6.9a**) (0.18 g, 3.08 mmol) were reacted to give 2-methyl-*N*-(2,2,4,7,8-pentamethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[*b*][1,4]diazepin-10-ylidene)propan-2-amine (**6.8e**); **Physical characteristics**: white crystalline solid; Yield: 0.34 g, 74%; **Mp**: 121-123 ºC, $\mathbf{R_f}$ : 0.7, hexane/ethyl acetate (8 : 2); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 6.59 (1H, s, Ar-H), 6.35 (1H, s, Ar-H), 3.71 (1H, br s, NH), 2.11 (6H, s, 2 × $CH_3$), 1.95 (1H, d, *J* = 13.2 Hz, $CH_2$), 1.74 (1H, d, *J* = 13.2 Hz, $CH_2$), 1.37 (3H, s, $CH_3$), 1.27 (9H, s, ($CH_3$)$_3$), 1.12 (3H, s, $CH_3$), 1.07 (3H, s, $CH_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 168.1 (C-10), 138.0 (Ar-C), 135.5 (Ar-C), 134.7 (Ar-C), 129.9 (Ar-CH) , 127.3(Ar-C), 118.4 (Ar-CH), 65.1, 60.9, 55.6, 52.9 (C-3), 32.4 ($CH_3$), 30.9 (($CH_3$)$_3$), 26.5 ($CH_3$), 21.5 ($CH_3$), 19.6 ($CH_3$), 19.1 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3277, 2923, 1723, 1596; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{19}H_{30}N_3^+$ 300.2434 found 300.2436.

*8.6.2.6 2,4,4-trimethyl-N-(2,2,4,7,8-pentamethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[b][1,4]diazepin-10-ylidene)pentan-2-amine (**6.8f**)*



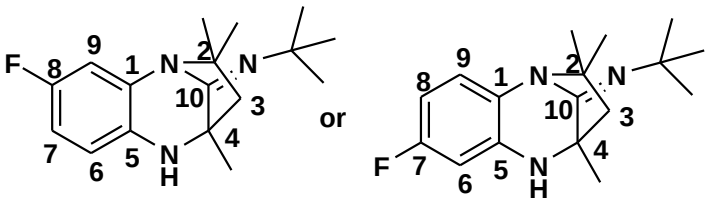

5,6-Dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3j**) (0.25 g, 1.54 mmol), 1,1,3,3-tetramethylbutyl isocyanide (**6.4b**) (0.21 g, 1.54 mmol) and acetone (**6.9a**) (0.18 g, 3.08 mmol) were reacted to give 2,4,4-trimethyl-*N*-(2,2,4,7,8-pentamethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[*b*][1,4]diazepin-10-ylidene)pentan-2-amine (**6.8f**); **Physical characteristics**: brown solid; Yield: 0.36 g, 67%; **Mp**: 91-93 ºC, $\mathbf{R_f}$ : 0.9, hexane/ethyl acetate (8 : 2); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 6.57 (1H, s, Ar-H), 6.33 (1H, s, Ar-H), 3.60 (1H, br s, NH), 2.09 (6H, s, 2 × $CH_3$), 1.93 (1H, d, *J* = 12.8 Hz, $CH_2$), 1.73 (1H, d, *J* = 13.2 Hz, $CH_2$), 1.60 (1H, d, *J* = 14 Hz, $CH_2$) 1.47-1.44 (1H, $CH_2$), 1.44 (3H, s, $CH_3$), 1.35 (3H, s, $CH_3$), 1.19 (3H, s, $CH_3$), 1.19 (3H, s, $CH_3$), 1.05 (3H, s, $CH_3$), 0.87 (9H, s, ($CH_3$)$_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 166.0 (C-10), 138.2 (Ar-C), 135.5 (Ar-C), 134.7 (Ar-C), 129.97 (Ar-CH), 127.29 (Ar-C), 118.29 (Ar-CH), 65.05, 61.21, 59.42, 57.39 (C-3), 52.89 ($CH_2$), 32.53 ($CH_3$), 31.84 (($CH_3$)$_3$), 31.06, 29.95 ($CH_3$), 26.64 ($CH_3$), 21.54 ($CH_3$), 19.60 ($CH_3$); 19.06 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3312, 2928, 1689, 1616; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{23}H_{38}N_3^+$ 356.3060 found 356.3057.

*8.6.2.7 2-Methyl-N-(2,2,4,8-tetramethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[b][1,4]diazepin-10-ylidene)propan-2-amine (**6.8g**) or 2-methyl-N-(2,2,4,7-tetramethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[b][1,4]diazepin-10-ylidene)propan-2-amine*



1*H*-benzo[*d*]imidazol-2(3*H*)-imine (**6.3h**) (0.25 g, 1.69 mmol), *tert*-butyl isocyanide (**6.4a**) (0.14 g, 1.69 mmol) and acetone (**6.9a**) (0.19 g, 3.38 mmol) were reacted to give 2-methyl-*N*-(2,2,4,8-tetramethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[*b*][1,4]diazepin-10-ylidene)propan-2-amine (**6.8g**) or 2-methyl-*N*-(2,2,4,7-tetramethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[*b*][1,4]diazepin-10-ylidene)propan-2-amine; **Physical characteristics**: white solid; Yield: 0.09 g, 19%; **Mp**: 105-107 ºC, **$R_f$** : 0.9, hexane/ethyl acetate (9 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 6.71 (1H, d, *J* = 8.0 Hz, Ar-CH), 6.47 (1H, d, *J* = 7.2 Hz, Ar-CH), 3.82 (1H, br s, NH), 2.19 (3H, s, $CH_3$), 1.99 (1H, d, *J* = 13.2 Hz, $CH_2$), 1.76 (1H, d, *J* = 13.2 Hz, $CH_2$), 1.38 (3H, s, $CH_3$), 1.27 (9H, s, ($CH_3$)$_3$), 1.14 (3H, s, $CH_3$), 1.08 (3H, s, $CH_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 167.4 (C-10), 140.3 (Ar-C), 136.4 (Ar-C), 134.8 (Ar-C), 128.9 (Ar-CH), 119.8 (Ar-CH), 117.1 (Ar-CH), 65.2, 61.01, 55.6, 53.3 (C-3), 32.5 ($CH_3$), 30.9 (($CH_3$)$_3$), 26.6 ($CH_3$), 21.3 ($CH_3$), 21.3 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3263, 2963, 2924, 2855, 1699, 1610; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{18}H_{28}N_3^+$ 286.2278 found 286.2278.

*8.6.2.8 N-(8-Fluoro-2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[b][1,4]diazepin-10-ylidene)-2-methylpropan-2-amine (**6.8h**) or N-(7-Fluoro-2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[b][1,4]diazepin-10-ylidene)-2-methylpropan-2-amine*

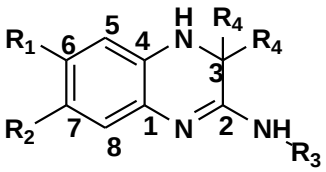


5-Fluoro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3d**) (0.25 g, 1.64 mmol), *tert*-butyl isocyanide (**6.4a**)(0.14 g, 1.64 mmol) and acetone (**6.9a**) (0.19 g, 3.28 mmol) were reacted to give *N*-(8-fluoro-2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[*b*][1,4]diazepin-10-ylidene)-2-methylpropan-2-amine (**6.8h**) or *N*-(7-Fluoro-2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-

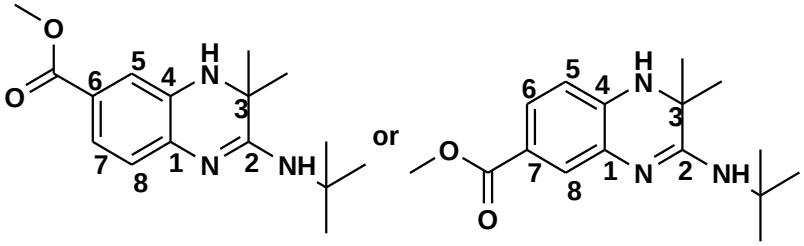
methanobenzo[*b*][1,4]diazepin-10-ylidene)-2-methylpropan-2-amine; **Physical characteristics**: yellow solid; Yield: 0.08 g, 17 %; **Mp**: 103-105 ºC, $\mathbf{R_f}$ : 0.7, hexane/ethyl acetate (9 : 1) ; **$^1$H NMR** (400 MHz, $CDCl_3$) δ 6.71-6.66 (1H, m, Ar-H), 6.60-6.57 (1H, m, Ar-H), 6.50-6.46 (1H, m, Ar-H), 3.77 (1H, br s, NH), 2.01 (1H, d, *J* = 13.6 Hz, $CH_2$), 1.78 (1H, d, *J* = 13.2 Hz, $CH_2$), 1.38 (3H, s, $CH_3$), 1.27 (9H, s, ($CH_3$)$_3$), 1.14 (3H, s, $CH_3$), 1.01 (3H, s, $CH_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 166.7 (C-10), 156.6 (d, $J_{CF}$ = 229.4 Hz, Ar-C), 138.7 (d, $J_{CF}$ = 10.1 Hz, Ar-C), 136.9, 117.6 (d, $J_{CF}$ = 9.1 Hz, Ar-CH), 115.6 (d, $J_{CF}$ = 22.1 Hz, Ar-CH), 113.5 (d, $J_{CF}$ = 23.1 4 Hz, Ar-CH), 65.7, 60.7, 55.8, 52.9 (C-3), 32.3 ($CH_3$), 30.9 (($CH_3$)$_3$), 26.5 ($CH_3$), 21.2 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3257, 2922, 1696, 1596, 1469.

### *8.6.3 General method for the synthesis of N-,3,3-Dimethyl-3,4-dihydroquinoxalin-2(1H)-ylidene)-2-methylpropan-2-amine derivatives (**6.14a-i**)*

In a round bottomed flask equipped with a magnetic stirrer bar, a mixture of 1H-benzo[*d*]imidazol-2(3*H*)-one derivatives (**6.3**), isocyanide derivative (**6.4**) and 2 equivalents of acetone/ acetone–D or 3-pentanone (**6.9**) was stirred at room temperature (rt) for 24 h, while being monitored by TLC. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using hexane-ethyl acetate as eluent to obtain the product.

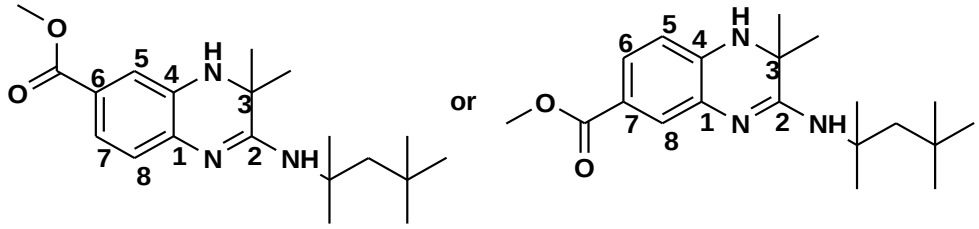



#### *8.6.3.1 (E)-Methyl 2-(tert-butylimino)-3,3-dimethyl-1,2,3,4-tetrahydroquinoxaline-6-carboxylate (**6.14a**) or (E)-methyl 3-(tert-butylimino)-2,2-dimethyl-1,2,3,4-tetrahydroquinoxaline-6-carboxylate*



Methyl 2-thioxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-5-carboxylate (**6.3i**) (0.25 g, 1.30 mmol), *tert*-butyl isocyanide (**6.4a**) (0.11 g, 1.30 mmol) and acetone (**6.9a**) (0.15 g, 2.60 mmol) were reacted to give (*E*)-methyl 2-(*tert*-butylimino)-3,3-dimethyl-1,2,3,4-tetrahydroquinoxaline-6-carboxylate (**6.14a**) or (*E*)-methyl 3-(tert-butylimino)-2,2-dimethyl-1,2,3,4-tetrahydroquinoxaline-6-carboxylate; **Physical characteristics**: white solid; Yield: 0.18 g, 48%; **Mp**: 130-132 ºC, **$R_f$** : 0.2, hexane/ethyl acetate (8 : 2); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.50 (1H, d, *J* = 8 Hz, Ar-H), 7.28 (1H, d, *J* = 1.6 Hz, Ar-H), 7.07 (1H, d, *J* = 8.4 Hz, Ar-H), 4.46 (1H, br s, NH), 3.89 (3H, s, O-$CH_3$), 3.60 (1H, br s, NH), 1.51 (9H, s, ($CH_3$)$_3$), 1.31 (6H, s, 2 × $CH_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) 167.8 (C=O), 159.4 (C-2), 140.2 (Ar-C), 134.5 (Ar-C), 123.9 (Ar-C), 123.5 (Ar-CH), 121.94 (Ar-CH), 114.8 (Ar-CH), 52.2, 51.8 ($CH_3$-O), 50.7, 29.1 (($CH_3$)$_3$), 26.23 (2 × $CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3675, 3448, 3351, 2901, 2971, 1693, 1616, 1571; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{16}H_{24}N_3O_2^+$ 290.1863 found 290.1865.

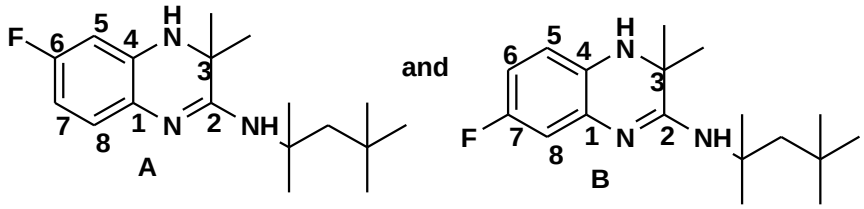
*8.6.3.2 (E)-methyl 3,3-Dimethyl-2-((2,4,4-trimethylpentan-2-yl)imino)-1,2,3,4-tetrahydroquinoxaline-6-carboxylate (**6.14b**) or (E)-methyl 2,2-dimethyl-3-((2,4,4-trimethylpentan-2-yl)imino)-1,2,3,4-tetrahydroquinoxaline-6-carboxylate*




Methyl 2-thioxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-5-carboxylate (**6.3i**) (0.25 g, 1.30 mmol), 1,1,3,3-tetramethylbutyl isocyanide (**6.4b**) (0.18 g, 1.30 mmol) and acetone (**6.9a**) (0.15 g, 2.60 mmol) were reacted to give (*E*)-methyl 3,3-dimethyl-2-((2,4,4-trimethylpentan-2-yl)imino)-1,2,3,4-tetrahydroquinoxaline-6-carboxylate (**6.14b**) or (*E*)-methyl 2,2-dimethyl-3-((2,4,4-trimethylpentan-2-yl)imino)-1,2,3,4-tetrahydroquinoxaline-6-carboxylate; **Physical characteristics**: white solid; Yield: 0.23 g, 52%; **Mp**: 150-152 ºC, **$R_f$** : 0.5, hexane/ethyl acetate (8: 2); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.51 (1H, d, *J* = 8 Hz, Ar-H), 7.29 (1H, d, *J* = 5.2 Hz, Ar-H), 7.06 (1H, d, *J* = 6.8 Hz, Ar-H), 4.52 (1H, br s, NH), 3.89 (3H, s, O-$CH_3$), 3.60 (1H, br s, NH), 1.93 (2H, s, $CH_2$), 1.58 (6H, s, 2 × $CH_3$), 1.30 (6H, s, 2$CH_3$), 1.07 (9H, s, ($CH_3$)$_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) 167.8 (C=O), 158.8 (C-2), 140.4 (Ar-C), 134.5 (Ar-C), 123.7 (Ar-C), 123.4 (Ar-CH), 121.9 (Ar-CH), 114.8 (Ar-CH), 56.2, 52.3 ($CH_2$), 51.8, 50.6, 31.9, 31.8

(($CH_3$)$_3$), 29.2, ($CH_3$), 26.2 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3675, 3478, 3346, 2971, 2901, 1696, 1620, 1567; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{20}H_{32}N_3O_2^+$ 346.2489 found 346.2492.

*8.6.3.3 (E)-N-(6-Fluoro-3,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-ylidene)-2,4,4-trimethylpentan-2-amine (**6.14c**) and (E)-N-(7-fluoro-3,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-ylidene)-2,4,4-trimethylpentan-2-amine (**6.14d**)*

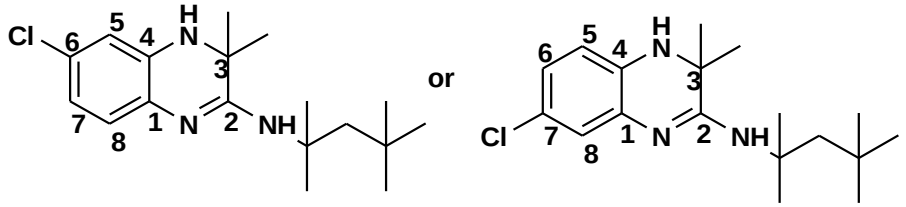



5-Fluoro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3d**) (0.25 g, 1.64 mmol), 1,1,3,3-tetramethyl butyl isocyanide (**6.4b**) (0.23 g, 1.64 mmol) and acetone (**6.9a**) (0.19 g, 3.28 mmol) were reacted to give (*E*)-*N*-(6-fluoro-3,3-dimethyl-3,4-dihydroquinoxalin-2(1*H*)-ylidene)-2,4,4-trimethylpentan-2-amine (**6.14c**) and (*E*)-*N*-(7-fluoro-3,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-ylidene)-2,4,4-trimethylpentan-2-amine (**6.14d**).

**Compound one: Physical characteristics**: yellow solid; Yield: 0.12 g, 30%; **Mp**: 108-110 ºC, **$R_f$** : 0.5, hexane/ethyl acetate (8 : 2); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 6.76 (1H, *J* = 6.8 Hz, Ar-H), 6.49-6.44 (2H, m, Ar-H), 4.36 (1H, br s, NH), 3.33 (1H, br s, NH), 1.89 (2H, s, $CH_2$), 1.52 (6H, s, 2 × $CH_3$), 1.24 (6H, s, 2 × $CH_3$), 1.03 (9H, s, (C*H*$_3$)$_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) 167.0 (C-2), 159.1 (d, $J_{CF}$ = 199.2. Hz, Ar-CH), 146.6 (Ar-C), 130.8 (Ar-C), 113.7 (d, $J_{CF}$ = 9.1 Hz, Ar-CH), 110.5 (d, $J_{CF}$ = 22.1 Hz, Ar-CH), 108.0 (d, $J_{CF}$ = 24.1 Hz, Ar-CH), 65.7, 56.0, 52.3 ($CH_2$), 31.9, 31.8 ((*C*$H_3$)$_3$), 29.2 ($CH_3$), 26.0 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3675, 3455, 3351, 2901, 2971,2921, 1770, 1622, 1577; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{18}H_{29}FN_3^+$ 306.2340 found 306.2348.

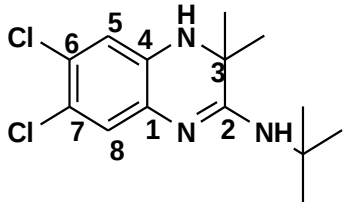
**Compound two**: **Physical characteristics**: yellow solid; Yield: 0.09 g, 22%; **Mp**: 105-107 ºC, **$R_f$** : 0.8, hexane/ethyl acetate (8 : 2); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 6.76 (1H, *J* = 6.8 Hz, Ar-H), 6.51-6.42 (2H, m, Ar-H), 4.36 (1H, br s, NH), 3.33 (1H, br s, NH), 1.89 (2H, s, $CH_2$), 1.52 (6H, s, 2$CH_3$), 1.24 (6H, s, 2 × $CH_3$), 1.03 (9H, s, (C*H*$_3$)$_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) 160.5 (C-2), 157.3 (d, $J_{CF}$ = 154.9 Hz, Ar-CH), 137.1 (Ar-C), 130.8 (Ar-C), 113.7 (d, $J_{CF}$ = 9.1 Hz, Ar-CH), 110.4 (d, $J_{CF}$ = 23.1 Hz, Ar-CH), 108.0 (d, $J_{CF}$ = 23.1 Hz, Ar-CH), 87.1, 56.0, 52.3 ($CH_2$), 50.7, 31.9, 31.8 ((*C*$H_3$)$_3$), 29.2 ($CH_3$), 26.0 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3675, 3455, 2987, 2971, 2904, 1770, 1622, 1515; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{18}H_{29}FN_3^+$ 306.2340 found 306.2345.

*8.6.3.4 (E)-N-(6-Chloro-3,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-ylidene)-2,4,4-trimethylpentan-2-amine (**6.14e**) or (E)-N-(7-chloro-3,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-ylidene)-2,4,4-trimethylpentan-2-amine*




5-Chloro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3e**) (0.25 g, 1.48 mmol), 1,1,3,3-tetramethyl butyl isocyanide (**6.4b**) (0.21 g, 1.48 mmol) and acetone (**6.9a**) (0.17 g, 2.96 mmol) were reacted to give (*E*)-*N*-(6-chloro-3,3-dimethyl-3,4-dihydroquinoxalin-2(1*H*)-ylidene)-2,4,4-trimethylpentan-2-amine (**6.14e**) or E)-N-(7-chloro-3,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-ylidene)-2,4,4-trimethylpentan-2-amine; **Physical characteristics**: yellow solid; Yield: 0.18 g, 46%; **Mp**: 105-107 ºC, $\mathbf{R_f}$ : 0.6, hexane/ethyl acetate (8 : 2); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.02 (1H, s, Ar-H), 6.73 (1H, d, *J* = 8 Hz, Ar-H), 6.44 (1H, d, *J* = 8 Hz, Ar-H), 4.34 (1H, br s, NH), 3.44 (1H, br s, NH), 1.89 (2H, s, $CH_2$), 1.52 (6H, s, $2CH_3$), 1.25 (6H, s, 2 × $CH_3$), 1.03 (9H, s, ($CH_3$)$_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) 157.9 (C-2), 147.0 (Ar-C), 139.3 (Ar-C), 128.4 (Ar-CH), 123.7 (Ar-CH), 121.9 (Ar-CH), 114.3 (Ar-CH), 56.1, 52.3 ($CH_2$), 50.7, 31.9, 31.8 (($CH_3$)$_3$), 29.3 ($CH_3$), 26.2 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3675, 2987, 2972, 2901, 1710, 1650; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{18}H_{29}ClN_3^+$ 322.2045 found 322.2055.

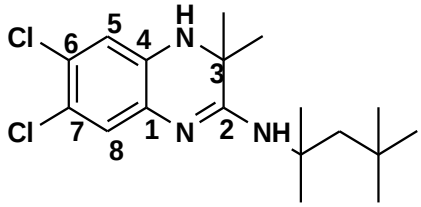
*8.6.3.5 (E)-N-(6,7-Dichloro-3,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-ylidene)-2-methylpropan-2-amine **(6.14f)***




5,6-Dichloro-1*H*-benzo[d]imidazol-2(3*H*)-one (**6.3k**) (0.25 g, 1.28 mmol), *tert*-butyl isocyanide (**6.4a**) (0.17 g, 1.28 mmol) and acetone (**6.9a**) (0.15 g, 2.56 mmol) were reacted to give (*E*)-*N*-(6,7-dichloro-3,3-dimethyl-3,4-dihydroquinoxalin-2(1*H*)-ylidene)-2-methylpropan-2-amine (**6.14f**); **Physical characteristics**: brown solid; Yield: 0.28 g, 74%; **Mp**: 144-146 ºC, $\mathbf{R_f}$ : 0.7, hexane/ethyl acetate (8 : 2); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.09 (1H, s, Ar-H), 6.58 (1H, s, Ar-H), 4.31 (1H, br s, NH), 3.62 (1H, br s, NH), 1.45 (9H, s, ($CH_3$)$_3$), 1.26 (6H, s, $2CH_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 158.2 (C-2), 135.4 (Ar-C), 134.5 (Ar-C),

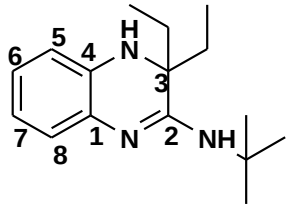
124.7 (Ar-CH), 121.6 (Ar-C), 114.2 (Ar-CH), 68.3, 51.9, 28.9 (($CH_3$)$_3$), 26.1 ($CH_3$), 18.9 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3675, 3460, 1700, 1605; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{14}H_{20}Cl_2N_3^+$ 300.1029 found 300.1039.

*8.6.3.6 (E)-N-(6,7-Dichloro-3,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-ylidene)-2,4,4-trimethylpentan-2-amine (**6.14g**)*




5,6-Dichloro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3k**) (0.25 g, 1.28 mmol), 1,1,3,3-tetramethylbutyl isocyanide (**6.4b**) (0.18 g, 1.28 mmol) and acetone (**6.9a**) (0.15 g, 2.56 mmol) were reacted to give (*E*)-*N*-(6,7-dichloro-3,3-dimethyl-3,4-dihydroquinoxalin-2(1*H*)-ylidene)-2,4,4-trimethylpentan-2-amine (**6.14g**); **Physical characteristics**: brown solid; Yield: 0.34 g, 76%; **Mp**: 157-159 ºC, **R$_f$** : 0.7, hexane/ethyl acetate (8 : 2); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.08 (1H, s, Ar-H), 6.59 (1H, s, Ar-H), 4.38 (1H, br s, NH), 3.62 (1H, br s, NH), 1.87 (2H, s, $CH_2$), 1.51 (6H, s, 2CH3), 1.25 (6H, s, 2 × $CH_3$), 1.02 (9H, s, ($CH_3$)$_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 157.8 (C-2), 135.7 (Ar-C), 134.6 (Ar-C), 124.7 (Ar-CH), 124.3 (Ar-C), 121.7 (Ar-C), 114.3 (Ar-CH), 68.2, 56.1, 52.1 ($CH_2$), 50.5, 31.8 (($CH_3$)$_3$), 29.2 ($CH_3$), 26.2 ($CH_3$), 19.0 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3456, 3356, 1609, 1573; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{18}H_{28}Cl_2N_3^+$ 356.1655 found 356.1660.

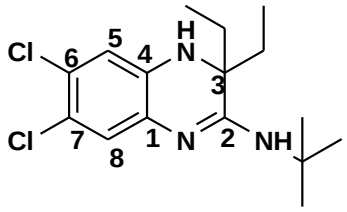
*8.6.3.7 (E)-N-(3,3-Diethyl-3,4-dihydroquinoxalin-2(1H)-ylidene)-2-methylpropan-2-amine (**6.14h**)*




1*H*-benzo[*d*]imidazol-2(3*H*)-imine (**6.3h**) (0.25 g, 1.87 mmol), *tert*-butyl isocyanide (**6.4a**) (0.16 g, 1.87 mmol) and 3 pentanone (**6.9b**) (0.32 g, 3.73 mmol) were reacted to give (*E*)-*N*-(3,3-diethyl-3,4-dihydroquinoxalin-2(1*H*)-ylidene)-2-methylpropan-2-amine (**6.14h**); **Physical characteristics**: yellow solid; Yield: 0.37 g, 76%; **Mp**: 138-140 ºC, **R$_f$** : 0.5,

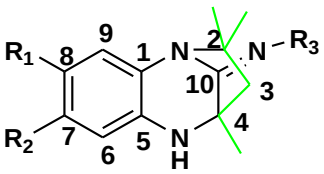
hexane/ethyl acetate (8 : 2); **$^{1}$H NMR** (400 MHz, $CDCl_3$) δ 6.96 (1H, d, *J* = 7.6 Hz, Ar-H), 6.75 (1H, d, *J* = 7.2 Hz, Ar-H), 6.63 (1H, d, *J* = 7.2 Hz, Ar-H), 6.43 (1H, d, *J* = 7.6 Hz, Ar-H), 4.09 (1H, br s, NH), 3.25 (1H, br s, NH), 1.69 (2H, q, *J* = 7.2 Hz, $CH_2$), 1.47 (9H, s, $(CH_3)_3$), 0.99 (3H, t, *J* = 7.6, $CH_3$), 1.08 (6H, s, $2CH_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 154.9 (C-2), 135.5 (Ar-C), 133.7 (Ar-C), 123.9 (Ar-C), 122.9 (Ar-C) , 118.1 (Ar-C), 111.8 (Ar-C), 57.9, 51.6, 32.4 ($2 \times CH_2$), 29.1 ($(CH_3)_3$), 8.2 ($2 \times CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3449, 3359, 2962, 1683, 1611, 1584; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{16}H_{26}N_3^+$ 260.2121 found 260.2125.

*8.6.3.8 (E)-N-(6,7-Dichloro-3,3-diethyl-3,4-dihydroquinoxalin-2(1H)-ylidene)-2-methylpropan-2-amine (**6.14i**)*



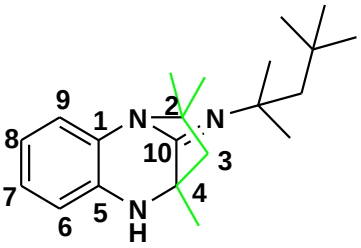

5,6-Dichloro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3h**) (0.25 g, 1.28 mmol), *tert*-butyl isocyanide (**6.4a**) (0.17 g, 1.28 mmol) and 3-pentanone (**6.9b**) (0.22 g, 2.56 mmol) were reacted to give (*E*)-*N*-(6,7-dichloro-3,3-diethyl-3,4-dihydroquinoxalin-2(1*H*)-ylidene)-2-methylpropan-2-amine (**6.14i**); **Physical characteristics**: brown solid; Yield: 0.08 g, 19%; **Mp**: 110-112 ºC, $R_f$ : 0.6, hexane/ethyl acetate (8 : 2);$^{1}$H NMR (400 MHz, $CDCl_3$) δ 6.99 (1H, s, Ar-H), 6.44 (1H, s, Ar-H), 4.22 (1H, br s, NH), 3.30 (1H, br s, NH), (2H, q, *J* = 7.2 Hz, $2 \times CH_2$), (1.44 (9H, s, $(CH_3)_3$), 0.97 (6H, s, $2 \times CH_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 155.7 (C-2), 134.4 (Ar-C), 122.4 (Ar-C), 117.6 (Ar-CH), 112.3 (Ar-C), 33.2 ($2 \times CH_2$), 29.2, 28.9 ($(CH_3)_3$), 8.3 ($2CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 2970, 1612, 1492; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{16}H_{24}C_{l2}N_3^+$ 328.1342 found 328.1343.

*8.6.4 General procedure for the synthesis of 2-methyl-N-(2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[b][1,4]diazepin-10-ylidene)propan-2-amine derivatives using* ***Acetone-d$_6$*** *(**6.8i-l**) (Green-deuterated-atoms)*




In a round bottomed flask equipped with a magnetic stirrer bar, a mixture of 1*H*-benzo[*d*]imidazol-2(3*H*)-one derivative (**6.3**), isocyanide derivative (**6.4**) and 2 equivalents of acetone–D (**6.9c**) was stirred at room temperature (rt) for 24 h, while being monitored by TLC. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using hexane-ethyl acetate as eluent to obtain the product.

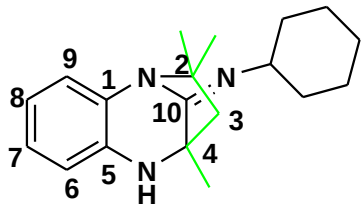
*8.6.4.1 2,4,4-Trimethyl-N-(2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[b][1,4]diazepin-10-ylidene)pentan-2-amine (**6.8i -D**)*




1*H*-Benzo[*d*]imidazol-2(3*H*)-imine (**6.3h**) (0.25 g, 1.87 mmol ), 1,1,3,3-tetramethylbutyl isocyanide (**6.4b**) (0.26 g, 1.87 mmol) and acetone-D (**6.9c**) (0.24 g, 3.74 mmol) were reacted to give 2,4,4-trimethyl-*N*-(2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[*b*][1,4]diazepin-10-ylidene)pentan-2-amine (**6.8i -D**); **Physical characteristics**: maroon paste; Yield: 0.36 g, 57%, **R$_f$** : 0.9, hexane/ethyl acetate (8 : 2); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 6.93 (1H, t, *J* = 7.6 Hz, Ar-CH), 6.82 (1H, d, *J* = 8 Hz, Ar-CH), 6.65 (1H, t, *J* = 7.2 Hz, Ar-CH), 6.51 (1H, d, *J* = 8.0 Hz, Ar-CH), 3.86 (1H, br s, NH), 1.61 (1H, d, *J* = 14.4 Hz, 2 × $CH_2$), 1.45 (3H, s, $CH_3$), 1.37 (1H, d, *J* = 7.6 Hz, 2$CH_2$), 1.22 (3H, s, $CH_3$), 0.86 (9H, s, ($CH_3$)$_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 164.9 (C-10), 140.9 (Ar-C), 137.2 (Ar-C), 129.3 (Ar-CH), 126.7 (Ar-CH), 118.9 (Ar-CH), 116.4 (Ar-CH), 65.3, 61.3, 59.6, 57.4

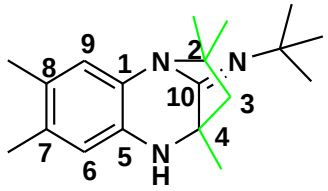
($CH_2$), 53.4, 32.7 ($CH_3$), 31.8 (($CH_3$)$_3$), 30.9 ($CH_3$), 30.2 ($CH_3$), 26.7 ($CH_3$), 21.4 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3675, 3256, 2986, 2969, 2901, 1687, 1599; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{21}H_{34}N_3^+$ 328.2747 and 339.2747 –D (11D) found isotopes 338.3384 and 339.3413.

*8.6.4.2 N-(2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[b][1,4]diazepin-10-ylidene)cyclohexanamine (**6.8j D**)*




1*H*-Benzo[*d*]imidazol-2(3*H*)-imine (**6.3h**) (0.25 g, 1.87 mmol), cyclohexyl isocyanide (**6.4b**) (0.21 g, 1.87 mmol) and acetone-D (**6.9c**) (0.24 g, 3.74 mmol) were reacted to give *N*-(2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[*b*][1,4]diazepin-10-ylidene)cyclohexanamine (**6.8i -D**); **Physical characteristics**: yellow crystalline solid; Yield: 0.35 g, 61%, **Mp**: 115-118 ºC, **R$_f$** : 0.5, hexane/ethyl acetate (8 : 2); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 6.95 (1H, t, *J* = 7.6 Hz, Ar-CH), 6.87 (1H, d, *J* = 7.6 Hz, Ar-CH), 6.66 (1H, t, *J* = 7.2 Hz, Ar-CH), 6.52 (1H, d, *J* = 8.0 Hz, Ar-CH), 4.03 (1H, s, CH), 3.87 (1H, br s, NH), 1.79-1.69 (6H, m, cyclohexyl) 1.44-1.33 (2H, m, cyclohexyl), 1.21-1.01 (2H, m, cyclohexyl), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 151.5 (C-10), 140.8 (Ar-C), 136.8 (Ar-C), 128.9 (Ar-CH), 126.9 (Ar-CH), 118.9 (Ar-CH), 116.1 (Ar-CH), 58.7 (CH), 33.9 ($CH_2$), 33.6 ($CH_2$), 29.9 ($CH_2$), 25.9 ($CH_2$), 25.1 ($CH_2$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3263, 2963, 2924, 2855, 1699, 1610; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{19}H_{28}N_3^+$ 298.2278 and 309.2278-D (11-D) found isotopes 306.2755 and 309.2884.

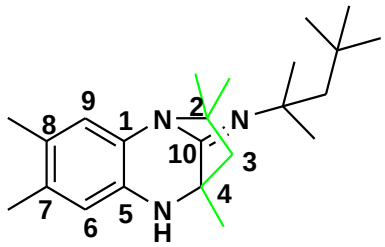
*8.6.4.3 2-Methyl-N-(2,2,4,7,8-pentamethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[b][1,4]diazepin-10-ylidene)propan-2-amine (**6.8k D**)*




5,6-Dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3j**) (0.25 g, 1.54 mmol), *tert*-butyl isocyanide (**6.4a**) (0.17 g, 1.54 mmol) and acetone-D (**6.9c**) (0.19g, 3.08 mmol) were reacted

to give 2-methyl-*N*-(2,2,4,7,8-pentamethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[*b*][1,4]diazepin-10-ylidene)propan-2-amine (**6.8k D**); **Physical characteristics**: white solid; Yield: 0.35 g, 73%; **Mp**: 95-97 ºC, **$R_f$** : 0.6, hexane/ethyl acetate (8 : 2); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 6.59 (1H, s, Ar-H), 6.35 (1H, s, Ar-H), 3.71 (1H, br s, NH), 2.11 (6H, s, 2 × $CH_3$), 1.26 (9H, s, ($CH_3$)$_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 168.5 (C-10), 137.9 (Ar-C), 135.6 (Ar-C), 134.7 (Ar-C), 129.8 (Ar-CH), 127.4 (Ar-C) 118.4 (Ar-CH), 55.5, 30.9 ($CH_3$), 19.6 ($CH_3$), 19.2 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3264, 2961, 2922, 2854, 1697, 1617.

*8.6.4.4 2,4,4-Trimethyl-N-(2,2,4,7,8-pentamethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[b][1,4]diazepin-10-ylidene)pentan-2-amine (**6.8l D**)*

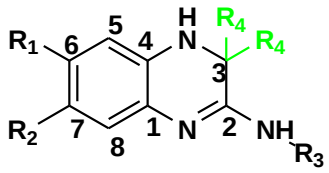



5,6-Dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3j**) (0.25 g, 1.54 mmol), 1,1,3,3-tetramethyl butyl isocyanide (**6.4b**) (0.21 g, 1.54 mmol) and acetone-D (**6.9c**) (0.19 g, 3.08 mmol) were reacted to give 2,4,4-trimethyl-*N*-(2,2,4,7,8-pentamethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[*b*][1,4]diazepin-10-ylidene)pentan-2-amine (**6.8l D**); **Physical characteristics**: white solid; Yield: 0.35 g, 61%; **Mp**: 147-149 ºC, **$R_f$** : 0.8, hexane/ethyl acetate (8: 2); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 6.58 (1H, s, Ar-H), 6.35 (1H, s, Ar-H), 3.68 (1H, br s, NH), 2.11 (6H, s, 2 × $CH_3$), 1.61 (1H, d, *J* = 14.4 Hz, $CH_2$) and 1.45-1.36 (1H, m $CH_2$), 1.45 (3H, s, $CH_3$), 0.87 (9H, s, ($CH_3$)$_3$, **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 166.1 (C-10), 138.1 (Ar-C), 135.6 (Ar-C), 134.7 (Ar-C), 129.9 (Ar-CH), 127.4 (Ar-C), 118.4 (Ar-CH), 59.4, 57.3 ($CH_2$), 31.9, 31.8 (($CH_3$)$_3$), 31.1 ($CH_3$), 29.9 ($CH_3$), 19.6 ($CH_3$); 19.1 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3312, 2988, 2973, 2928, 2959, 2862, 1699, 1616.

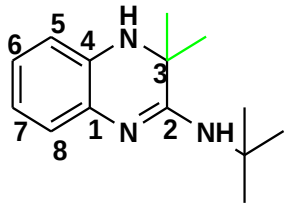
*8.6.5 General method for the synthesis of N-,3,3-Dimethyl-3,4-dihydroquinoxalin-2(1H)-ylidene)-2-methylpropan-2-amine derivatives using Acetone-$d_6$ (**6.14h-j**)*

In a round bottomed flask equipped with a magnetic stirrer bar, a mixture of 1*H*-Benzo[*d*]imidazol-2(3*H*)-one derivatives (**6.3**), isocyanide derivative (**6.4**) and 2 equivalents of acetone–D (**6.9c**) was stirred at room temperature (rt) for 24 h, while being monitored by

TLC. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using hexane-ethyl acetate as eluent to obtain the product.

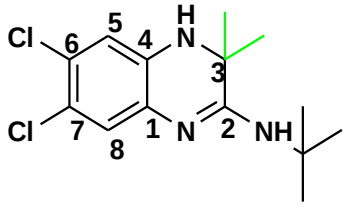



*8.6.5.1 (E)-N-(3,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-ylidene)-2-methylpropan-2-amine (**6.14h D**)*




1*H*-Benzo[*d*]imidazol-2(3*H*)-imine (**6.3h**) (0.25 g, 1.87 mmol), *tert*-butyl isocyanide (**6.4a**) (0.17 g, 1.87 mmol) and acetone-D (**6.9c**) (0.24 g, 3.74 mmol) were reacted to give (*E*)-*N*-(3,3-dimethyl-3,4-dihydroquinoxalin-2(1*H*)-ylidene)-2-methylpropan-2-amine (**6.14h-D**); **Physical characteristics**: white solid; Yield: 0.43 g, 70%; **Mp**: 86-88 ºC, **$R_f$** : 0.8, hexane/ethyl acetate (8 : 2) ; **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.04 (1H, d, *J* = 7.2 Hz, Ar-H), 6.81 - 6.75 (2H, m, Ar-H), 6.53 (1H, d, *J* = 7.2 Hz, Ar-H), 4.19 (1H, br s, NH), 3.43 (1H, br s, NH), 1.47 (9H, s, $(CH_3)_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 157.6 (C-2), 135.6 (Ar-C), 134.9 (Ar-C), 124.1 (Ar-CH), 122.7 (Ar-CH), 119.6 (Ar-CH), 113.6 (Ar-CH), 51.7, 29.1 ($(CH_3)_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3438, 3371, 3261, 3047, 2953, 1697, 1617, 1582; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated $C_{14}H_{22}N_3^+$ 232.1808 and 6-D found isotope 238.2188.

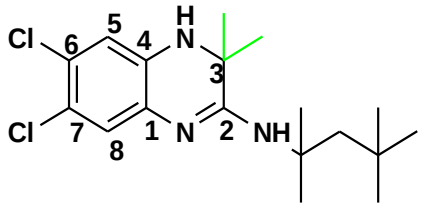
*8.6.5.2 (E)-N-(6,7-Dichloro-3,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-ylidene)-2-methylpropan-2-amine (**6.14i D**)*




5,6-Dichloro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3k**) (0.25 g, 1.28 mmol), *tert*-butyl isocyanide (**6.4a**) (0.17 g, 1.28 mmol) and acetone-D (**6.9c**) (0.16 g, 2.56 mmol) were reacted

to give (*E*)-*N*-(6,7-dichloro-3,3-dimethyl-3,4-dihydroquinoxalin-2(1*H*)-ylidene)-2-methylpropan-2-amine (**6.12i D**); **Physical characteristics**: brown solid; Yield: 0.28 g, 72%; **Mp**: 141-142 ºC, **$R_f$** : 0.7, hexane/ethyl acetate (8 : 2); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.09 (1H, Ar-H), 6.59 (1H, s, Ar-H), 4.31 (1H, br s, NH), 3.49 (1H, br s, NH), 1.45 (9H, s, ($CH_3$)$_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 158.36 (C-2), 135.6 (Ar-C), 134.6 (Ar-C), 124.9 (Ar-CH), 124.6 (Ar-C), 121.8 (Ar-C), 114.4 (Ar-CH), 52.09, 29.04 (($CH_3$)$_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3675, 3460, 3354, 2972, 2901, 1770, 1605, 1574; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{14}H_{20}Cl_2N_3^+$ 300.1029 and 306.1029-D (6-D) found isotope 306.1372.

*8.6.5.3 (E)-N-(6,7-Dichloro-3,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-ylidene)-2,4,4-trimethylpentan-2-amine (**6.14j-D**)*



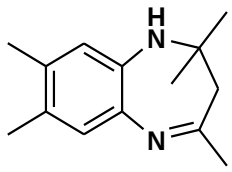

5,6-Dichloro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3k**) (0.25 g, 1.28 mmol), 1,1,3,3-tetramethyl butyl isocyanide (**6.4b**) (0.18 g, 1.28 mmol) and acetone-D (**6.9c**) (0.16 g, 2.56 mmol) were reacted to give (*E*)-*N*-(6,7-dichloro-3,3-dimethyl-3,4-dihydroquinoxalin-2(1*H*)-ylidene)-2,4,4-trimethylpentan-2-amine (**6.12j D)**; **Physical characteristics**: brown solid; Yield: 0.37 g, 80%; **Mp**: 157-159 ºC, **$R_f$** : 0.7, hexane/ethyl acetate (8 : 2); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.08 (1H, s, Ar-H), 6.59 (1H, s, Ar-H), 4.36 (1H, br s, NH), 3.49 (1H, br s, NH), 1.87 ($CH_2$), 1.51 (6H, s, 2 × CH3), 1.02 (9H, s, ($CH_3$)$_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 158.2 (C-2), 127.1 (Ar-C), 124.8 (Ar-C), 121.0 (Ar-C), 114.4 (2 × Ar-CH), 56.2, 52.2, 31.9, 31.8 (($CH_3$)$_3$), 29.9 ($CH_3$), 26.2 × ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3675, 3357, 2987, 2971, 2922, 1657, 1608, 1573; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{18}H_{28}Cl_2N_3^+$ 356.1655 and 6-D 362.1655 found isotope 362.2024.

*8.6.6 General method for the synthesis of 2,2,4-Trimethyl-2,3-dihydro-1H-benzo[b][1,4]diazepine derivatives (**6.12a-b**)*

In a round bottomed flask equipped with a magnetic stirrer bar, a mixture of 5,6-dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3j**) or 5,6-dichloro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3k**),

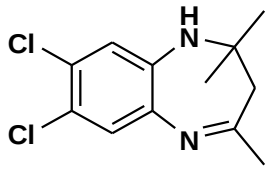
urea and acetone (**6.9a**) (2eq) were set for reflux for 12h, while being monitored by TLC. After completion the reaction was cooled to room temperature. Excess acetone was removed under reduced pressure to obtain a residue that was washed with water to remove urea, then ethyl acetate to obtain the product.

*8.6.6.1 2,2,4,7,8-Pentamethyl-2,3-dihydro-1H-benzo[b][1,4]diazepine (**6.12a**)*



Urea (0.11 g, 1.84 mmol) **(6.2a**), 4,5-dimethyl-1,2-phenylenediamine (**6.1a**) (0.25 g, 1.84 mmol) and acetone (**6.4a**) (0.16 g, 2.82 mmol) were reacted to give 2,2,4,7,8-pentamethyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine (**6.12a**); **Physical characteristics**: brown solid; Yield: 0.39 g, 85%; **Mp**: 88-90 ºC; **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 6.70 (1H, s, Ar-H), 6.59 (1H, s, Ar-H), 4.43 (1H, s, N-H); 2.19 (3H, s, $CH_3$), 2.09 (6H, s, 2 × $CH_3$), 1.20 (6H, s, 2 × $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 170.0 (C=N), 137.5 (Ar-C), 136.9 (Ar-C), 132.6 (Ar-C), 127.7 (Ar-C), 127.4 (Ar-CH), 122.2 (Ar-CH), 66.7, 45.3 ($CH_2$), 29.9 ($CH_3$), 29.4 ($CH_3$), 18.9 ($CH_3$), 18.5 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3314, 2914, 1633, 1477; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated $C_{14}H_{21}N_2^+$ 217.1699 found 217.1702.

*8.6.6.2 7,8-Dichloro-2,2,4-trimethyl-2,3-dihydro-1H-benzo[b][1,4]diazepine (**6.12b**)*



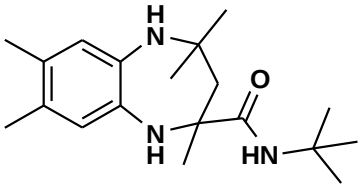
Urea (**6.2a**) (0.08 g, 1.41 mmol), 4,5-Dichloro-1,2-phenylenediamine (**6.1h**) (0.25 g, 1.41 mmol) and acetone (**6.4a**) ( 0.16 g, 2.82 mmol) were reacted to give 7,8-dichloro-2,2,4-trimethyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine (**6.12b**); **Physical characteristics**: purple solid; Yield: 0.29 g, 80%; **Mp**: 113-115 ºC; **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 7.09 (1H, s, Ar-H), 7.03 (1H, s, Ar-H), 5.35 (H, s, N-H), 2.26 (2H, s, $CH_2$), 2.22 (3H, s, $CH_3$), 1.23 (6H, 2 × $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 173.2 (C=N), 139.9 (Ar-C), 138.1 (Ar-C), 128.5 (Ar-CH), 126.4 (Ar-C), 121.1 (Ar-CH), 120.1 (Ar-C), 65.5, 45.6 ($CH_2$), 30.1 ($CH_3$), 29.7 ($CH_3$);

**FT-IR $\nu_{max}$/cm$^{-1}$**: 3299, 2960, 2923, 1733, 1641; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated $C_{12}H_{15}Cl_2N_2^+$ 257.0607 found 257.0612.

*8.6.7 General procedure for the synthesis of **6.13a-b***

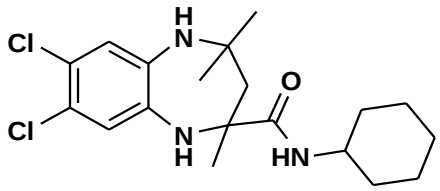
In a round bottomed flask equipped with a magnetic stirrer bar, isocyanide (**6.4**) was added to a mixture of 2,2,4,7,8-pentamethyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine derivative (**6.11**), ethanol and montmorillonite k-10 clay or *p*-toluenesulfonic acid. The reaction mixture was allowed to stir at room temperature for 24 h while being monitored by TLC. After completion, the reaction was cooled and the solvent removed under reduced pressure to obtain a crude residue, which was then purified by silica gel column chromatography using hexane-ethyl acetate as eluent, to obtain the product.

*8.6.7.1 7.8-Dimethyl-N-(tert-butyl)-2,4,4-trimethyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepine-2-carboxamide (**6.13a**)*



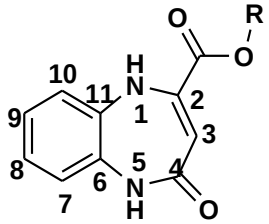

2,2,4,7,8-Pentamethyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine (**6.10a**) (0.10 g, 0.46 mmol), isocyanide (**6.4a**) (0.04 g, 0.46 mmol) and *p*-toluenesulfonic acid (0.01 g 0.46 mmol) were reacted to give 7.8-dimethyl-*N*-(*tert*-butyl)-2,4,4-trimethyl-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxamide (**6.13a**); **Physical characteristics**: yellow solid; Yield: 0.11 g, 75%, **Mp**: 200-202 ºC, **$R_f$** : 0.6, hexane/ethyl acetate (1 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.19 (1H, d, *J* = 5.2 Hz, NH-C=O), 6.39 (2H, s, Ar-H), 2.39 (1H, br s, NH), 2.15 (1H, d, *J* = 14.8 Hz, $CH_2$), 2.07 and 2.06 (6H, 2 × s, 2 × $CH_3$), 1.53 (1H, d, *J* = 14.8 Hz, $CH_2$), 1.29 (3H, s, $CH_3$), 1.25 (9H, s, ($CH_3$)$_3$), 1.21 (1H, br s, NH), 1.14 (3H, s, $CH_3$), 1.06 (3H, s, $CH_3$), **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 175.6 (C=O), 135.0 (Ar-C), 134.4 (Ar-C), 130.6 (Ar-C), 129.9 (Ar-C), 124.2 (Ar-CH) , 121.3 (Ar-CH), 60.6, 52.9, 50.7, 47.5 ($CH_2$), 32.5 ($CH_3$), 30.9 ($CH_3$), 29.3 ($CH_3$), 28.7 (($CH_3$)$_3$), 19.0 ($CH_3$), 18.9 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3380, 3286, 2926, 1642, 1591; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{19}H_{32}N_3O^+$ 318.2540 found 318.2521.

*8.6.7.2 7,8-dichloro-N-cyclohexyl-2,4,4-trimethyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepine-2-carboxamide (**6.13b**)*




7,8-Dichloro-2,2,4-trimethyl-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepine (**6.11b**) (0.14 g, 0.62 mmol), isocyanide (**6.4c**) (0.07 g, 0.62 mmol) and *p*-toluenesulfonic acid (0.12 g 0.62 mmol) were reacted to give 7,8-dichloro-*N*-cyclohexyl-2,4,4-trimethyl-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxamide (**6.11b**); **Physical characteristics**: brown solid; Yield: 0.11 g, 83%, **Mp**: 168-170 ºC (lit.[241] 169-172 ºC), $\mathbf{R_f}$ : 0.5, hexane/ethyl acetate (1 : 1); **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.03 (1H, d, *J* = 8.4 Hz, NH-C=O), 6.72 (1H, s, Ar-H), 6.69 (1H, s, Ar-H), 3.78-3.69 (1H, m, CH), 3.47 (1H, br s, NH), 2.82 (1H, br s, NH), 2.32 (1H, d, *J* = 14.8 Hz, $CH_2$), 1.87-1.84 (1H, d, *J* =12Hz $CH_2$), 1.68-1.55 (4H, m), 1.38-1.37 (5H, m), 1.32 (5H, m), 1.21-1.22 (5H, m); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 174.4 (C=O), 137.4 (Ar-C), 135.8 (Ar-C), 125.1 (Ar-C), 123.4 (Ar-C), 123.2 (Ar-CH) , 119.7 (Ar-CH), 60.6, 53.6, 48.3 (CH), 47.1 ($CH_2$), 33.2 ($CH_2$, cyclohexyl), 32.6 ($CH_2$, cyclohexyl), 32.3 ($CH_3$, ), 30.8 ($CH_3$), 29.9 ($CH_3$), 25.7 ($CH_2$, cyclohexyl), 24.9 ($CH_2$, cyclohexyl), 24.8 ($CH_2$, cyclohexyl); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3299, 2960, 2923, 2853, 1642, 1591;

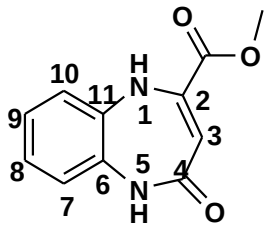
*8.6.8 General method for the synthesis of 4-oxo-4,5-dihydro-benzodiazepine-2-carboxylates (**6.16a-j**)*




In a round bottomed flask equipped with a magnetic stirrer bar, DMAD / DEtAD /DTAD (1 eq) was added to 1*H*-benzo[*d*]imidazol-2(3*H*)-one derivatives in ethanol (15 ml). The reaction mixture was refluxed for 8 h while being monitored by TLC. After completion, the reaction was cooled and the solvent removed under reduced pressure to obtain a residue, which was then purified by silica gel column chromatography using hexane- ethyl acetate as eluent, to

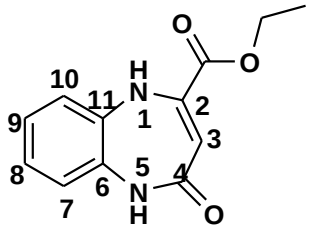
obtain the product. Alternatively, the precipitate was washed with cold ethanol and filtered to give solid product.

*8.6.8.1 Methyl 4-oxo-4,5-dihydro-1H-benzo[b][1,4]diazepine-2-carboxylate (**6.16a**)*




1*H*-Benzo[*d*]imidazol-2(3*H*)-one (**6.3a**) (0.25 g, 1.89 mmol) and dimethyl acetylenedicarboxylate (**6.5a**) (0.27 g, 1.89 mmol) were reacted to give methyl 4-oxo-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxylate (**6.16a**); **Physical characteristics**: yellow solid; Yield: 0.34 g, 83%, **Mp**: 224-226 ºC, **$R_f$** : 0.8, hexane/ethyl acetate (1 : 1); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 11.74 (1H, s, NH), 11.03 (1H, s, NH), 7.39 (1H, d, *J* = 7.2 Hz, Ar-H), 7.07-6.99 (3H, m, Ar-H), 5.52 (1H, s, H-3), 3.68 (3H, s, $CH_3$), **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 169.3 (C=O), 155.4 (C=O), 143.9 (Ar-C), 125.0 (Ar-C), 124.7 (Ar-C), 123.3 (Ar-CH), 122.4 (Ar-CH), 115.2 (Ar-CH), 115.1 (Ar-CH), 83.4 (C-3), 50.5; **FT-IR $\nu_{max}$/cm$^{-1}$**: 3215, 2902, 1686, 1614, 1501, 1598, 1429; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{11}H_{11}N_2O_3^+$ 219.0764 found 219.0752.

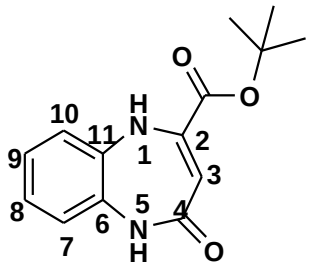
*8.6.8.2 Ethyl 4-oxo-4,5-dihydro-1H-benzo[b][1,4]diazepine-2-carboxylate (**6.16b**)*




1*H*-Benzo[*d*]imidazol-2(3*H*)-one (**6.3a**) (0.25 g, 1.89 mmol) and diethyl acetylenedicarboxylate (**6.5b**) (0.32 g, 1.89 mmol) were reacted to give ethyl 4-oxo-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxylate (**6.13b**); **Physical characteristics**: yellow solid; Yield: 0.34 g, 79%, **Mp**: 209-211 ºC, **$R_f$** : 0.9, hexane/ethyl acetate (1 : 1) ; **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 11.74 (1H, s, NH), 11.05 (1H, s, NH), 7.39 (1H, d, *J* = 7.2 Hz, Ar-H), 7.08-7.00 (3H, m, Ar-H), 5.49 (1H, s, H-3), 4.15 (2H, q, *J* = 7.2 Hz, $CH_2$), 1.24 (3H, t, *J* = 6.8

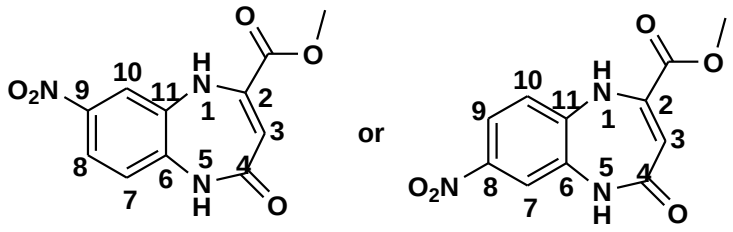
Hz, $CH_3$), **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 169.1 (C=O), 158.1 (C=O), 155.5 (Ar-C), 143.9 (Ar-C), 125.1 (Ar-C) , 124.8 (Ar-C), 123.4 (Ar-CH), 122.5 (Ar-CH), 115.4 (Ar-CH), 115.2 (Ar-CH), 83.7 (C-3), 59.1 ($CH_2$), 14.3 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3204, 2969, 1683, 1642, 1613, 1460, 1434; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{12}H_{13}N_2O_3^+$ 233.0921 found 233.0920.

*8.6.8.3 Tert-butyl 4-oxo-4,5-dihydro-1H-benzo[b][1,4]diazepine-2-carboxylate (**6.16c**)*



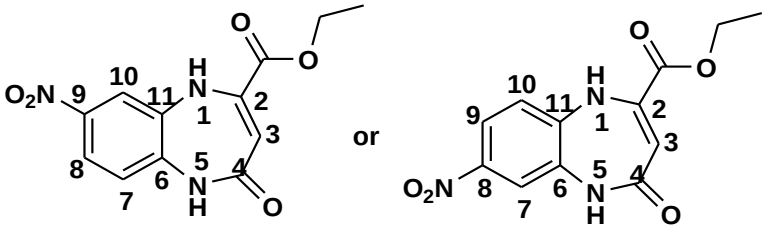
1*H*-Benzo[*d*]imidazol-2(3*H*)-one (**6.3a**) (0.21 g, 1.49 mmol) and di-*tert*-butyl acetylenedicarboxylate (**6.5c**) (0.34 g, 1.49 mmol) were reacted to give *tert*-butyl 4-oxo-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxylate (**6.13c**); **Physical characteristics**: yellow solid; Yield: 0.29 g, 75%, **Mp**: 210-212 ºC, **R$_f$** : 0.9, hexane/ethyl acetate (1 : 1); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 11.64 (1H, s, NH), 11.97 (1H, s, NH), 7.40 (1H, d, $J$ = 7.6 Hz, Ar-H), 7.05-6.99 (3H, m, Ar-H), 5.42 (1H, s, H-3), 1.48 (9H, s, $((CH_3)_3)$ **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 169.1 (C=O), 155.7 (C=O), 143.5 (Ar-C), 125.0 (Ar-C), 124.9 (Ar-C), 123.4 (Ar-CH), 122.3 (Ar-CH), 115.3 (Ar-CH), 115.1 (Ar-CH), 95.6 (C-3), 79.2 $C(CH_3)_3$, 28.1 $(CH_3)_3$; **FT-IR $\nu_{max}$/cm$^{-1}$**: 2971, 2881, 1737, 1680, 1640, 1624; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{14}H_{17}N_2NaO_3^+$ 283.1059 found 283.1051.

*8.6.8.4 Methyl 7-nitro-4-oxo-4,5-dihydro-1H-benzo[b][1,4]diazepine-2-carboxylate(**6.16d**) or methyl 8-nitro-4-oxo-4,5-dihydro-1H-benzo[b][1,4]diazepine-2-carboxylate*



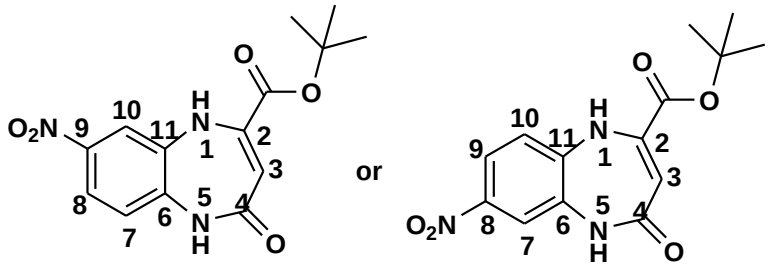
5-Nitro-1*H*-benzo[d]imidazol-2(3H)-one (**6.1i**) (0.25 g, 1.39 mmol) and dimethylacetylenedicarboxylate (**6.5a**) (0.19 g, 1.39 mmol) were reacted to give methyl 7-nitro-4-oxo-4,5-dihydro-1H-benzo[*b*][1,4]diazepine-2-carboxylate (**6.16d**) or methyl 8-nitro-4-oxo-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxylate; **Physical characteristics**: yellow solid; Yield: 0.34 g, 92%, **Mp**: 288-290 ºC, **$R_f$** : 0.9 ; **$^1$H NMR** (400 MHz, $CDCl_3$) δ 12.19 (1H, NH), 11.09 (1H, NH), 8.53 (1H, Ar-H), 7.89-7.86 (1H, d, *J* = 8.8 Hz, Ar-H), 7.17-7.15 (1H, d, *J* = 8.8 Hz, Ar-H), 5.58 (1H, CH), 3.71 (3H, s, $CH_3$); **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 168.52, 156.01 (2 × C=O), 142.01, 142.52, 130.97, 125.50, 117.82, 115.34, 111.18, 86.04, 50.85; **FT-IR *ν*max/cm$^{-1}$**: 3193, 3092, 2949, 1695, 1668, 1641, 1603, 1535; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{11}H_{10}N_3O_5^+$ 264.0615 found 264.0611.

*8.6.8.5 Ethyl 7-nitro-4-oxo-4,5-dihydro-1H-benzo[b][1,4]diazepine-2-carboxylate (**6.16e**) or ethyl 8-nitro-4-oxo-4,5-dihydro-1H-benzo[b][1,4]diazepine-2-carboxylate*



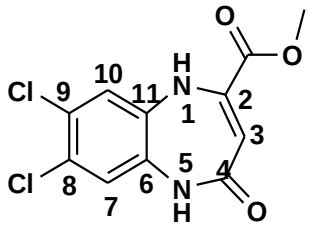

4-Nitro-*o*-phenylenediamine (**6.1i**) (0.25 g, 1.39 mmol) and diethyl acetylenedicarboxylate (**6.5b**) (0.24 g, 1.39 mmol) were reacted to give ethyl 8-nitro-4-oxo-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxylate (**6.16e**) or ethyl 8-nitro-4-oxo-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxylate; **Physical characteristics**: yellow solid; Yield: 0.33 g, 85%, **Mp**: 279-281, **$R_f$** :0.8, hexane/ethyl acetate (5 :5); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 12.17 (1H, br s, NH), 11.13 (1H, s, NH), 8.53 (1H, s, Ar-H), 7.87 (1H, d, *J* = 8.4 Hz, Ar-H), 7.16 (1H, d, *J* = 8.8 Hz, Ar-H), 5.57 (1H, s, H-3), 4.18 (2H, q, *J* = 7.2 Hz, $CH_2$), 1.25 (3H, t, *J* = 7.2 Hz, $CH_3$); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 168.3 (C=O), 156.0 (C=O), 142.7 (Ar-C), 142.6 (Ar-C), 130.9 (Ar-C), 125.5 (Ar-C), 117.8 (Ar-CH), 115.3 (Ar-CH), 111.2 (Ar-CH), 86.3 (C-3), 59.3 ($CH_2$), 14.3 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3187, 3136, 3084, 2904, 1695, 1633, 1602, 1530, 1493, 1487; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{12}H_{12}N_3O5^+$ 278.0771 found 278.0762.

*8.6.8.6 Tert-butyl 7-nitro-4-oxo-4,5-dihydro-1H-benzo[b][1,4]diazepine-2-carboxylate (**6.16f**) or tert-butyl 8-nitro-4-oxo-4,5-dihydro-1H-benzo[b][1,4]diazepine-2-carboxylate*



4-Nitro-*o*-phenylenediamine (**6.1i**) (0.20 g, 1.17 mmol) and di-*tert*-butyl acetylenedicarboxylate (**6.5c**) (0.25 g, 1.17 mmol) were reacted to give *tert*-butyl 7-nitro-4-oxo-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxylate (**6.16f**) or tert-butyl 8-nitro-4-oxo-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxylate; **Physical characteristics**: yellow solid; Yield: 0.28 g, 80%, **Mp**: 208-210 ºC, **$R_f$**: 0.5, hexane/ethyl acetate (1: 1); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 12.08 (1H, s, NH), 11.10 (1H, s, NH), 8.55 (1H, s, Ar-H), 7.86 (1H, d, *J* = 8.8 Hz, Ar-H), 7.15 (1H, d, *J* = 8.8 Hz, Ar-H), 5.51 (1H, s, H-3), 1.49 (9H, s, $((CH_3)_3)$, **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 168.2 (C=O), 156.1 (C=O), 142.7 (Ar-C), 142.1 (Ar-C), 130.9 (Ar-C), 125.7 (Ar-C), 117.7 (Ar-CH), 115.3 (Ar-CH), 111.1 (Ar-CH), 88.1 (C-3), 79.5 $C(CH_3)_3$, 28.0 $(CH_3)_3$; **FT-IR $\nu_{max}$/cm$^{-1}$**: 2975, 1690, 1616, 1541; **HRMS (ESI-TOF)** m/z: $[M+Na]^+$ Calculated for $C_{14}H_{16}N_3NaO_5^+$ 328.0909 found 328.1013.

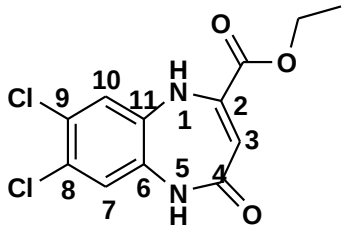
*8.6.8.7 Methyl 7,8-dichloro-4-oxo-4,5-dihydro-1H-benzo[b][1,4]diazepine-2-carboxylate (**6.16g**)*



5,6-Dichloro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3k**) (0.25 g, 1.24 mmol) and dimethyl acetylenedicarboxylate (**6.5a**) (0.18 g, 1.24 mmol) were reacted to give methyl 7,8-dichloro-4-oxo-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxylate (**6.16g**); **Physical characteristics**: grey solid; Yield: 0.29 g, 83%, **Mp**: 270-272 ºC, **$R_f$**: 0.5, hexane/ethyl acetate (1 : 1) ; **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 11.84 (1H, s, NH), 10.97 (1H, s, NH), 7.88 (1H, s, Ar-H), 7.15 (1H,

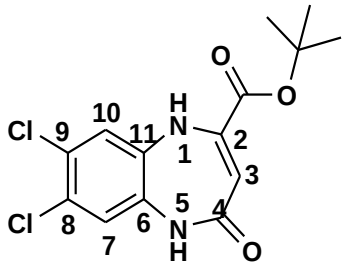
s, Ar-H), 5.56 (1H, s, H-3), 3.69 (3H, s, $CH_3$), **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 168.9 (C=O), 155.5 (C=O), 142.8 (Ar-C), 125.5 (Ar-C), 125.4 (Ar-C), 124.8 (Ar-C), 123.5 (Ar-C), 116.9 (Ar-CH), 115.9 (Ar-CH), 85.6 (C-3), 50.9 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 2991, 1688, 1622, 1500; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{11}H_9Cl_2N_2O_3^+$ 286.9985 found 286.9982.

*8.6.8.8 Ethyl 7,8-dichloro-4-oxo-4,5-dihydro-1H-benzo[b][1,4]diazepine-2-carboxylate (**6.16h**)*




5,6-Dichloro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3k**) (0.20 g, 1.24 mmol) and diethyl acetylenedicarboxylate (**6.5b**) (0.21 g, 1.24 mmol) were reacted to give ethyl 7,8-dichloro-4-oxo-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxylate (**6.16h**); **Physical characteristics**: green solid; Yield: 0.28 g, 76%, **Mp**: 265-267 ºC, **$R_f$** : 0.9, hexane/ethyl acetate (1 : 1); **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 11.81 (1H, s, NH), 10.99 (1H, s, NH), 7.85 (1H, s, Ar-H), 7.14 (1H, s, Ar-H), 5.54 (1H, s, H-3), 4.15 (2H, q, $J$ = 6.8 Hz, $CH_2$) 1.24 (3H, t, $J$ = 6.8 Hz, $CH_3$), **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 168.6 (C=O), 155.5 (C=O), 142.8 (Ar-C), 125.5 (Ar-C), 125.4 (Ar-C), 124.8 (Ar-C), 123.4 (Ar-C), 116.9 (Ar-CH), 115.9 (Ar-CH), 85.9 (C-3), 59.4 ($CH_2$), 14.3 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 3182, 3072, 1693, 1650, 1625, 1615; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{12}H_{11}Cl_2N_2O_3^+$ 301.0141 found 301.0139.

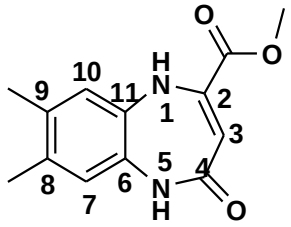
*8.6.8.9 Tert-butyl 7,8-dichloro-4-oxo-4,5-dihydro-1H-benzo[b][1,4]diazepine-2-carboxylate (**6.16i**)*




5,6-Dichloro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (**6.3k**) (0.20 g, 0.99 mmol) and dimethyl acetylenedicarboxylate (**6.5c**) (0.22 g, 0.99 mmol) were reacted to give *tert*-butyl 7,8-dichloro-

4-oxo-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxylate (**6.16i**); **Physical characteristics**: green solid; Yield: 0.29 g, 75%, **Mp**: 216-218 ºC, **$R_f$** : 0.9, hexane/ethyl acetate (1 : 1) ; **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 11.73 (1H, s, NH), 10.95 (1H, s, NH), 7.87 (1H, s, Ar-H), 7.14 (1H, s, Ar-H), 5.48 (1H, s, H-3), 1.48 (9H, s, (9H, (($CH_3$)$_3$), **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 168.5 (C=O), 155.5 (C=O), 142.3 (Ar-C), 125.5 (Ar-C), 125.4 (Ar-C), 124.7 (Ar-C), 123.2 (Ar-C), 116.8 (Ar-CH), 115.8 (Ar-CH), 87.8 (C-3), 79.5 *C*($CH_3$)$_3$, 28.0 (*C*$H_3$)$_3$; **FT-IR $\nu_{max}$/cm$^{-1}$**: 3226, 2970, 1693, 1653,1626; **HRMS (ESI-TOF)** m/z: $[M+Na]^+$ Calculated for $C_{14}H_{15}Cl_2N_2NaO_3^+$ 351.0454 found 351.0366.

*8.6.8.10 Methyl 7,8-dimethyl-4-oxo-4,5-dihydro-1H-benzo[b][1,4]diazepine-2-carboxylate (**6.16j**)*




5,6-Dimethyl-1*H*-benzo[*d*]imidazol-2(3H)-one (**6.3j**) (0.25 g, 1.54 mmol) and dimethyl acetylenedicarboxylate (**6.5a**) (0.22 g, 1.54 mmol) were reacted to give methyl 7,8-dimethyl-4-oxo-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepine-2-carboxylate (**6.16j**); **Physical characteristics**: yellow solid; Yield: 0.23 g, 62%, **Mp**: 225-227 ºC, **$R_f$** : 0.9, hexane/ethyl acetate (5 : 5) ; **$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 11.67 (1H, s, NH), 10.98 (1H, s, NH), 7.17 (1H, s, Ar-H), 6.82 (1H, s, Ar-H), 5.46 (1H, s, H-3), 3.67 (3H, s, $CH_3$), 2.16 (6H,s, 2 × $CH_3$) **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 169.7 (C=O), 155.6 (C=O), 144.2 (Ar-C), 131.7 (Ar-C), 130.9 (Ar-C), 122.9 (Ar-C), 122.6 (Ar-C), 116.1 (Ar-CH), 115.9 (Ar-CH), 82.6 (C-3), 50.7 ($CH_2$), 19.0 ($CH_3$); **FT-IR $\nu_{max}$/cm$^{-1}$**: 2948, 1679, 1615, 1508; **HRMS (ESI-TOF)** m/z: $[M+H]^+$ Calculated for $C_{13}H_{15}N_2O_3^+$ 247.1077 found 247.1075.

## 8.7. X-ray crystallography

For the X-ray crystallographic analysis of compound **6.8a** a single crystal was mounted on a Bruker Venture Photon II CMOS diffractometer or mounted on an Apex-II CCD diffractometer. Unit cell, and data collection were performed at -100(2) ºC and processed with *Apex 3* (Venture) or *Apex 2* (Apex-II). Using Olex2,[197] the structure was solved with the *ShelXT*

structure solution program[198] using Intrinsic Phasing and refined with the *ShelXL* refinement package[199] using Least Squares minimisation.

*Table 8.7.1(Chapter 3) Crystal data and structure refinement for **3.20a**, **3.20e**, **2.21a**, **3.22a** and **2.23b***

| **Crystal data** | **3.20a** | **3.20e** | **3.21a** | **3.22a** | **3.23b** |
|---|---|---|---|---|---|
| CCDC | 1981633 | 1981634 | 1981635 | 1986041 | 1986042 |
| Empirical formula | $C_{17}H_{21}N_3O_7S$ | $C_{19}H_{23}N_3O_7S$ | $C_{15}H_{21}N_3O_5$ | $C_{13}H_{19}N_5O_4$ | $C_7H_5N_3O_3S$ |
| Formula weight | 411.43 | 437.46 | 323.35 | 309.33 | 211.20 |
| Temperature/K | 173.15 | 173.15 | 173.15 | 173.15 | 173.15 |
| Crystal system | monoclinic | orthorhombic | monoclinic | triclinic | orthorhombic |
| Space group | $P2_1/c$ | $Pna2_1$ | $P2_1/n$ | P-1 | Pbca |
| a/Å | 10.9954(9) | 17.1233(5) | 9.5486(7) | 9.8469(8) | 14.1861(8) |
| b/Å | 12.9096(11) | 6.5690(2) | 10.0227(8) | 11.8782(10) | 6.4531(4) |
| c/Å | 13.3590(12) | 35.6586(11) | 16.2563(12) | 14.4773(11) | 18.4965(9) |
| α/° | 90 | 90 | 90 | 73.074(4) | 90 |
| β/° | 98.507(3) | 90 | 90.512(3) | 78.287(5) | 90 |
| γ/° | 90 | 90 | 90 | 83.256(4) | 90 |
| Volume/Å$^3$ | 1875.4(3) | 4011.0(2) | 1555.7(2) | 1583.2(2) | 1693.25(16) |
| Z | 4 | 8 | 4 | 4 | 8 |
| $\rho_{calc}$g/cm$^3$ | 1.457 | 1.449 | 1.381 | 1.298 | 1.657 |
| μ/mm$^{-1}$ | 0.219 | 0.210 | 0.105 | 0.098 | 0.365 |
| F(000) | 864.0 | 1840.0 | 688.0 | 656.0 | 864.0 |
| Crystal size/mm$^3$ | 0.26 × 0.202 × 0.108 | 0.325 × 0.111 × 0.033 | 0.44 × 0.326 × 0.282 | 0.229 × 0.083 × 0.06 | 0.287 × 0.145 × 0.055 |
| Radiation | MoKα (λ = 0.71073) | MoKα (λ = 0.71073) | MoKα (λ = 0.71073) | MoKα (λ = 0.71073) | MoKα (λ = 0.71073) |
| 2Θ range for data collection/° | 6.076 to 56.686 | 4.57 to 49.998 | 4.928 to 56.73 | 2.988 to 50 | 4.404 to 49.968 |
| Index ranges | -14 ≤ h ≤ 14, -17 ≤ k ≤ 17, -17 ≤ l ≤ 17 | -20 ≤ h ≤ 20, -7 ≤ k ≤ 7, -42 ≤ l ≤ 39 | -12 ≤ h ≤ 12, -13 ≤ k ≤ 13, -21 ≤ l ≤ 21 | -8 ≤ h ≤ 11, -14 ≤ k ≤ 14, -17 ≤ l ≤ 16 | -15 ≤ h ≤ 16, -7 ≤ k ≤ 7, -21 ≤ l ≤ 21 |
| Reflections collected | 34233 | 32358 | 25806 | 12163 | 8226 |
| Independent reflections | 4669 [$R_{int}$ = 0.0350, $R_{sigma}$ = 0.0185] | 5970 [$R_{int}$ = 0.1239, $R_{sigma}$ = 0.1138] | 3891 [$R_{int}$ = 0.0631, $R_{sigma}$ = 0.0481] | 5573 [Rint = 0.0932, Rsigma = 0.2651] | 1494 [Rint = 0.0750, Rsigma = 0.0713] |
| Data/restraints/parameters | 4669/0/259 | 5970/1/547 | 3891/0/213 | 5573/0/407 | 1494/0/128 |
| Goodness-of-fit on F$^2$ | 1.026 | 0.879 | 1.043 | 0.694 | 0.858 |
| Final R indexes [I>=2σ (I)] | $R_1$ = 0.0338, $wR_2$ = 0.0829 | $R_1$ = 0.0501, $wR_2$ = 0.0985 | $R_1$ = 0.0532, $wR_2$ = 0.1102 | R1 = 0.0492, wR2 = 0.0631 | R1 = 0.0362, wR2 = 0.0540 |
| Final R indexes [all data] | $R_1$ = 0.0425, $wR_2$ = 0.0874 | $R_1$ = 0.0982, $wR_2$ = 0.1110 | $R_1$ = 0.0867, $wR_2$ = 0.1227 | R1 = 0.1798, wR2 = 0.0810 | R1 = 0.0704, wR2 = 0.0603 |
| Largest diff. peak/hole / e Å$^{-3}$ | 0.34/-0.25 | 0.61/-0.28 | 0.28/-0.26 | 0.25/-0.29 | 0.20/-0.24 |

*Table 8.7.2 (Chapter5) Crystal data and structure refinement for **5.17b**, **5.17c**, **5.17e**, **5.17g** and **5.6a***

| **Crystal data** | **5.17b** | **5.17c** | **5.17e** | **5.17g** | **5.6b** |
|---|---|---|---|---|---|
| CCDC | | | | | |
| Empirical formula | $C_9H_8N_2O_3S$ | $C_{10}H_{12}N_2O_4S$ | $C_{15}H_{21}N_3O_5$ | $C_{14}H_9ClN_2O_3S$ | $C_8H_7N_3O_3S$ |
| Formula weight | 224.23 | 256.28 | 323.35 | 320.74 | 225.23 |
| Temperature/K | 173.15 | 173.15 | 173.15 | 173.15 | 173.15 |
| Crystal system | orthorhombic | monoclinic | monoclinic | orthorhombic | triclinic |
| Space group | Pbca | $P2_1/c$ | $P2_1/n$ | Pbca | P-1 |
| a/Å | 12.9902(2) | 7.4587(3) | 9.5486(7) | 15.167(2) | 8.6289(5) |

| Crystal data | 5.17b | 5.17c | 5.17e | 5.17g | 5.6b |
|---|---|---|---|---|---|
| b/Å | 6.9810(2) | 20.1251(9) | 10.0227(8) | 8.9457(14) | 9.9003(6) |
| c/Å | 21.2269(4) | 8.0128(3) | 16.2563(12) | 19.944(3) | 11.9343(7) |
| α/° | 90 | 90 | 90 | 90 | 76.618(2) |
| β/° | 90 | 108.8923(15) | 90.512(3) | 90 | 72.127(2) |
| γ/° | 90 | 90 | 90 | 90 | 89.767(2) |
| Volume/Å$^3$ | 1924.95(7) | 1137.98(8) | 1555.7(2) | 2706.0(7) | 941.51(10) |
| Z | 8 | 4 | 4 | 8 | 4 |
| $\rho_{calc}$g/cm$^3$ | 1.547 | 1.496 | 1.381 | 1.575 | 1.589 |
| μ/mm$^{-1}$ | 0.323 | 0.290 | 0.105 | 0.447 | 0.333 |
| F(000) | 928.0 | 536.0 | 688.0 | 1312.0 | 464.0 |
| Crystal size/mm$^3$ | 0.452 × 0.112 × 0.074 | 0.467 × 0.091 × 0.051 | 0.44 × 0.326 × 0.282 | 0.435 × 0.048 × 0.04 | 0.38 × 0.351 × 0.18 |
| Radiation | MoKα (λ = 0.71073) | MoKα (λ = 0.71073) | MoKα (λ = 0.71073) | MoKα (λ = 0.71073) | MoKα (λ = 0.71073) |
| 2Θ range for data collection/° | 3.838 to 56.662 | 4.048 to 56.708 | 4.928 to 56.73 | 4.084 to 50 | 3.696 to 56.728 |
| Index ranges | -17 ≤ h ≤ 17, -9 ≤ k ≤ 9, -28 ≤ l ≤ 27 | -9 ≤ h ≤ 7, -26 ≤ k ≤ 26, -8 ≤ l ≤ 10 | -12 ≤ h ≤ 12, -13 ≤ k ≤ 13, -21 ≤ l ≤ 21 | -18 ≤ h ≤ 18, -10 ≤ k ≤ 10, -23 ≤ l ≤ 23 | -11 ≤ h ≤ 11, -13 ≤ k ≤ 13, -15 ≤ l ≤ 15 |
| Reflections collected | 16732 | 11779 | 25806 | 16592 | 16340 |
| Independent reflections | 2400 [$R_{int}$ = 0.0437, $R_{sigma}$ = 0.0295] | 2839 [$R_{int}$ = 0.0356, $R_{sigma}$ = 0.0325] | 3891 [$R_{int}$ = 0.0631, $R_{sigma}$ = 0.0481] | 2381 [$R_{int}$ = 0.1235, $R_{sigma}$ = 0.1070] | 4715 [$R_{int}$ = 0.0461, $R_{sigma}$ = 0.0464] |
| Data/restraints/parameters | 2400/0/138 | 2839/0/160 | 3891/0/213 | 2381/0/191 | 4715/0/273 |
| Goodness-of-fit on F$^2$ | 1.063 | 1.051 | 1.043 | 0.795 | 0.951 |
| Final R indexes [I>=2σ (I)] | $R_1$ = 0.0341, $wR_2$ = 0.0820 | $R_1$ = 0.0359, $wR_2$ = 0.0900 | $R_1$ = 0.0532, $wR_2$ = 0.1102 | $R_1$ = 0.0362, $wR_2$ = 0.0599 | $R_1$ = 0.0333, $wR_2$ = 0.0732 |
| Final R indexes [all data] | $R_1$ = 0.0480, $wR_2$ = 0.0899 | $R_1$ = 0.0474, $wR_2$ = 0.0973 | $R_1$ = 0.0867, $wR_2$ = 0.1227 | $R_1$ = 0.0910, $wR_2$ = 0.0696 | $R_1$ = 0.0444, $wR_2$ = 0.0771 |
| Largest diff. peak/hole / e Å$^{-3}$ | 0.28/-0.27 | 0.28/-0.29 | 0.28/-0.26 | 0.22/-0.26 | 0.24/-0.37 |

*Table 8.7.3 (Chapter 6)Crystal data and structure refinement for **5.8a**, **5.8k**, **5.14f** and **5.14g***

| Crystal data | 6.8a | 6.8k | 6.14f | 6.14g |
|---|---|---|---|---|
| CCDC | | | | |
| Empirical formula | | $C_{19}H_{29}N_3$ | $C_{14}H_{19}Cl_2N_3$ | $C_{18}H_{27}Cl_2N_3$ |
| Formula weight | | 299.45 | 300.22 | 356.32 |
| Temperature/K | | 173.15 | 173.15 | 173.15 |
| Crystal system | | monoclinic | orthorhombic | orthorhombic |
| Space group | | $P2_1/n$ | $P2_12_12_1$ | $P2_12_12_1$ |
| a/Å | | 12.5443(4) | 8.4799(2) | 8.1778(2) |
| b/Å | | 10.8032(3) | 11.2725(3) | 10.8600(3) |
| c/Å | | 13.1304(4) | 16.0259(5) | 21.2146(6) |
| α/° | | 90 | 90 | 90 |
| β/° | | 90.6124(19) | 90 | 90 |
| γ/° | | 90 | 90 | 90 |
| Volume/Å$^3$ | | 1779.31(9) | 1531.91(7) | 1884.09(9) |
| Z | | 4 | 4 | 4 |
| $\rho_{calc}$g/cm$^3$ | | 1.118 | 1.302 | 1.256 |
| μ/mm$^{-1}$ | | 0.066 | 0.415 | 0.348 |
| F(000) | | 656.0 | 632.0 | 760.0 |

| **Crystal data** | **6.8a** | **6.8k** | **6.14f** | **6.14g** |
|---|---|---|---|---|
| Crystal size/$mm^3$ | | 0.526 × 0.295 × 0.197 | 0.411 × 0.321 × 0.134 | 0.269 × 0.256 × 0.196 |
| Radiation | | MoKα (λ = 0.71073) | MoKα (λ = 0.71073) | MoKα (λ = 0.71073) |
| 2Θ range for data collection/° | | 4.466 to 56 | 4.418 to 56.616 | 3.84 to 55.996 |
| Index ranges | | -16 ≤ h ≤ 16, -14 ≤ k ≤ 14, -17 ≤ l ≤ 17 | -11 ≤ h ≤ 11, -12 ≤ k ≤ 15, -21 ≤ l ≤ 20 | -10 ≤ h ≤ 10, -14 ≤ k ≤ 14, -28 ≤ l ≤ 28 |
| Reflections collected | | 28352 | 20413 | 36147 |
| Independent reflections | | 4297 [$R_{int}$ = 0.0405, $R_{sigma}$ = 0.0291] | 3823 [$R_{int}$ = 0.0299, $R_{sigma}$ = 0.0247] | 4542 [$R_{int}$ = 0.0462, $R_{sigma}$ = 0.0307] |
| Data/restraints/parameters | | 4297/0/211 | 3823/0/185 | 4542/0/223 |
| Goodness-of-fit on $F^2$ | | 1.055 | 1.045 | 1.069 |
| Final R indexes [I>=2σ (I)] | | $R_1$ = 0.0421, $wR_2$ = 0.0994 | $R_1$ = 0.0269, $wR_2$ = 0.0618 | $R_1$ = 0.0298, $wR_2$ = 0.0665 |
| Final R indexes [all data] | | $R_1$ = 0.0617, $wR_2$ = 0.1092 | $R_1$ = 0.0311, $wR_2$ = 0.0638 | $R_1$ = 0.0372, $wR_2$ = 0.0692 |
| Largest diff. peak/hole / e $Å^{-3}$ | | 0.23/-0.19 | 0.17/-0.16 | 0.19/-0.16 |

## 8.8 Preliminary molecular modeling

### *8.8.1 Ligand preparation*

The synthesised ligands were prepared from 2-dimensional to 3-dimensional before docking by "LigPrep protocol" for generation of possible combination of stereoisomers and conformers using Schrödinger Maestro 11.2.

### *8.8.2 The absorption, distribution, metabolism and excretion (ADME) properties*

The absorption, distribution, metabolism and excretion (ADME) which describes the disposition of pharmaceutical compounds for application were also determined. The synthesised ligands were subjected for ADME calculations using the "QikProp process" from the Schrödinger Maestro 11.2. The ADME calculations were carried out in order to analyse solubility, intestinal absorption, skin permeability, central nervous system activity (CNS), blood-brain barrier (BBB) penetration.

### *8.8.3 Preparation of HIV IN-1 protein*

The HIV-1-IN-LEDGF/p75 used for modelling was obtained from Protein Data Bank (PDB) as an X-ray crystal structure code 2B4J.[149] Using "Protein Preparation Wizard" from Schrödinger Maestro 11.2 chain C and D (LEDGF/p75) were removed to obtain HIV-IN (Chain A and B). Water molecules and hydrogens were removed and locations of new assigned hydrogens have been minimized using the OPLS force field.[156] The missing residues and loop regions of chain A and B were constructed and the cap terminals were considered.[157] Receptor Grid Generation process was followed to modify and detect the binding site for docking purpose.[158]

### *8.8.4 Molecular docking of synthesised compounds into HIV IN binding site*

The molecular docking studies were performed to estimate the binding affinity of the National Institutes of Health (NIH) Clinical Collection library consisting of **727** drugs and ChemBridge Diverse Collection compound library containing **20000** ligands.

The NIH and ChemBridge compounds were positioned in the same binding sites of the HIV-1-IN were LEDGF binds using "ligand docking" protocol from the Schrödinger Maestro 11.2.

During docking the program takes ligand-receptor poses as input and provided rankings or the binding affinity of the pose. These poses gave ligand docking scores, glide scores and glide emodel scores and then their binding energy was then calculated using MM-GBSA method.

## 8.9 Biological evaluation methods

### *8.9.1 Development of a LEDGF/p75-HIV-1 IN AlphaScreen assay*

Alpha (amplified luminescent proximity homogenous assay) technology is an alternative to the traditional ELISA. In this assay no wash steps are needed and it is a bead based assay. This type of assay can be used for an array of interactions but in our case will be used for protein-protein interactions. Two types of beads are needed for each assay. The first bead is known as a donor bead and contains phthalcyanine which is a photosensitiser that converts ambient oxygen to a reactive form of oxygen when illuminated at 620 nm. The second bead is an acceptor bead and consists of a thioxene derivative that emits light. In solution, when the donor and acceptor bead are brought in close enough proximity by way of protein-protein interaction, the energy that is transferred from the singlet oxygen to the thioxene derivative results in the production of light which is measurable. An AlphaScreen assay previously described by Christ and co-workers was used to screen the compounds.[146] A stock solution of 1.5 µM GST tagged HIV-1 IN subtype B or C was made up in AlphaScreen assay buffer (150 mM NaCl, 25 mM Tris, 2 mM $MgCl_2$, 1 mM DTT, and 0.01% Tween 20 and 0.02% BSA, pH 7.4). A final concentration of 300 nM was added to a ½ area optiplate and incubated with each inhibitor at single doses and then later in doses ranging from 200 µM down to 0.3 µM for 30 minutes at room temperature with gentle shaking. Subsequently 100 nM His-tagged LEDGF/p75 was added and incubated for one hour at room temperature with gentle shaking. A final concentration of 10 µg/ml nickel chelating acceptor beads (Perkin Elmer, USA) and GST donor beads (Perkin Elmer, USA) were added and incubated in the dark at 30°C with gentle shaking. The plate was subsequently read between 520-620 nm on the EnSpire® plate reader (Perkin Elmer, USA). The control ran on the plate to ensure accurate reading of the compounds effects were: beads alone, HIV-1 IN subtype B or C alone, LEDGF/p75 alone, HIV-1 IN subtype B or C and LEDGF/p75 alone, each protein in isolation with beads alone. Once single dose inhibition by the inhibitor was determined, a dose response curve was determined to obtain a half maximal inhibitor concentration ($IC_{50}$) for all inhibitors under investigation. The percentage inhibition for each dose was determined with use of the controls and plotted against

the log of the concentration. The data obtained was plotted on a sigmoidal curve using Origin 8.1 (Origin Lab Corporation, UK) and an $IC_{50}$ and hills coefficient was determined. Statistics were calculated using the SISA t-test found at, http://www.quantitativeskills.com/sisa/.

*8.9.2 Development of a Dimerization AlphaScreen Assay*

For the initial HIV-1 IN-IN Alpha Screen assay, HIS tagged HIV-1 IN was incubated with GST tagged HIV-1 IN at varying concentrations to determine the optimal concentration for use. It was determined that 300 nM of each protein produced sufficient signal for detection. We then used know HIV-1 IN ALLINI compounds to test if the assay worked. MUT101 and CX05168 were used to determine if the assay accurately determined multimerization of HIV-1 IN. a final concentration of 300 nM for both GST and HIS tagged HIV-1 IN was incubated together with an initial concentration of 100 µM MUT101 and CX05168 and placed in the fridge overnight to allow for crosslinking. Ssubsequently the following morning nickel chelating acceptor beads (Perkin Elmer, USA) and GST donor beads (Perkin Elmer, USA) were added to a final concentration of 10 µg/ml and incubated at room temperature for 2 hours followed by 30°C in the dark with gentle shaking for 30 minutes. Once incubation was complete the plate was read between 520-620 nm on the EnSpire® plate reader (Perkin Elmer, USA). Subsequent to the single dose testing a range of concentrations were tested to determine the increase of multimerization with increase in compound concentration.

*8.9.3 Alphascreen assays*

*8.9.3.1 LEDGF/p75-HIV-1 IN assay*

A stock solution of 1.5 µM GST tagged HIV-1 IN subtype B or C was made up in AlphaScreen assay buffer (150 mM NaCl, 25 mM Tris, 2 mM MgCl2, 1 mM DTT, and 0.01% Tween 20 and 0.02% BSA, pH 7.4). A final concentration of 300 nM was added to a ½ area optiplate and incubated with each inhibitor at single doses of 100 uM for 30 minutes at room temperature with gentle shaking. Subsequently 100 nM His-tagged LEDGF/p75 was added and incubated for one hour at room temperature with gentle shaking. A final concentration of 10 µg/ml nickel chelating acceptor beads (Perkin Elmer, USA) and Glutathione donor beads (Perkin Elmer, USA) were added and incubated in the dark at 30°C with gentle shaking. The plate was subsequently read between 520-620 nm on the EnSpire® plate reader (Perkin Elmer, USA).

The control ran on the plate to ensure accurate reading of the compounds effects were: beads alone, HIV-1 IN subtype B or C alone, LEDGF/p75 alone, HIV-1 IN subtype B or C and LEDGF/p75 alone, each protein in isolation with beads alone.

*8.9.3.2 Multimerization assay*

The published HIV-1 IN-LEDGF/p75 inhibitors known as ALLINIs show drastic effects on the dimerization of HIV-1 IN. As such we ran a dimerization experiment to determine if the inhibitors had an effect on the dimerization of HIV-1 IN.

An AlphaScreen assay previously described by Christ and co-workers was adjusted to screen the compounds.[146] A stock solution of 500 nM GST tagged HIV-1 IN subtype B or C was made up in AlphaScreen assay buffer (150 mM NaCl, 25 mM Tris, 2 mM MgCl2, 1 mM DTT, and 0.01% Tween 20 and 0.02% BSA, pH 7.4) and also 500 nM HIS tagged HIV-1 IN subtype B or C was made up in the same assay buffer. A final concentration of 300 nM of both the GST tagged and HIS tagged HIV-1 IN was added to a ½ area optiplate and incubated with each inhibitor at single doses of 100 uM over night at 4°C. Subsequently the following morning a final concentration of 10 µg/ml nickel chelating acceptor beads (Perkin Elmer, USA) and glutathione donor beads (Perkin Elmer, USA) were added and incubated in the dark for 2 hours ar room temperature and then at 30°C for 30 minutes. The plate was subsequently read between 520-620 nm on the EnSpire® plate reader (Perkin Elmer, USA).

*8.9.4 Cytotoxicity assay*

The cytotoxicity test were done by Dr Qasim Fish at Mintek, AMD at Biomed group laboratory. The day of cytotoxicity testing, MT-4 cells (obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: MT-4 catalogue number 120) were counted and seeded at $5\times10^4$ cells per well. A total of 100 µl of cells were added to each well of a 96-well cell culture plates (TTP) for testing. The volume in the last row of wells of each plate was further increased to 197 µl of media containing MT-4 cells. The plate was placed into the incubator to equilibrate to 37 $^oC$ and 5% $CO_2$. Test compounds were prepared in plate format and each compound was at 25 mM stock concentration in DMSO. A total of 3.2 µL compound was added to the final row of wells containing 197µL cells and mixed to ensure the solution was homogeneous with the cells. A 2 fold serial dilution of an 8 times series was performed and the final 100 µL was discarded. The plate was placed at 37 °C and 5% $CO_2$ for 5 days. On the 5th day the cell

viability was assessed using the Cell Titre 96® Aqueous One Solution Cell Proliferation assay (Promega) as per manufactures instructions. Briefly, 20 µL of MTS solution was added to each well and incubated at 37 ºC for 4 hours. The absorbance of the plates were then read at 490 nm on a Spectra Max spectrophotometer (Molecular Devices). The data analysis was completed on Origin 8.1 with the log value of the compounds molar concentration plotted against the percentage cell viability determined by the absorbance level by sigmoidal fitting. From the curve, a half maximal cytotoxicity $CC_{50}$ for compounds was determined. Auranofin was used as a cytotoxic control compound starting at 100 µM across a 2 fold serial dilution of a 8 dilution series. DMSO was used a background control and 2 fold serially diluted from 1.5%.

*8.9.5 Antimicrobial assay*

This study was carried out by Ms Mapula Makgoba and Dr Qasim Fish at the Biomed group laboratory at Mintek. The MIC of the synthesised compounds was determined by broth microdilution assay using sterile 96-well flat-bottomed plate. Ampicillin was used as a control for *E.coli* and *S. aureus*, while Gentamycin was used as a controls for *B. subtilis* and *P. aeruginosa*.[201] The third plate was coming from the synthesised compounds. The bacterial strains used were acquired from Kwik stick microbiologics. TSB (Tryptic Soy Broth) medium was used as the media of choice and INT (*p*-iodonitrotetrazolium chloride) was used as growth indicator.

Preparation of inoculation medium, microbial cells and growth indication

TSB medium [Tryptone (10 g), yeast extract (5 g), and sodium chloride (10 g)] was dissolved in 1000 ml of distilled water. The medium was autoclaved for two hours and later cooled at room temperature. Microbial cells were prepared by inoculating the cells into 50 ml tubes filled with 10 ml of TSB medium. The tubes were incubated for 24 hr at 37 ºC with shaking at 200 rpm. 10 mg of INT was dissolved into 50 ml of distilled water. 4 fold dilution was performed using TBS media on the overnight microbial cells to obtain the dilution factor at which the microbial cells are adjusted to 0.1 McFarland standard at OD600 reading under spectrophotometer by cuvettes.

Broth microdilution method

The control plates were prepared by adding 100 µl of TBS media into the wells and 192 µL into the first wells. 0.4 mg/ml of 10 mg/ml gentamycin stock was added and 2-fold serial dilution was performed with concentration ranging from 0.4 mg/ml to 0.0013mg/ml. 4 mg/ml of 100 mg/ml ampicillin stock was added followed by a 2-fold serial dilution with concentration ranging from 4 mg/ml to 0.03 mg/ml. 100 µL of adjusted microbial suspension was added into each well and the plate was incubated for 24hrs at 37ºC. 40 µL of INT (indicator) was added into each well after 24h incubation and again incubated for 30 min at 37 ºC. The growth of bacterial strains was determined by observing the colour change and measuring absorbance at 600 nm using a microplate reader.

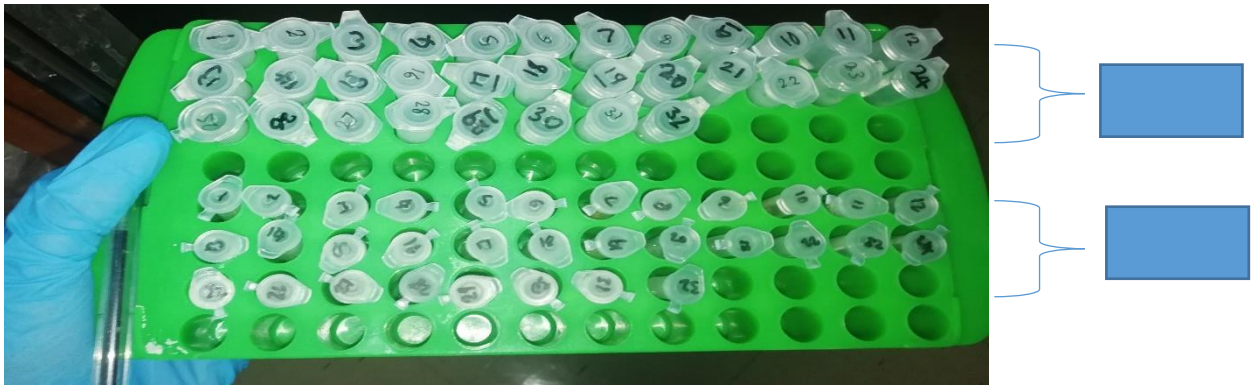


**Figure 8.1**: First 64 synthesised compounds stored at -20 °C

All synthesised compounds as shown on Figure 8.1 have been tested against *E. coli*, *B. subtilis*, *S. aureus* and *P. aeruginosa*. The compounds at 10mM stock concentration were tested at 0.4 mM first, followed by testing of the higher concentrated (100 mM) at 4 mM. The plates were divided into half, the first half was for testing against *E. coli/ S. aureus* and another half for *B. subtilis/ S. aeruginosa*. Three compounds per plate were tested and each compound was tested in duplicates. The synthesised compounds at 192 µL of TBS was added into the first wells, followed by inoculation of compounds. 2-fold serial dilution was performed with 100 µL medium inoculated in the other wells. 100 µL of adjusted bacterial suspension was added into each well and the plate was incubated for 24 hr at 37ºC. The growth of bacterial strains was determined by measuring absorbance at 600 nm using a microplate reader.

*8.9.6 Antiviral assay*

An HIV-1 chronically infected ACH-2 clone was obtained through the NIH HIV Reagent Program, Division of AIDS, NIAID, NIH: ACH-2 Cells, ARP-349, contributed by Dr. Thomas Folks. The cells were grown and maintained in RPMI 1640 (Sigma) supplemented with 10 mM HEPES, 2 mM L-glutamine, non-essential amino acids and 10% heat inactivated fetal bovine serum at 37 $^{o}$C and 5% $CO_2$. The ACH-2 cells were activated by 1 µM phorbol myristate acetate (PMA).[250] Supernatants were harvested at 48 hr by centrifugation at 4000 x g and stored at -80 $^{o}$C until use. MT4 cells were grown and maintained in RPMI (Gibco) supplemented with 2 mM L-glutamine, non-essential amino acids and 10% heat inactivated fetal bovine serum at 37 $^{o}$C and 5% $CO_2$. Harvested supernatant containing HIV-1 was added to MT4 cells and viral absorption was allowed for 24hr similar to the method by Rosenwirth and co-workers.[251] After viral absorption, the cells were washed once with fresh media, a sample was taken, and HIV-1 infection was allowed to develop. Seven days after infection, MT4 supernatant samples were tested for the presence of p24 antigen using the bioelisa HIV-1+2 Ag/Ab kit (biokit) as per manufacturer's instructions. After HIV-1 infection was confirmed, supernatant samples were isolated by centrifugation at 4000 x g and immediately used to infect $1x10^5$ cells/ml of MT4 cells. Virus was allowed to adsorb and 24 h later the cells were washed with fresh media and 100 µl of cells, $1x10^4$ cells, were aliquoted into a round bottom 96 well plate (TPP). Uninfected cells were also prepared. A day 1 sample was taken and stored at -80$^{o}$C until final testing. Compounds were diluted in fresh media to 200 µM concentrations and 100 µl was added to each test well, the final concentration of compounds was 100 µM. Control compounds include Dolutegravir and Raltegravir and were used at 10 µM each, blank wells contained equal volumes of DMSO. Upper and lower asymptotes were determined by untreated MT4 cells either infected with HIV (100%) or not infected with HIV (0%). Four days after infection, p24 presence was determined using the bioelisa HIV-1+2 Ag/Ab kit as per manufacturer's instructions. The data was used to determine if the selected concentration of compounds would reduce the amount of p24 and thereby indicate a reduction in HIV infectivity.

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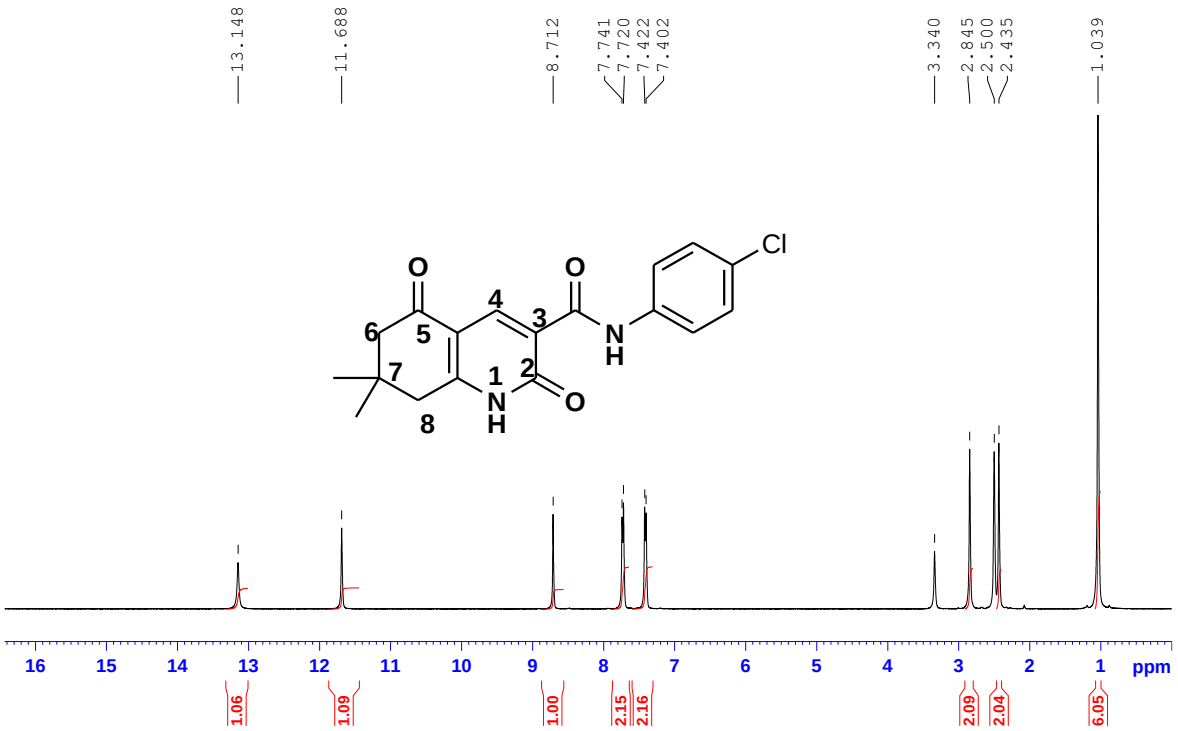
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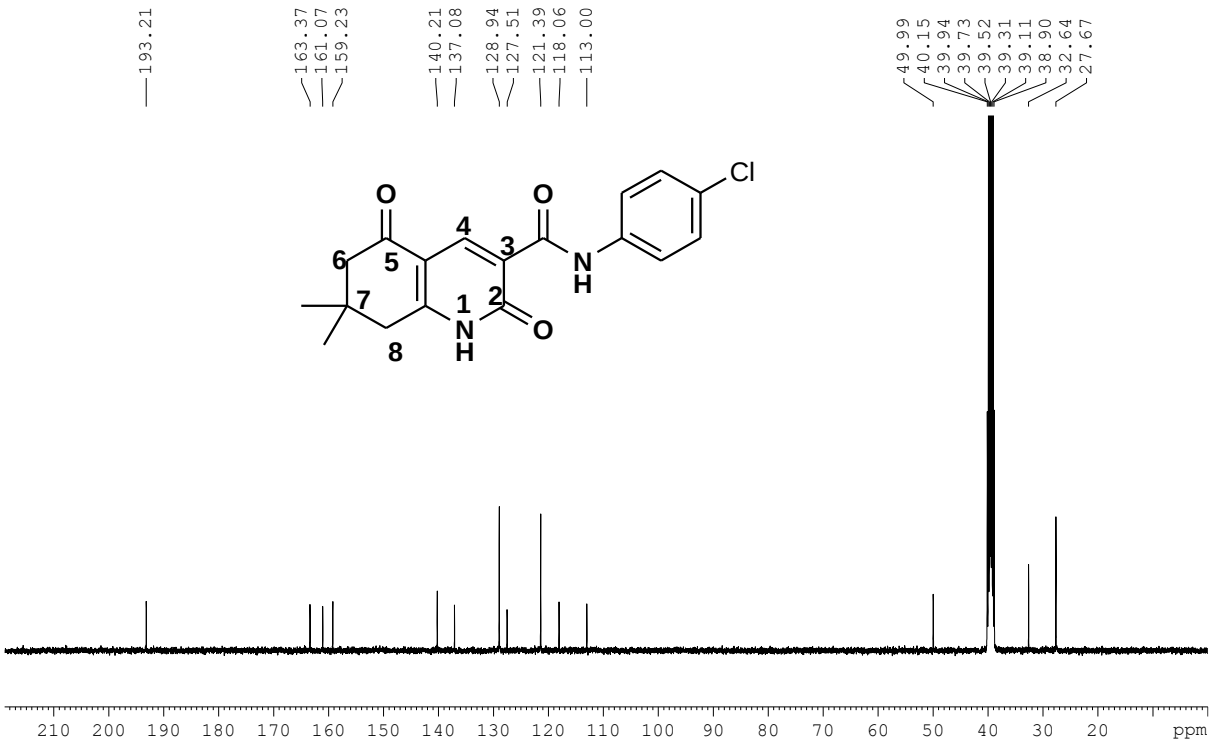
# Appendix, presentation of selected data

## CHAPTER 2: selected spectroscopic data

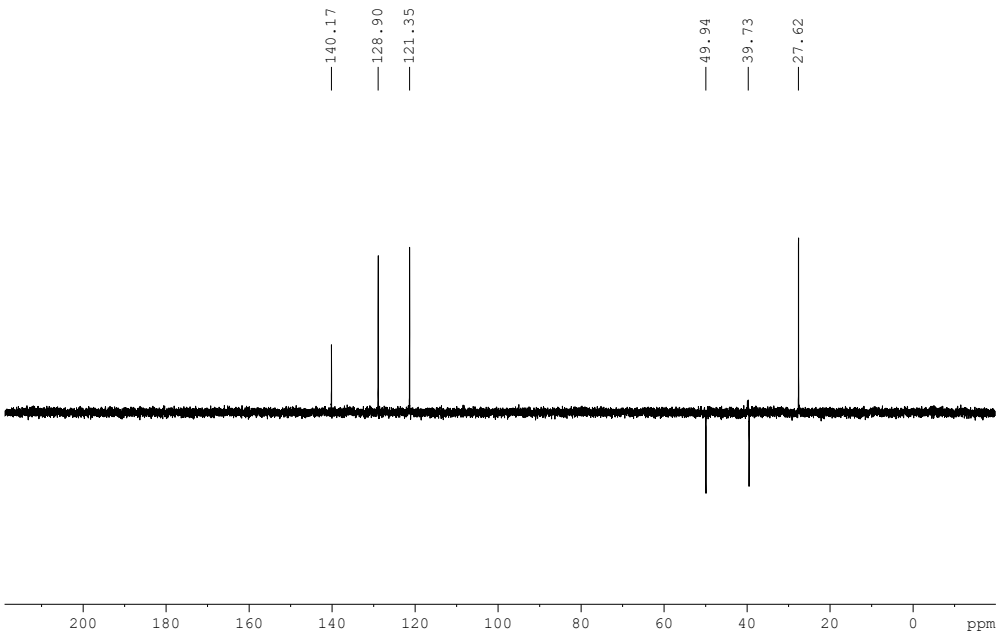
### 2.8a



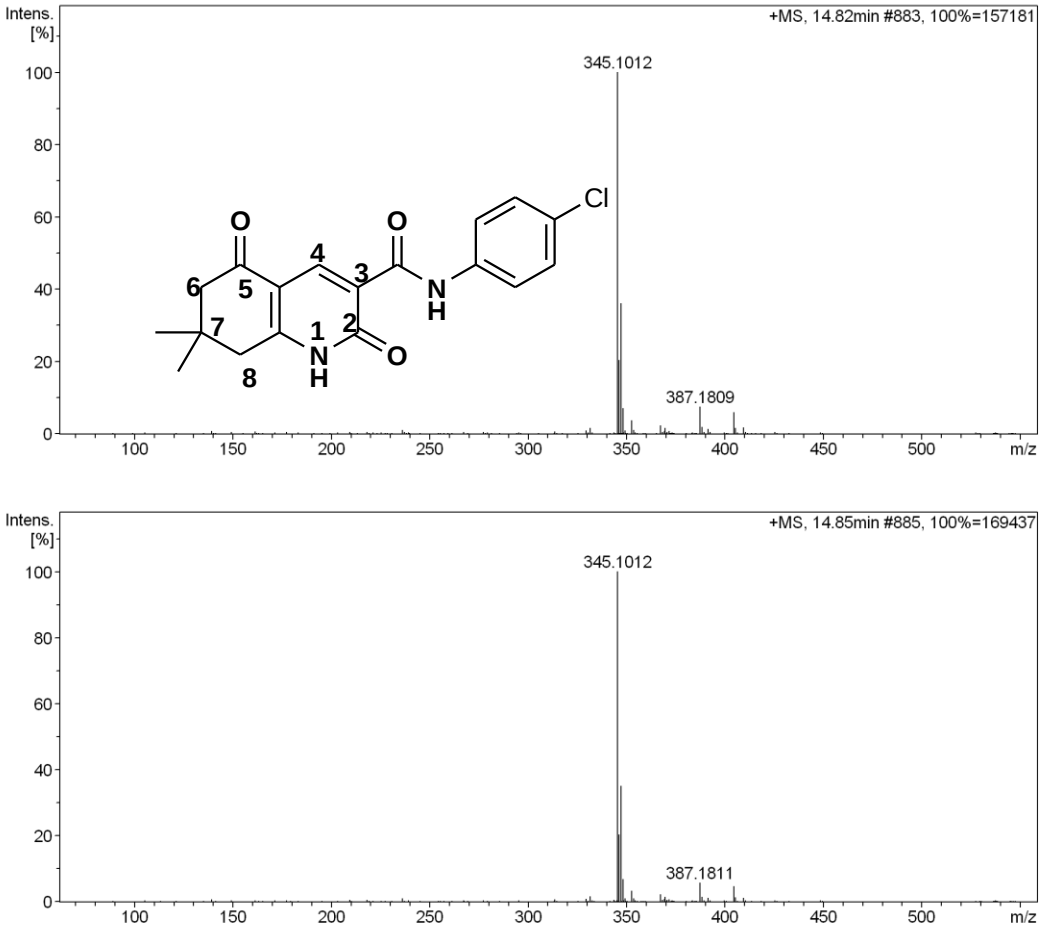

### 2.8a



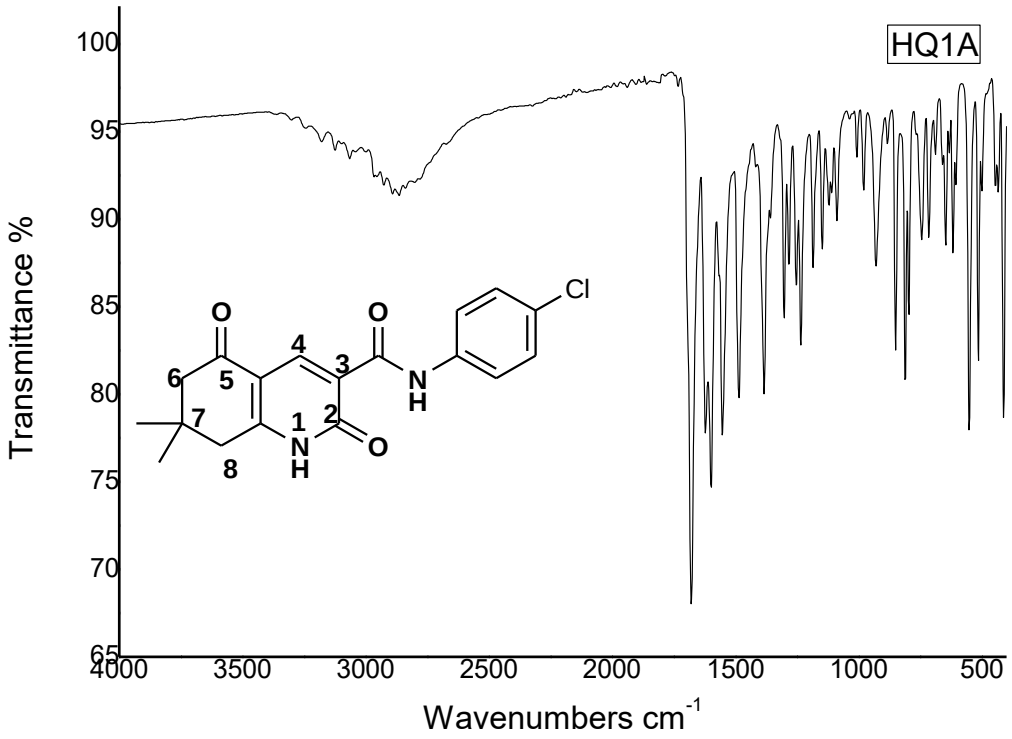

### 2.8a


140.17
128.90
121.35
49.94
39.73
27.62
200
180
160
140
120
100
80
60
40
20
0
ppm

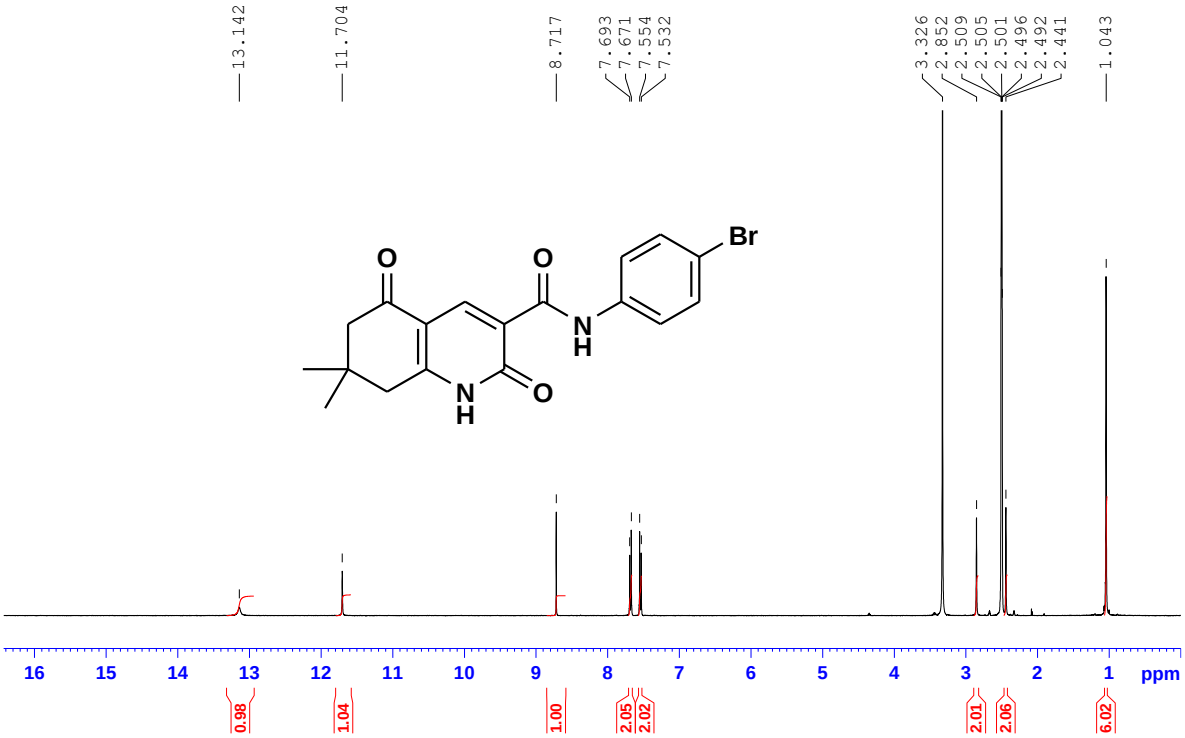
**2.8a**


Intens.
[%]
+MS, 14.82min #883, 100%=157181
345.1012
387.1809
Cl
O
O
N
H
O
N
H
m/z
Intens.
[%]
+MS, 14.85min #885, 100%=169437
345.1012
387.1811
m/z

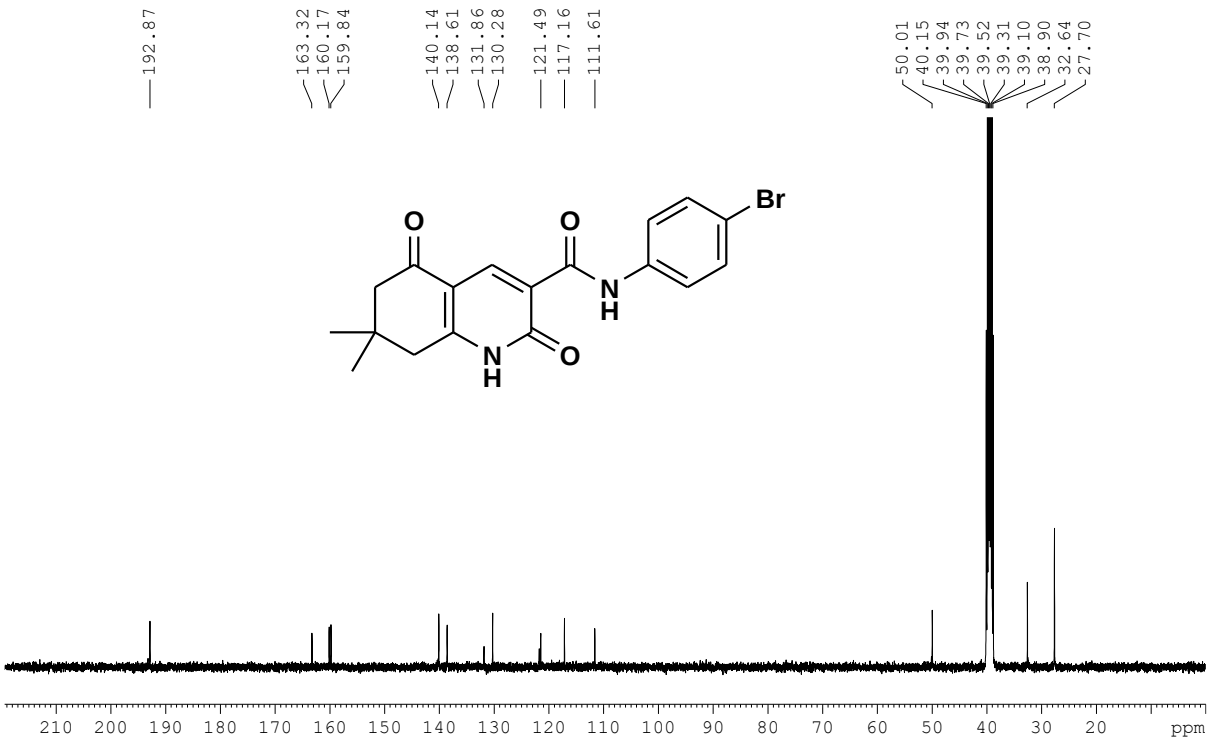
**2.8a**


HQ1A
Transmittance %
Wavenumbers cm-1
100
95
90
85
80
75
70
65
4000
3500
3000
2500
2000
1500
1000
500
O
O
Cl
N
H
O
N
H
O

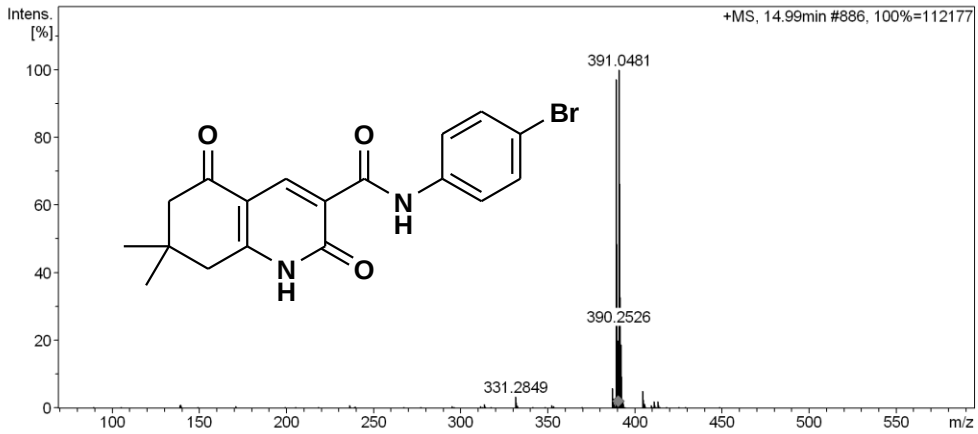
**2.8b**

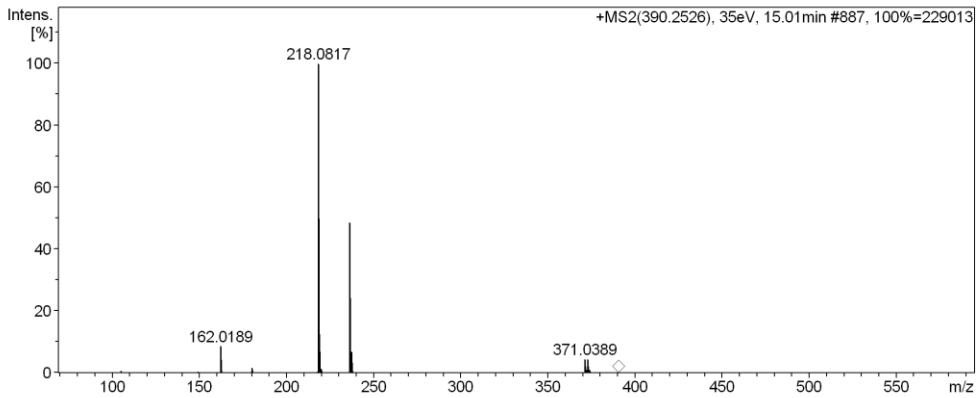

13.142
11.704
8.717
7.693
7.671
7.554
7.532
3.326
2.852
2.509
2.505
2.501
2.496
2.492
2.441
1.043
Br
O
O
N
H
N
H
O
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.98
1.04
1.00
2.05
2.02
2.01
2.06
6.02

**2.8b**

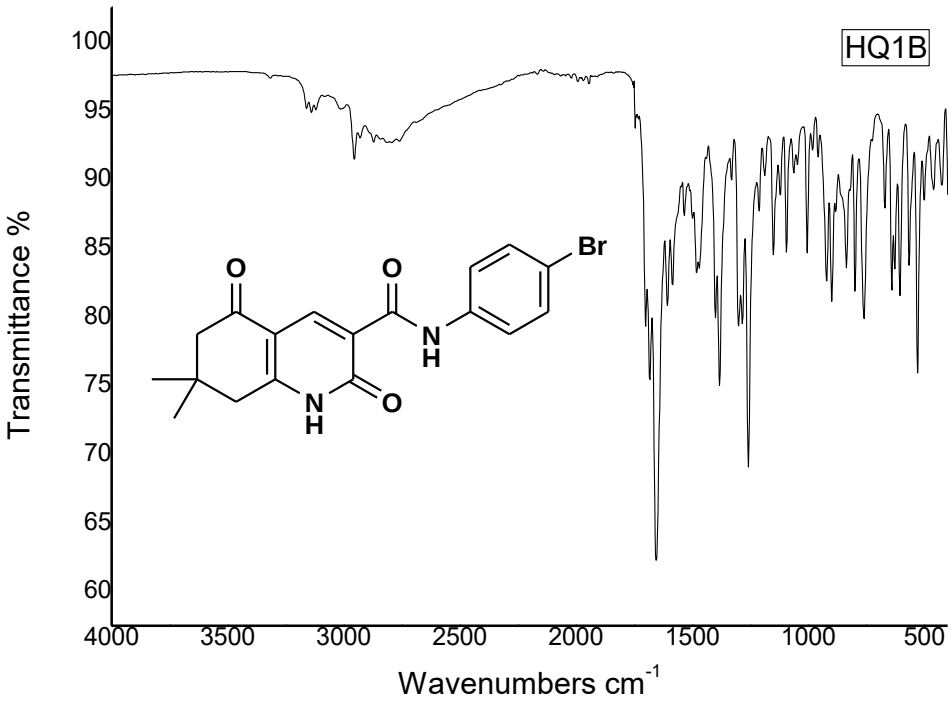

192.87
163.32
160.17
159.84
140.14
138.61
131.86
130.28
121.49
117.16
111.61
50.01
40.15
39.94
39.73
39.52
39.31
39.10
38.90
32.64
27.70
Br
O
O
N
H
N
H
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

**2.8b**

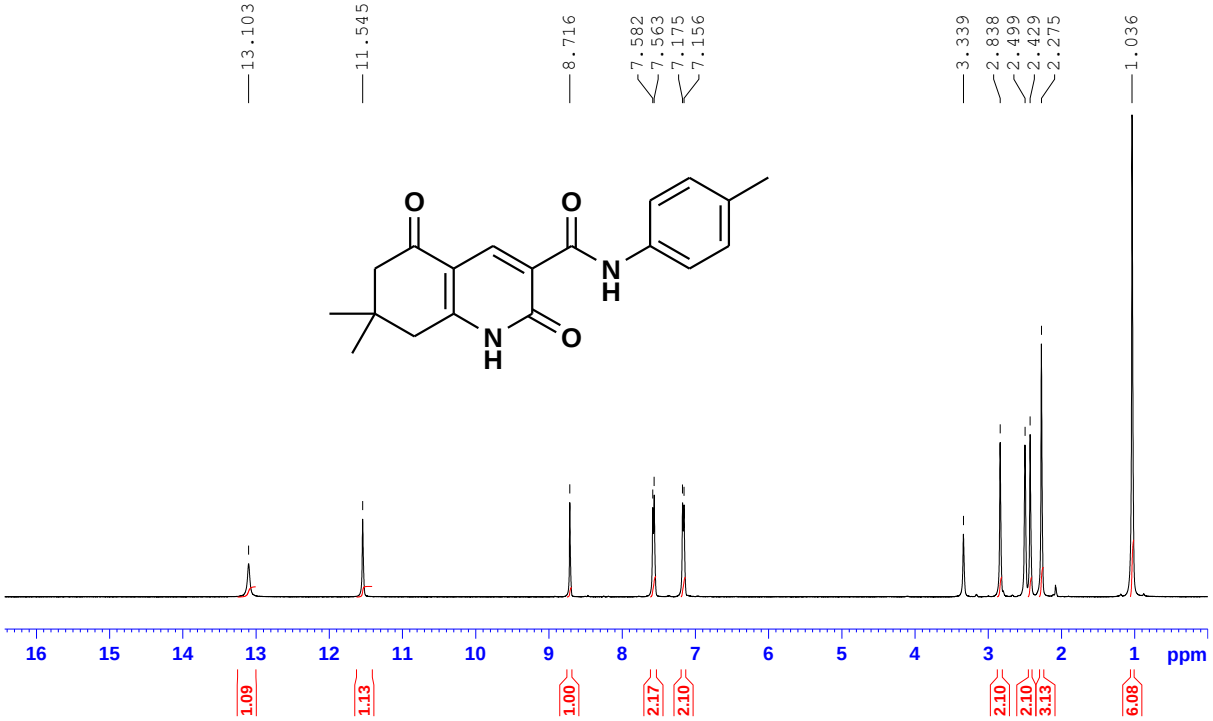

Intens.
[%]
+MS, 14.99min #886, 100%=112177
391.0481
390.2526
331.2849
Br
O
O
N
H
N
H
O
100 150 200 250 300 350 400 450 500 550 m/z


Intens.
[%]
+MS2(390.2526), 35eV, 15.01min #887, 100%=229013
218.0817
162.0189
371.0389
100 150 200 250 300 350 400 450 500 550 m/z

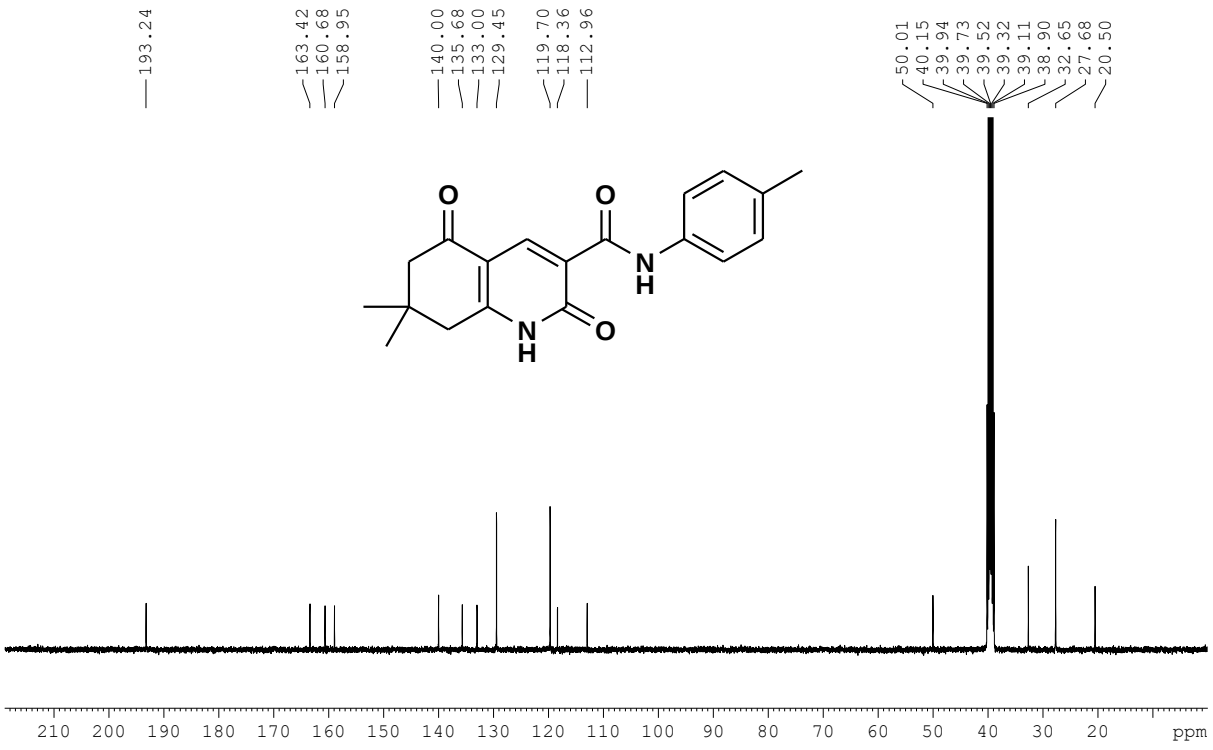
**2.8b**


HQ1B
Transmittance %
100
95
90
85
80
75
70
65
60
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers cm-1
Br
O
O
N
H
N
H
O

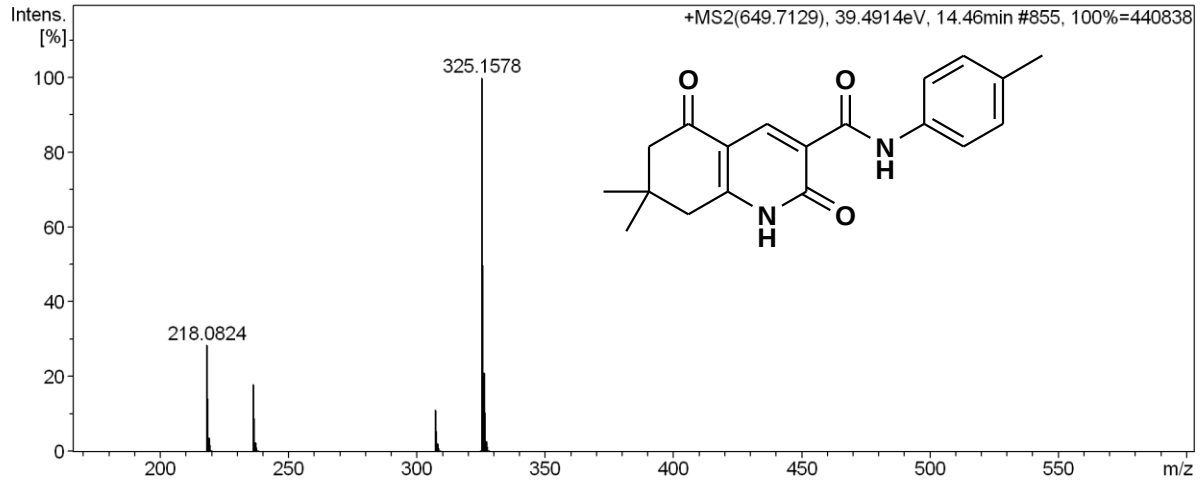
**2.8c**


13.103
11.545
8.716
7.582
7.563
7.175
7.156
3.339
2.838
2.499
2.429
2.275
1.036
O
O
N
H
N
H
O
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.09
1.13
1.00
2.17
2.10
2.10
2.10
3.13
6.08

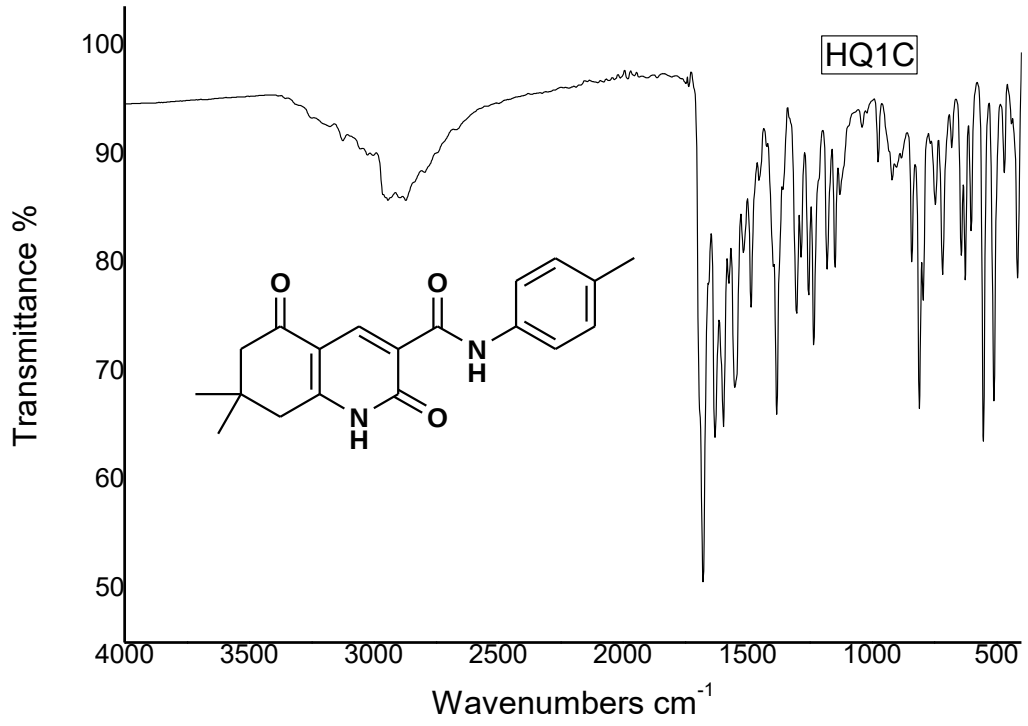
**2.8c**


193.24
163.42
160.68
158.95
140.00
135.68
133.00
129.45
119.70
118.36
112.96
50.01
40.15
39.94
39.73
39.52
39.32
39.11
38.90
32.65
27.68
20.50
O
O
N
H
N
H
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

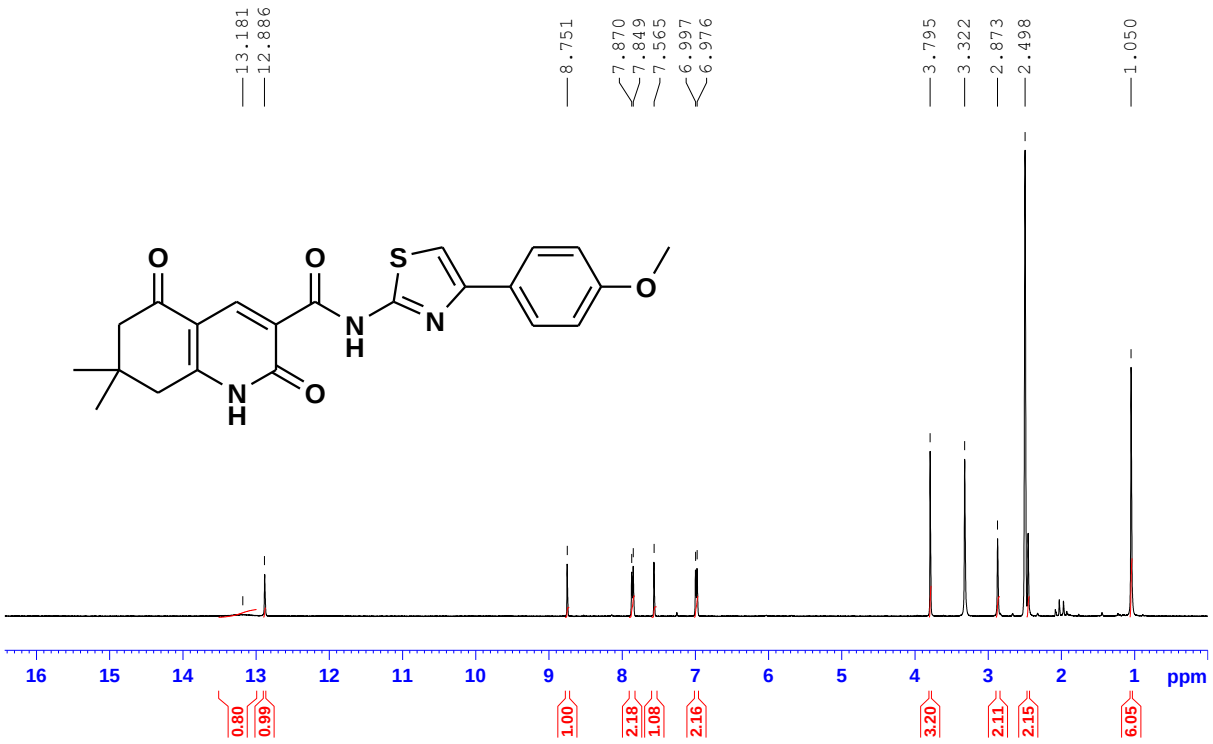
**2.8c**


Intens.
[%]
+MS2(649.7129), 39.4914eV, 14.46min #855, 100%=440838
325.1578
218.0824
O
O
N
H
N
H
O
100
80
60
40
20
0
200 250 300 350 400 450 500 550 m/z

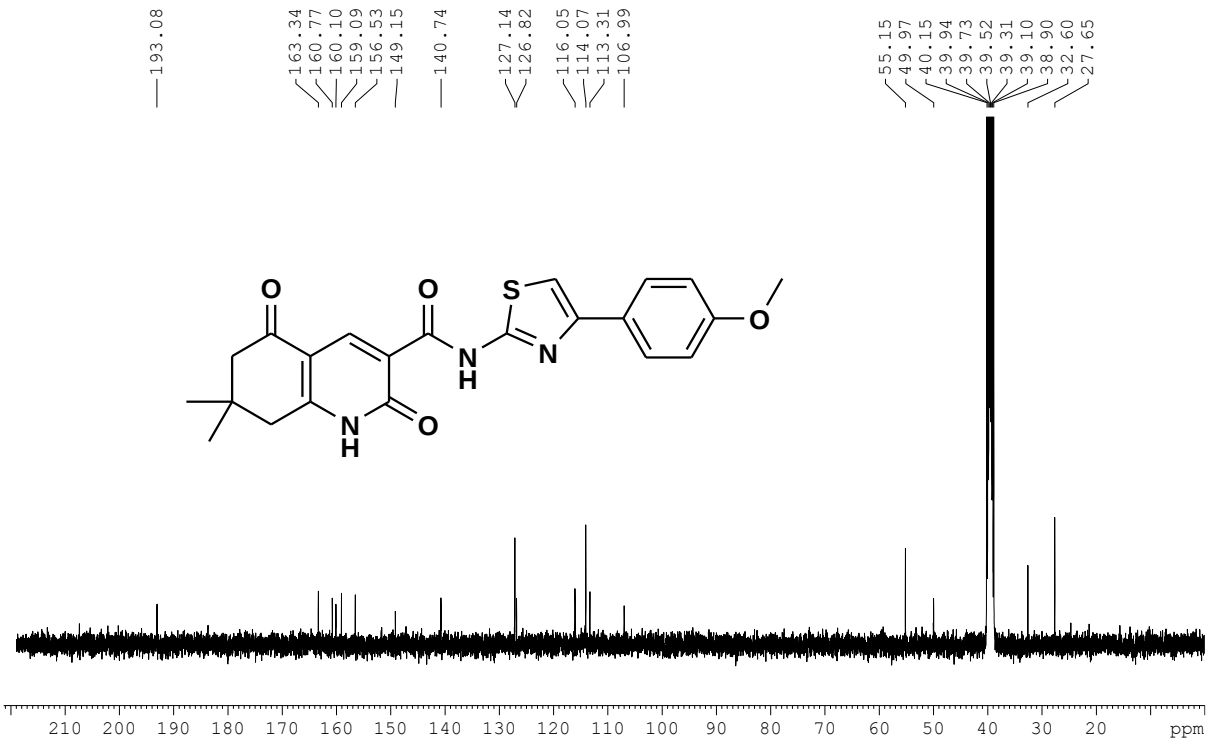
**2.8c**


HQ1C
Transmittance %
100
90
80
70
60
50
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers cm-1
O
O
N
H
N
H
O

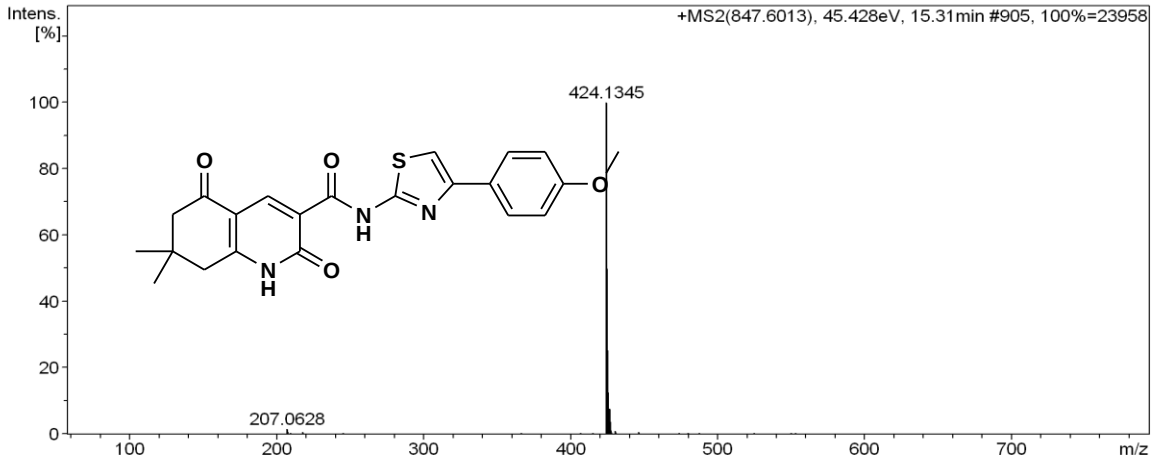
**2.11b**

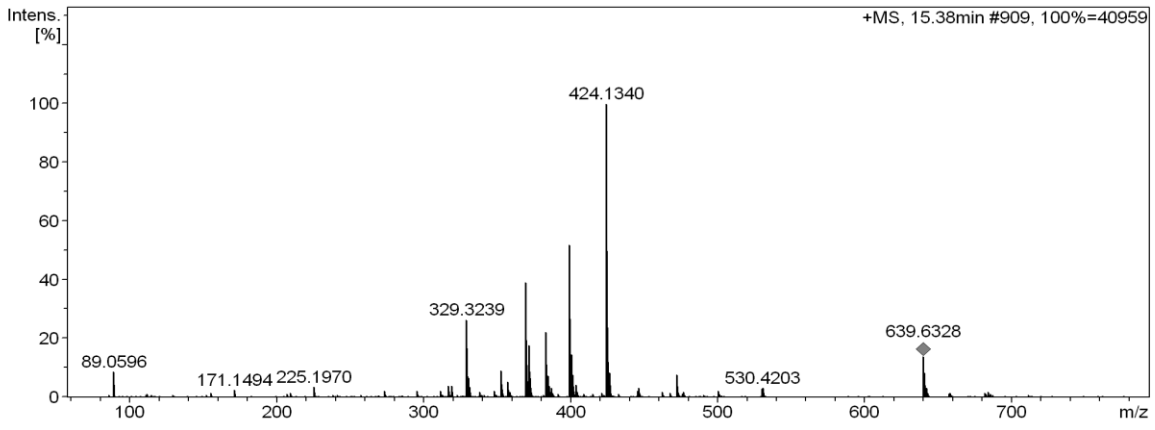

13.181
12.886
8.751
7.870
7.849
7.565
6.997
6.976
3.795
3.322
2.873
2.498
1.050
O
O
S
N
H
N
O
N
H
O
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.80
0.99
1.00
2.18
1.08
2.16
3.20
2.11
2.15
6.05

**2.11b**

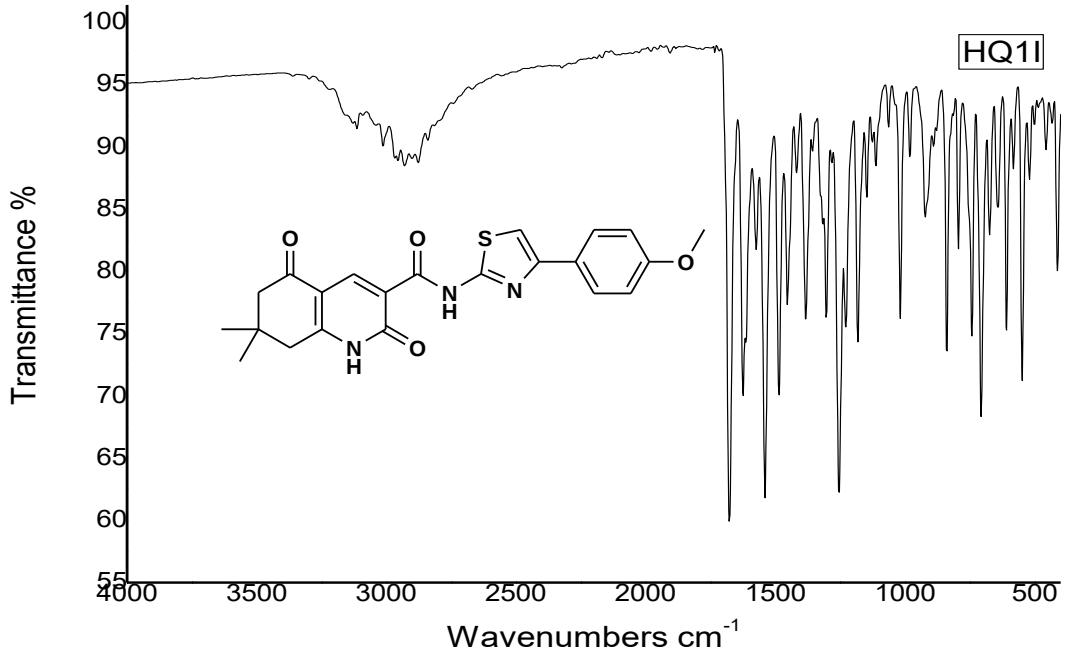

193.08
163.34
160.77
160.10
159.09
156.53
149.15
140.74
127.14
126.82
116.05
114.07
113.31
106.99
55.15
49.97
40.15
39.94
39.73
39.52
39.31
39.10
38.90
32.60
27.65
O
O
S
N
H
N
O
O
N
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

**2.11b**


Intens.
[%]
+MS2(847.6013), 45.428eV, 15.31min #905, 100%=23958
424.1345
207.0628
100
80
60
40
20
0
100 200 300 400 500 600 700 m/z

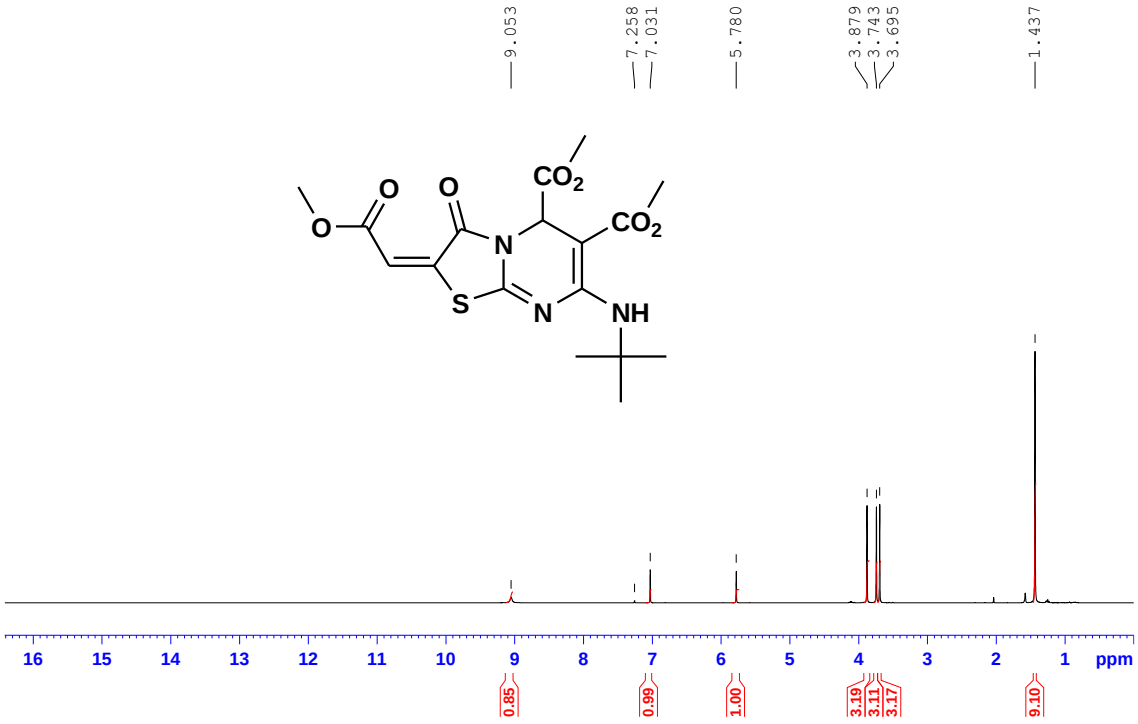

Intens.
[%]
+MS, 15.38min #909, 100%=40959
424.1340
329.3239
639.6328
89.0596
171.1494
225.1970
530.4203
100
80
60
40
20
0
100 200 300 400 500 600 700 m/z

**2.11b**

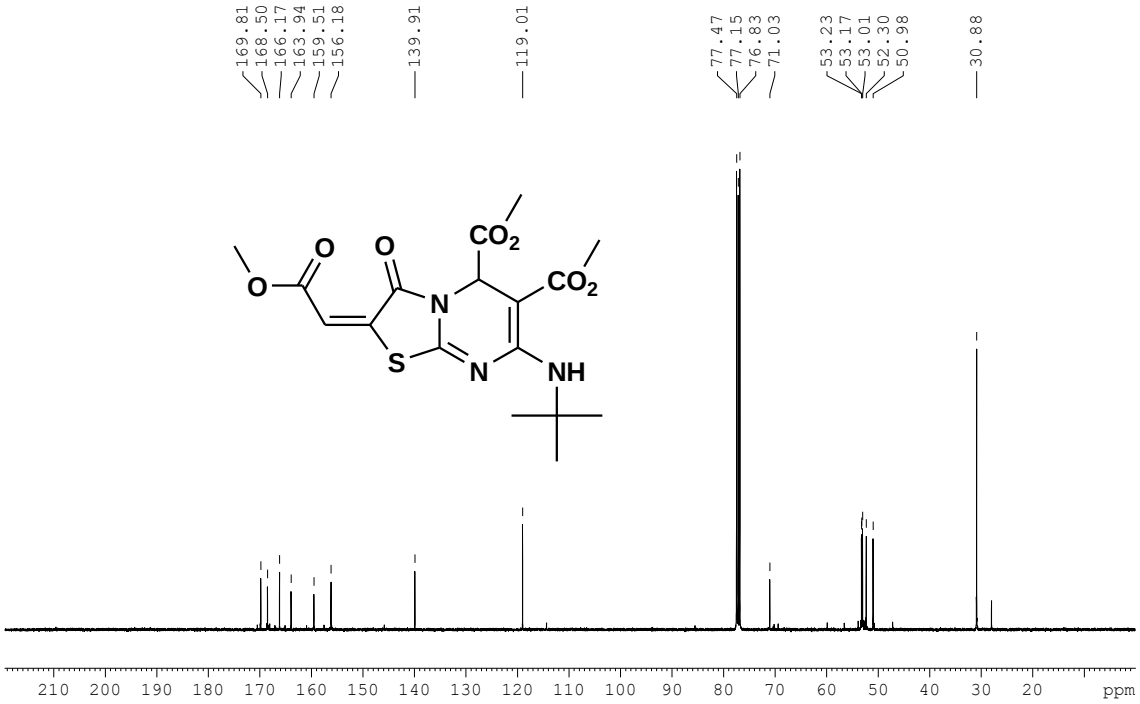



**CHAPTER 3: selected spectroscopic data**

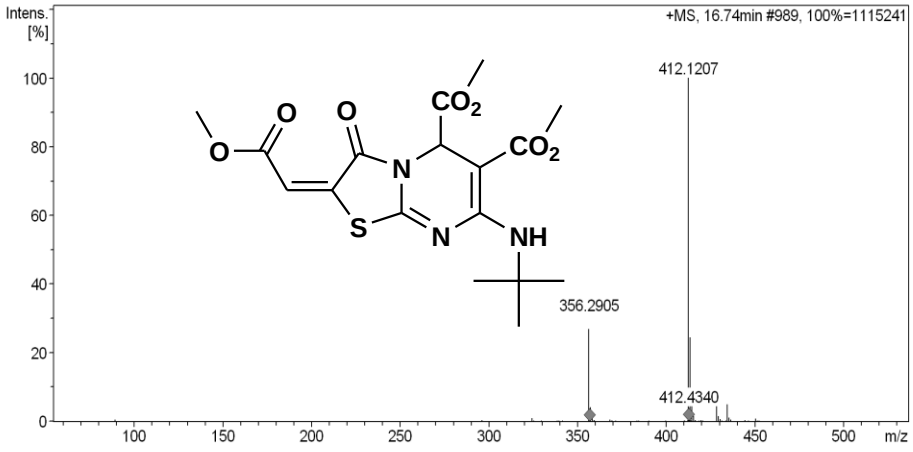
**3.20a**

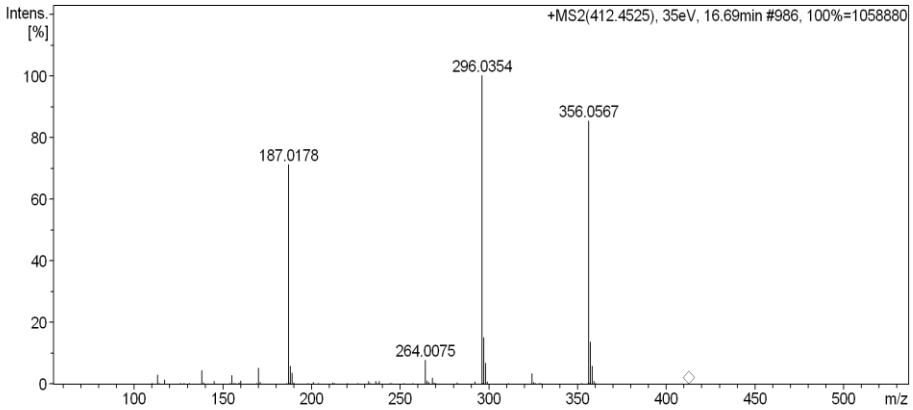



**3.20a**

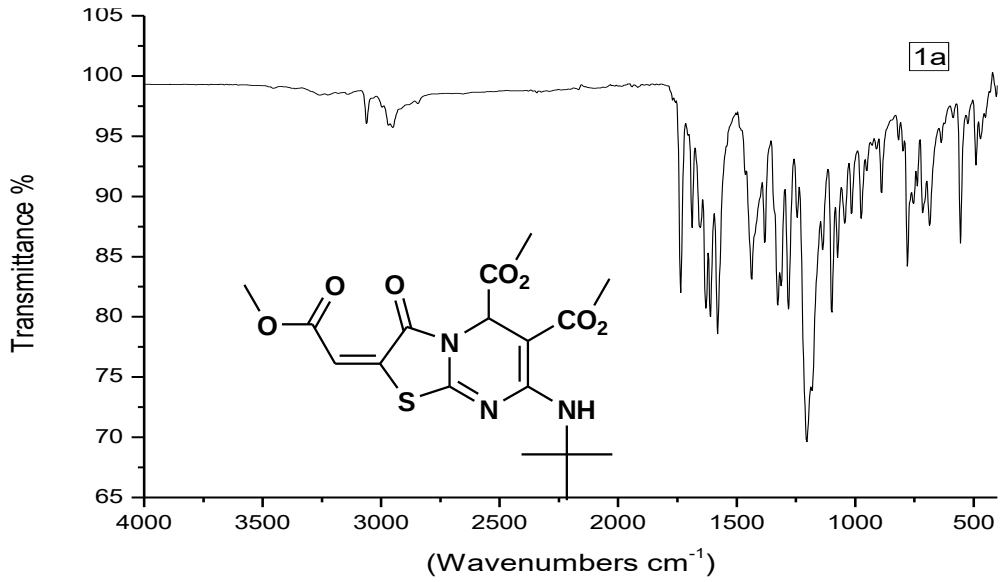

169.81
168.50
166.17
163.94
159.51
156.18
139.91
119.01
77.47
77.15
76.83
71.03
53.23
53.17
53.01
52.30
50.98
30.88
O
O
O
CO2
CO2
N
S
N
NH
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

**3.20a**

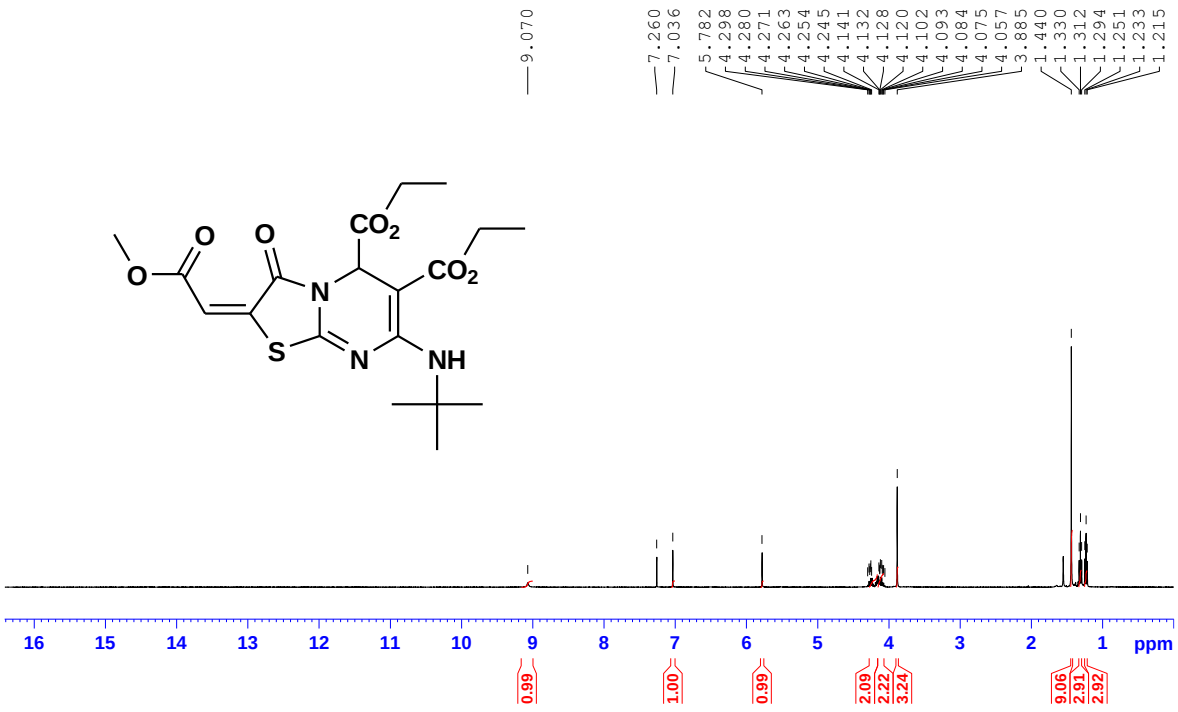

Intens.
[%]
+MS, 16.74min #989, 100%=1115241
412.1207
356.2905
412.4340
O
O
O
CO2
CO2
N
S
N
NH
100 150 200 250 300 350 400 450 500 m/z


Intens.
[%]
+MS2(412.4525), 35eV, 16.69min #986, 100%=1058880
296.0354
356.0567
187.0178
264.0075
100 150 200 250 300 350 400 450 500 m/z

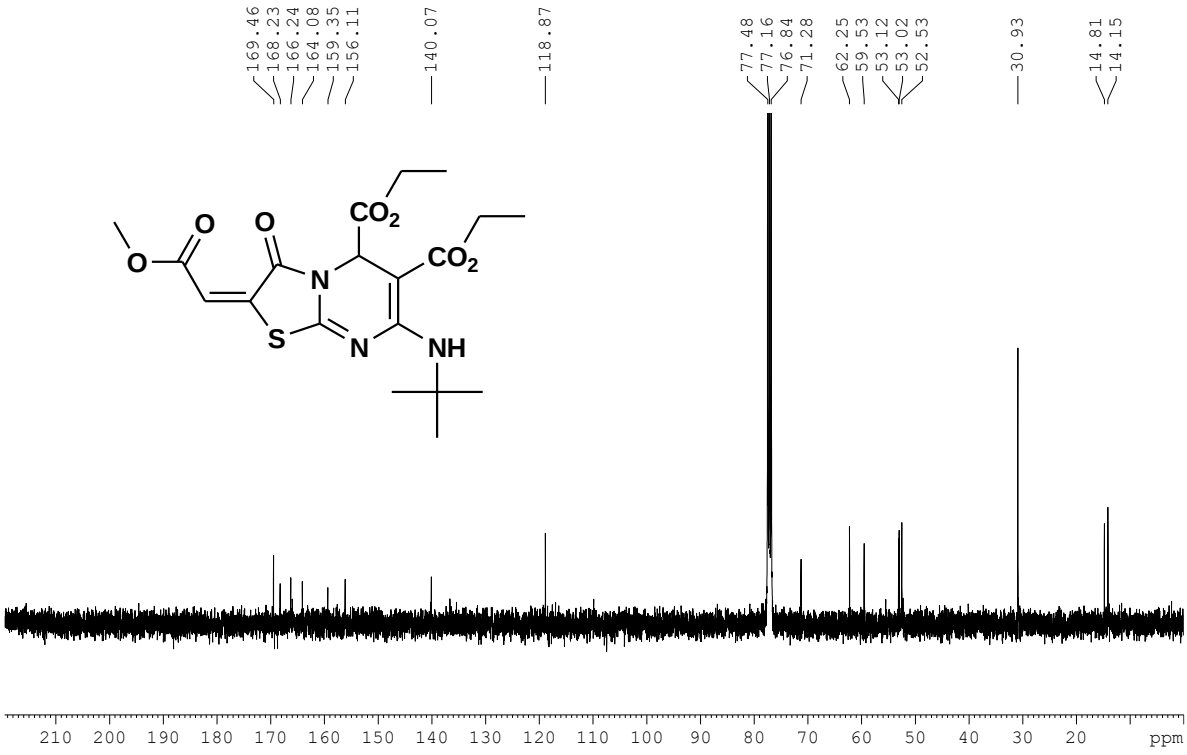
**3.20a**


1a
Transmittance %
(Wavenumbers cm-1)
105
100
95
90
85
80
75
70
65
4000
3500
3000
2500
2000
1500
1000
500
O
O
O
N
S
N
NH
CO2
CO2

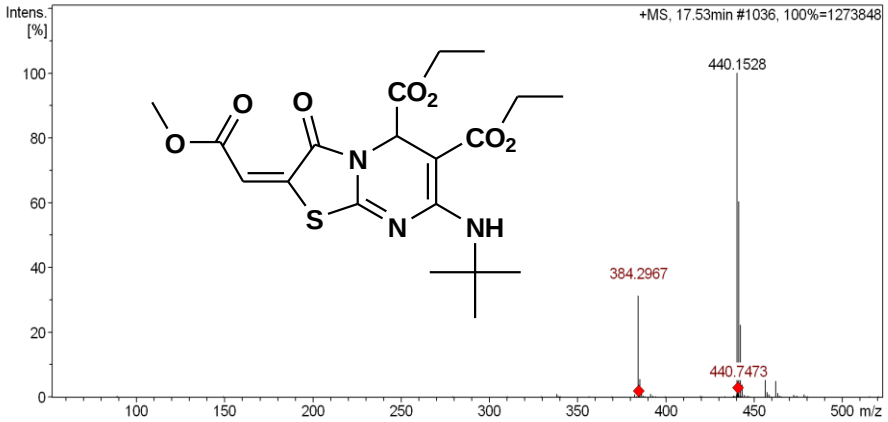
**3.20b**

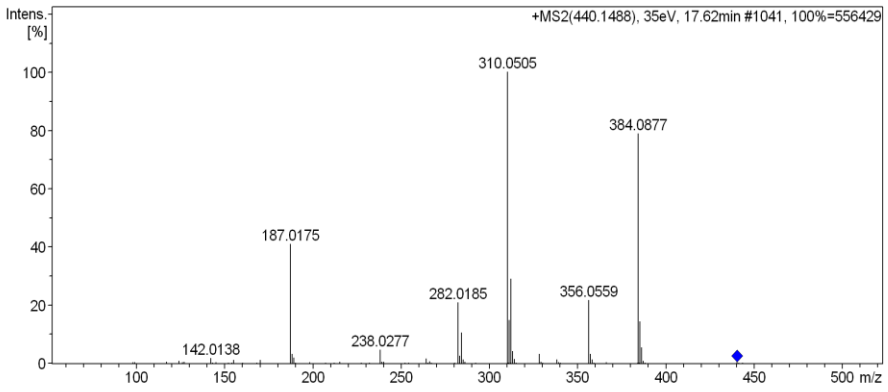

9.070
7.260
7.036
5.782
4.298
4.280
4.271
4.263
4.254
4.245
4.141
4.132
4.128
4.120
4.102
4.093
4.084
4.075
4.057
3.885
1.440
1.330
1.312
1.294
1.251
1.233
1.215
O
O
O
N
S
N
NH
CO2
CO2
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.99
1.00
0.99
2.09
2.22
3.24
9.06
2.91
2.92

**3.20b**

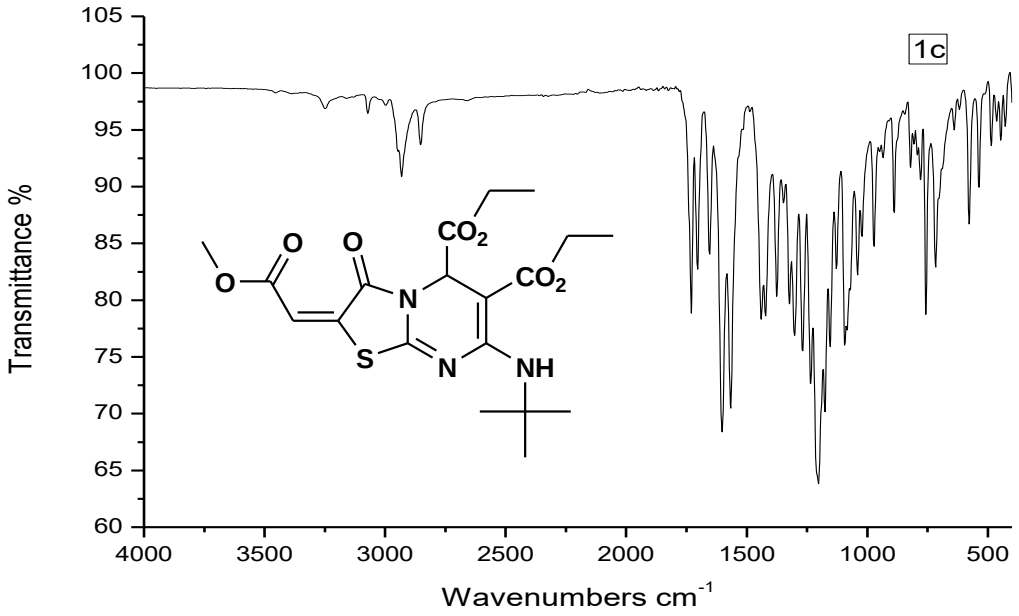

169.46
168.23
166.24
164.08
159.35
156.11
140.07
118.87
77.48
77.16
76.84
71.28
62.25
59.53
53.12
53.02
52.53
30.93
14.81
14.15
CO2
CO2
O
O
O
N
S
N
NH
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

**3.20b**

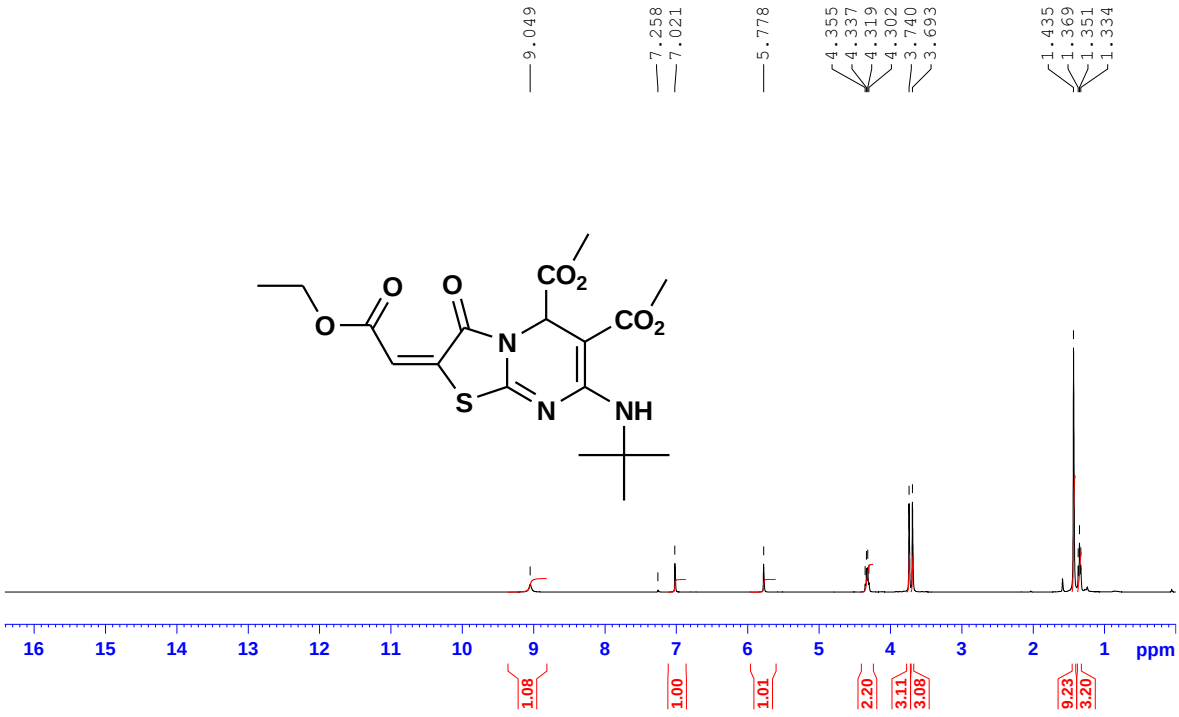

Intens.
[%]
+MS, 17.53min #1036, 100%=1273848
440.1528
384.2967
440.7473
CO2
CO2
O
O
O
N
S
N
NH
100 150 200 250 300 350 400 450 500 m/z


Intens.
[%]
+MS2(440.1488), 35eV, 17.62min #1041, 100%=556429
310.0505
384.0877
187.0175
282.0185
356.0559
142.0138
238.0277
100 150 200 250 300 350 400 450 500 m/z

**3.20b**


1c
Transmittance %
Wavenumbers cm-1
105
100
95
90
85
80
75
70
65
60
4000
3500
3000
2500
2000
1500
1000
500
O
O
O
CO2
CO2
N
S
N
NH

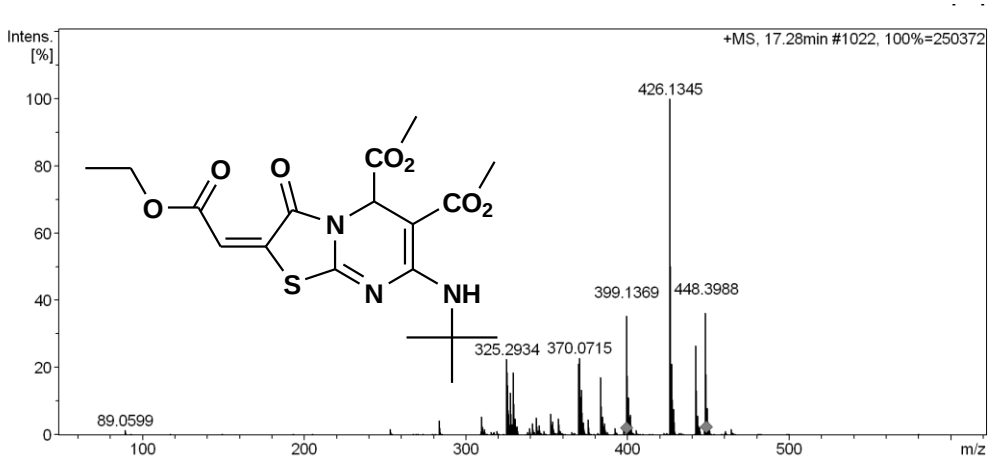
**3.20c**

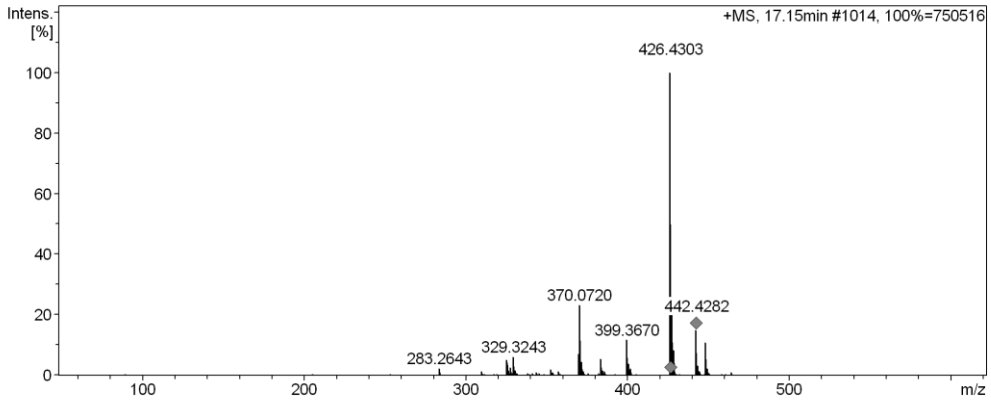

9.049
7.258
7.021
5.778
4.355
4.337
4.319
4.302
3.740
3.693
1.435
1.369
1.351
1.334
O
O
O
CO2
CO2
N
S
N
NH
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.08
1.00
1.01
2.20
3.11
3.08
9.23
3.20

**3.20c**

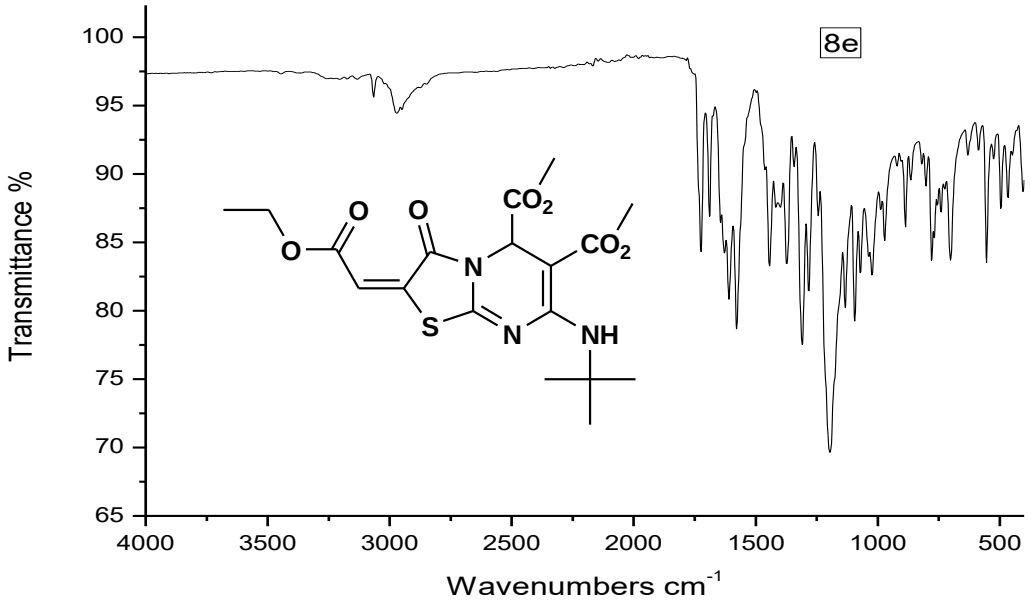

169.83
168.51
165.69
164.00
159.64
156.22
139.54
119.56
77.45
77.14
76.82
71.01
62.26
53.22
53.15
52.27
50.97
30.87
14.28
O
O
O
CO2
CO2
N
S
N
NH
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

**3.20c**

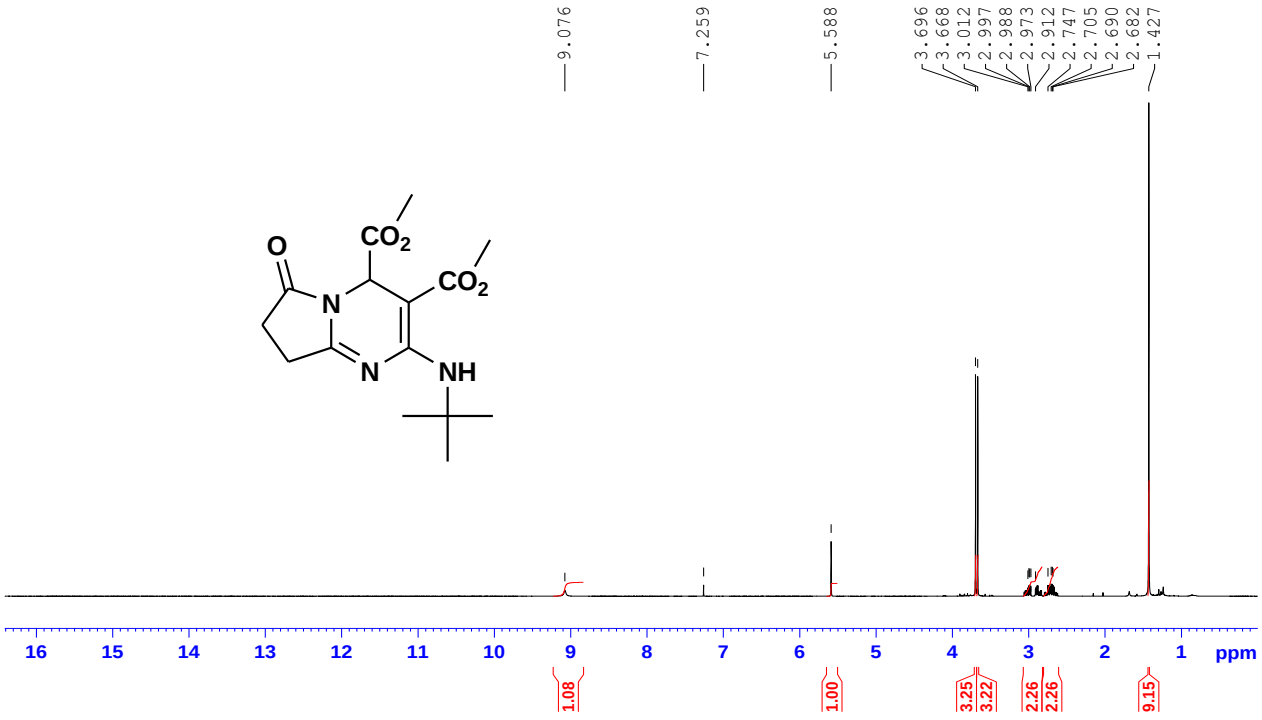

Intens.
[%]
+MS, 17.28min #1022, 100%=250372
426.1345
399.1369
448.3988
325.2934
370.0715
89.0599
O
O
O
CO2
CO2
N
S
N
NH
100 200 300 400 500 m/z


Intens.
[%]
+MS, 17.15min #1014, 100%=750516
426.4303
370.0720
442.4282
399.3670
283.2643
329.3243
100 200 300 400 500 m/z

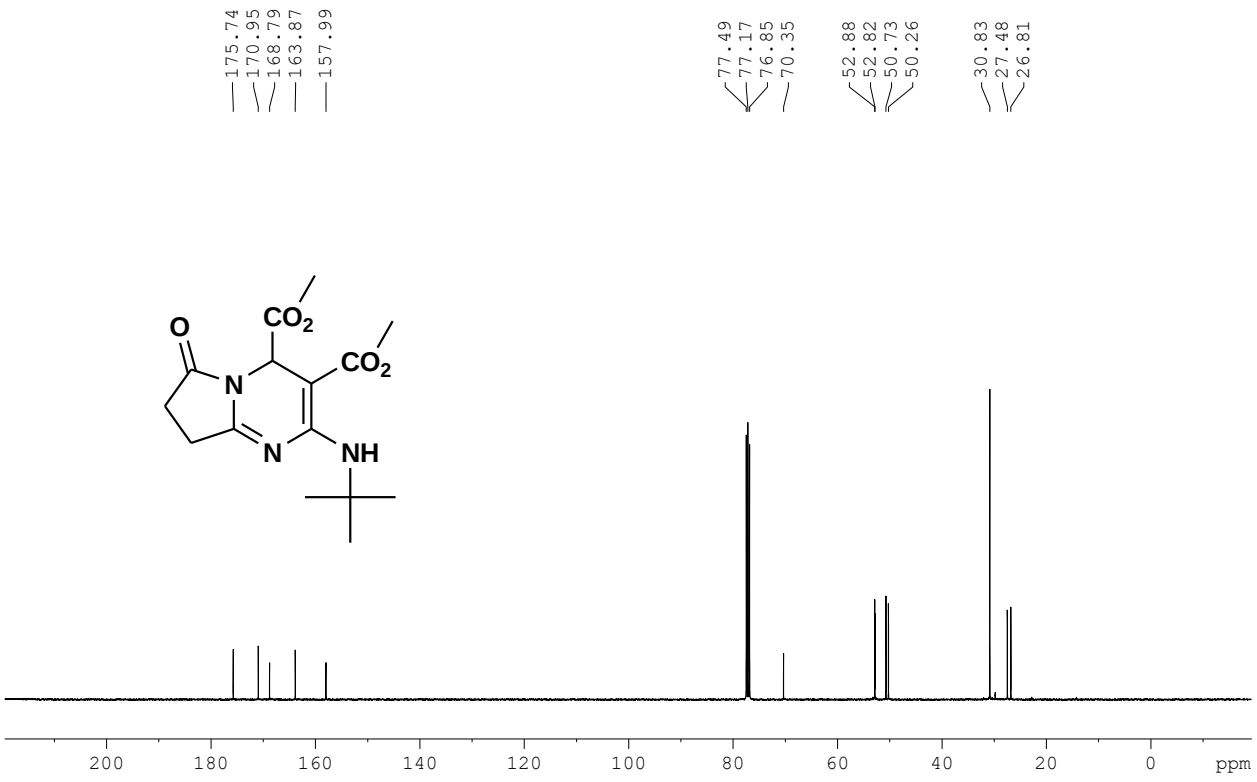
**3.20c**


8e
Transmittance %
Wavenumbers cm-1
100
95
90
85
80
75
70
65
4000
3500
3000
2500
2000
1500
1000
500
O
O
O
CO2
CO2
N
S
N
NH

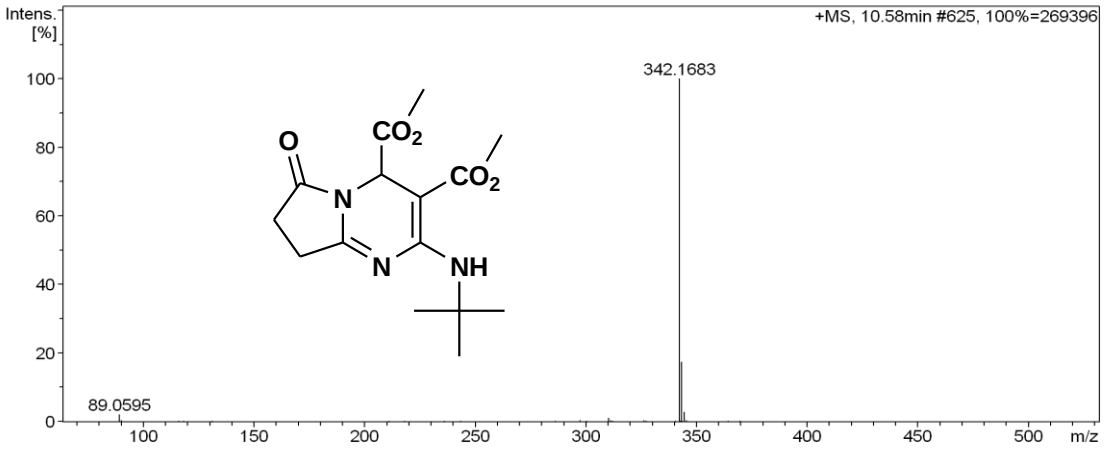
**3.21a**

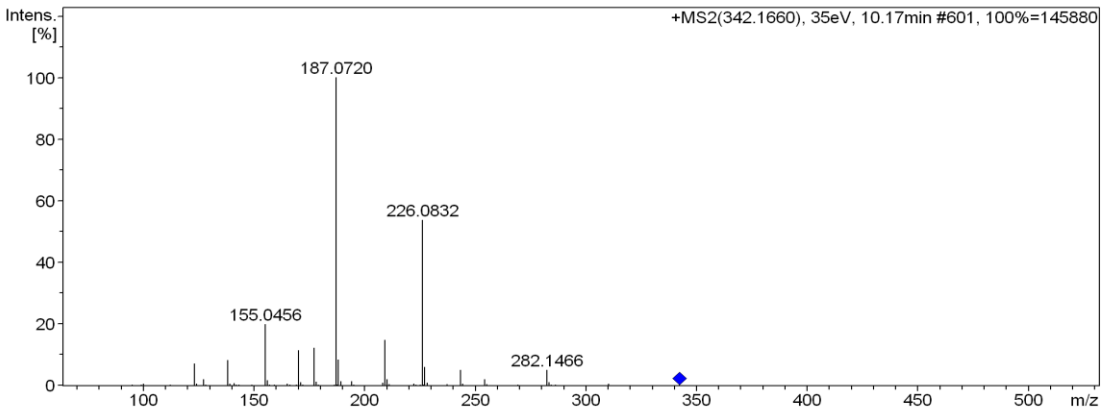

9.076
7.259
5.588
3.696
3.668
3.012
2.997
2.988
2.973
2.912
2.747
2.705
2.690
2.682
1.427
O
CO2
CO2
N
N
NH
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.08
1.00
3.25
3.22
2.26
2.26
9.15

**3.21a**

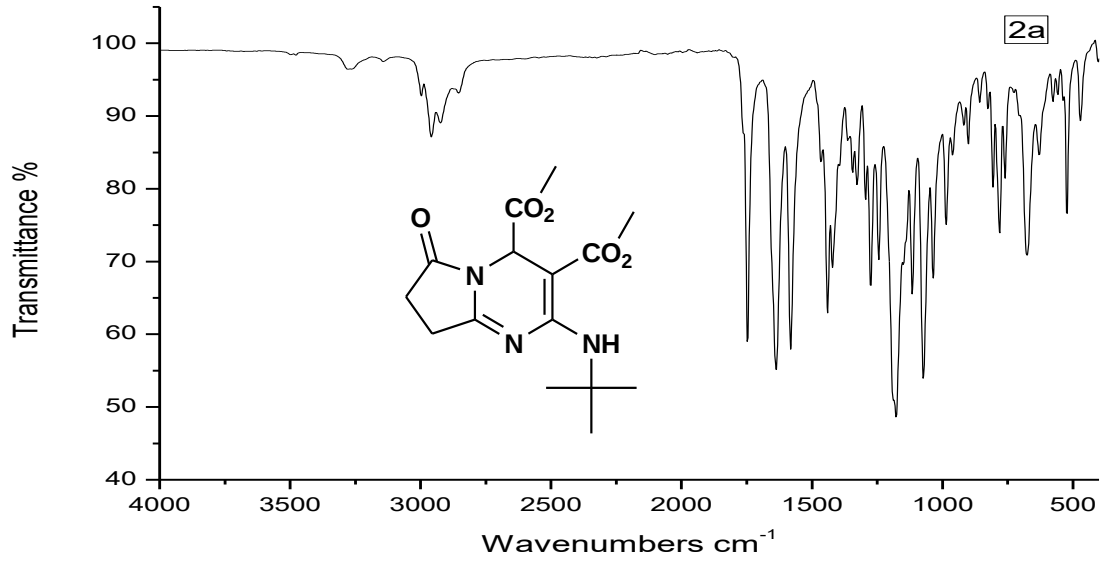

175.74
170.95
168.79
163.87
157.99
77.49
77.17
76.85
70.35
52.88
52.82
50.73
50.26
30.83
27.48
26.81
O
CO2
CO2
N
N
NH
200
180
160
140
120
100
80
60
40
20
0
ppm

**3.21a**

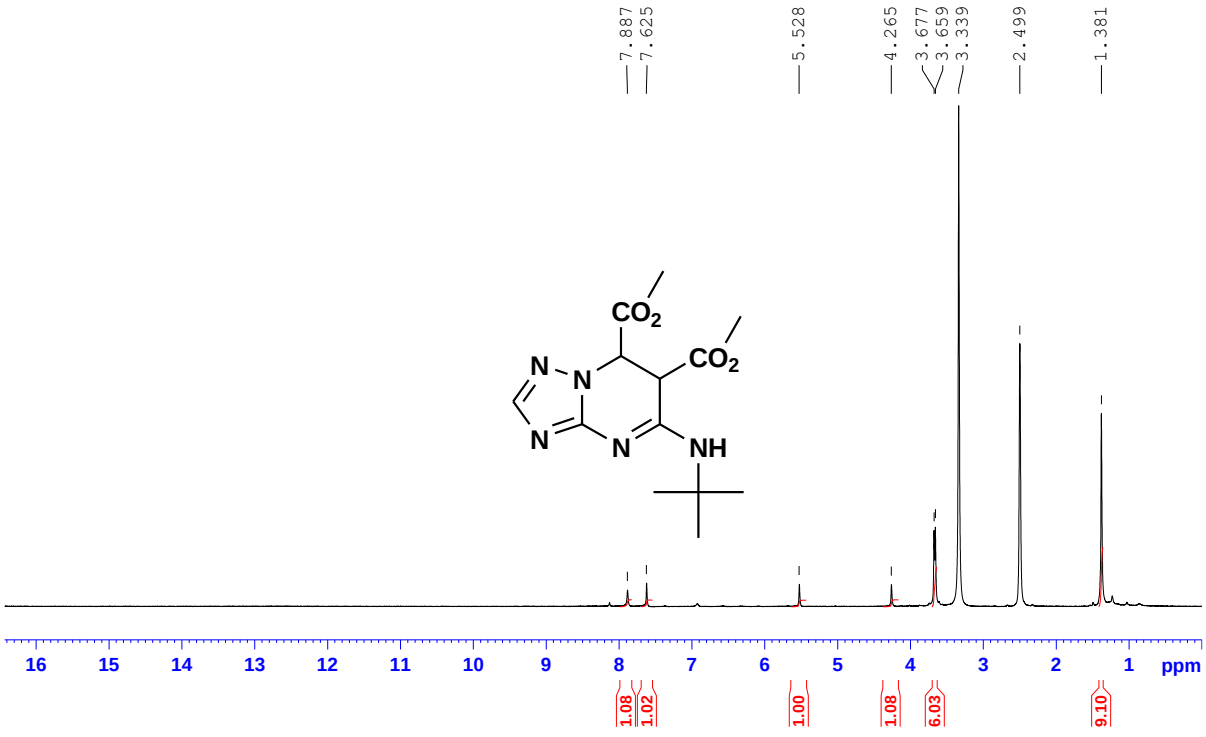

Intens.
[%]
+MS, 10.58min #625, 100%=269396
342.1683
89.0595
O
CO2
CO2
N
N
NH
100
80
60
40
20
0
100
150
200
250
300
350
400
450
500
m/z


Intens.
[%]
+MS2(342.1660), 35eV, 10.17min #601, 100%=145880
187.0720
226.0832
155.0456
282.1466
100
80
60
40
20
0
100
150
200
250
300
350
400
450
500
m/z

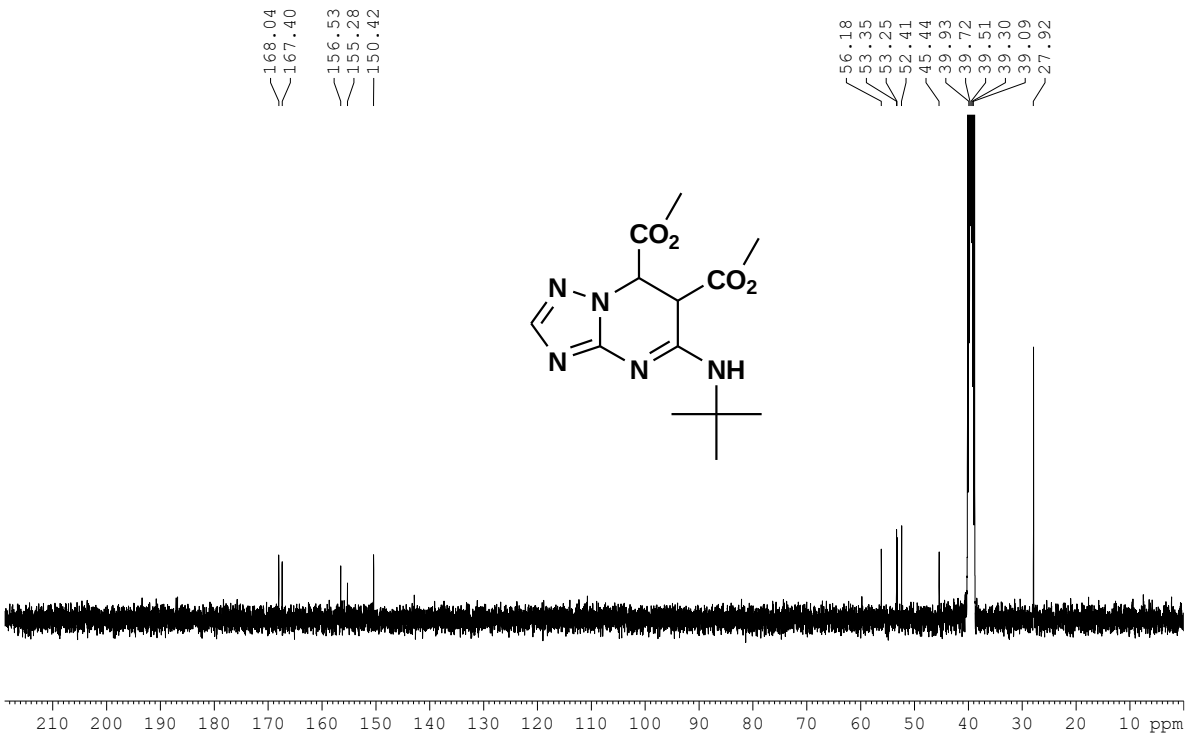
**3.21a**



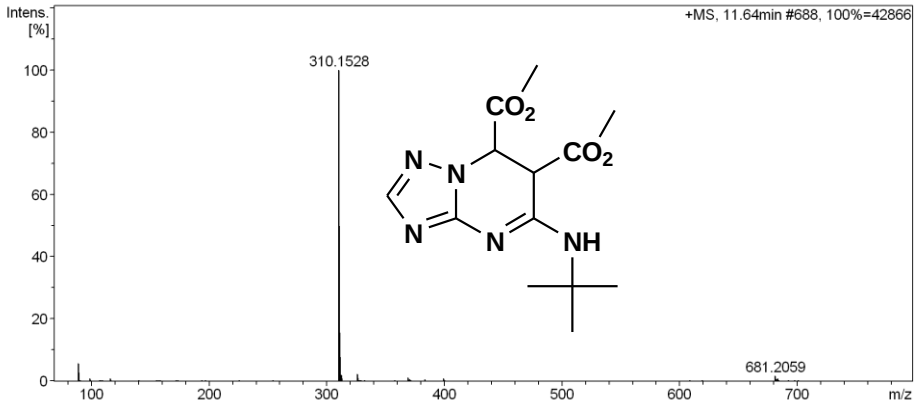

**3.22a**

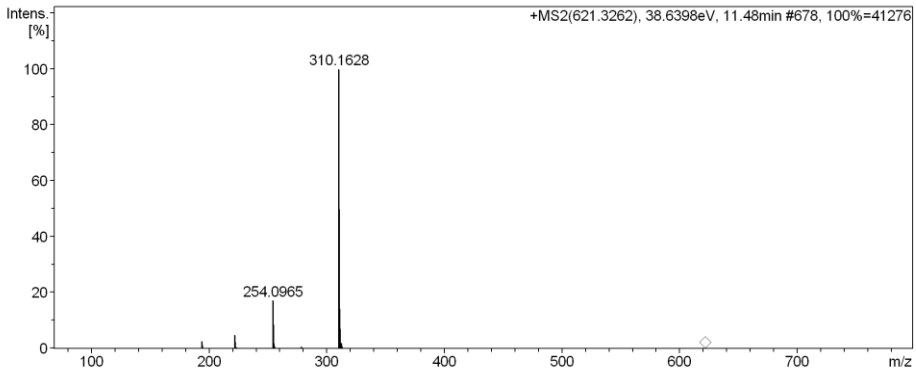



**3.22a**

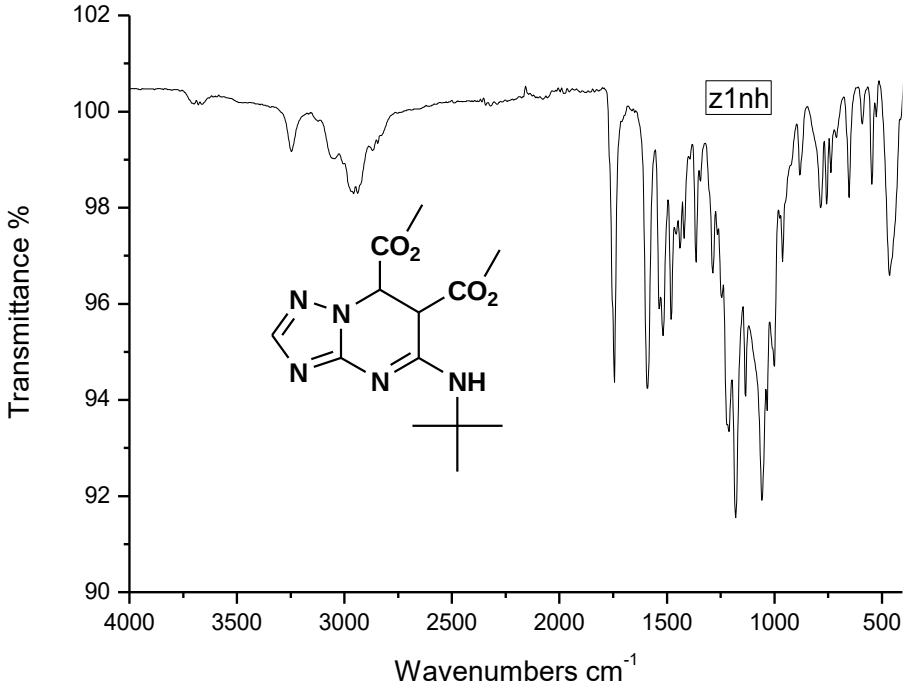

168.04
167.40
156.53
155.28
150.42
56.18
53.35
53.25
52.41
45.44
39.93
39.72
39.51
39.30
39.09
27.92
CO2
CO2
N
N
N
N
NH
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

**3.22a**

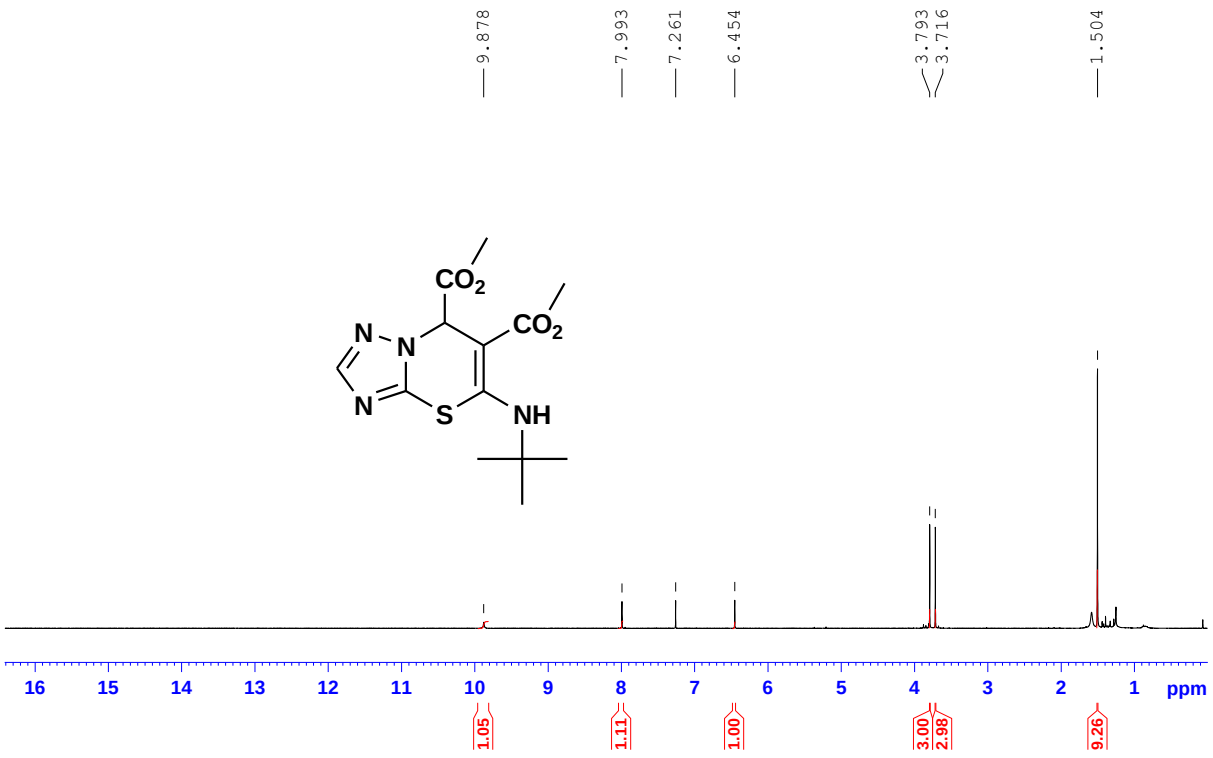

Intens.
[%]
+MS, 11.64min #688, 100%=42866
310.1528
681.2059
CO2
CO2
N
N
N
N
NH
100
80
60
40
20
0
100 200 300 400 500 600 700 m/z


Intens.
[%]
+MS2(621.3262), 38.6398eV, 11.48min #678, 100%=41276
310.1628
254.0965
100
80
60
40
20
0
100 200 300 400 500 600 700 m/z

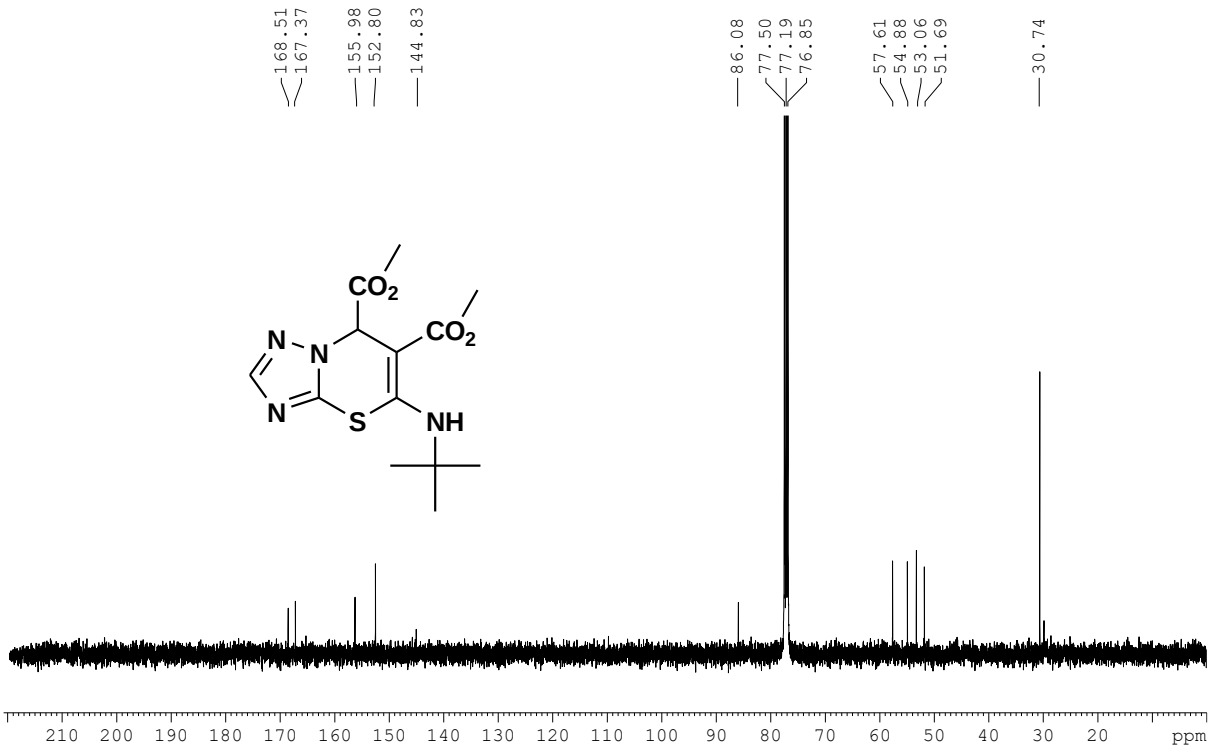
**3.22a**


z1nh
Transmittance %
Wavenumbers cm-1
CO2
CO2
NH

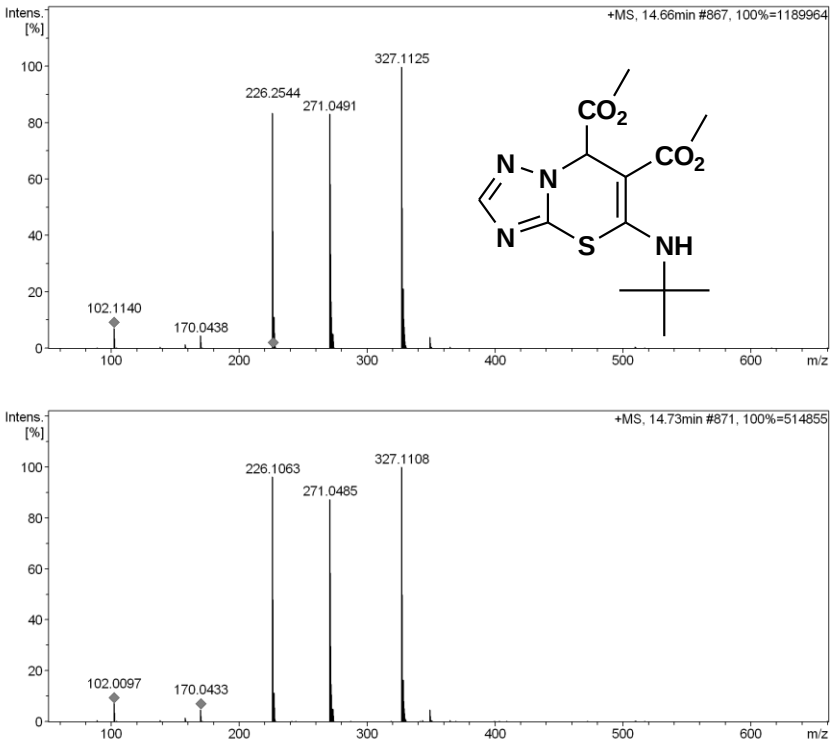
**3.23a**


9.878
7.993
7.261
6.454
3.793
3.716
1.504
CO2
CO2
NH
ppm
1.05
1.11
1.00
3.00
2.98
9.26

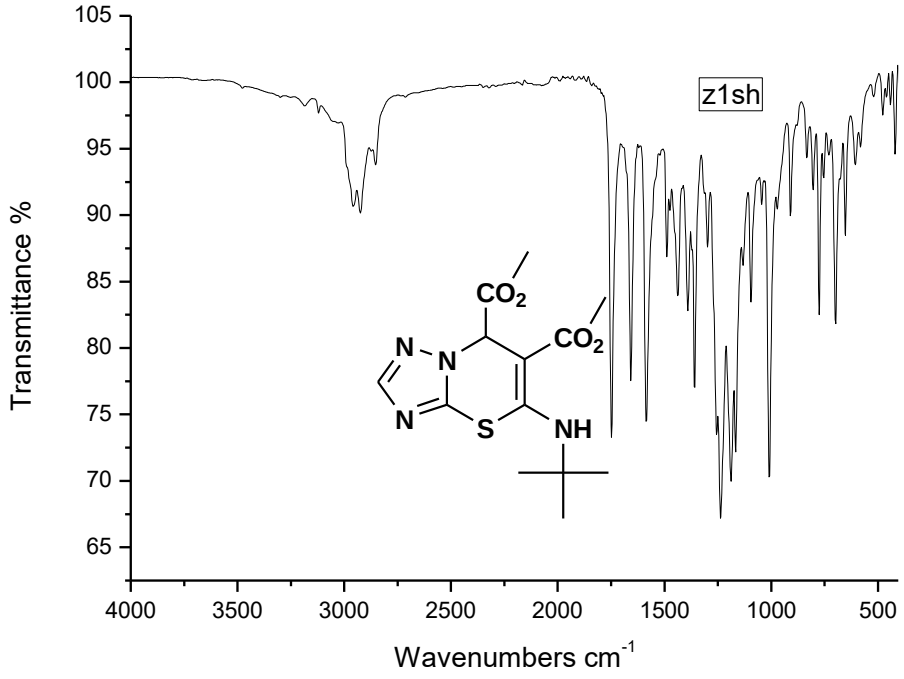
**3.23a**




**3.23a**

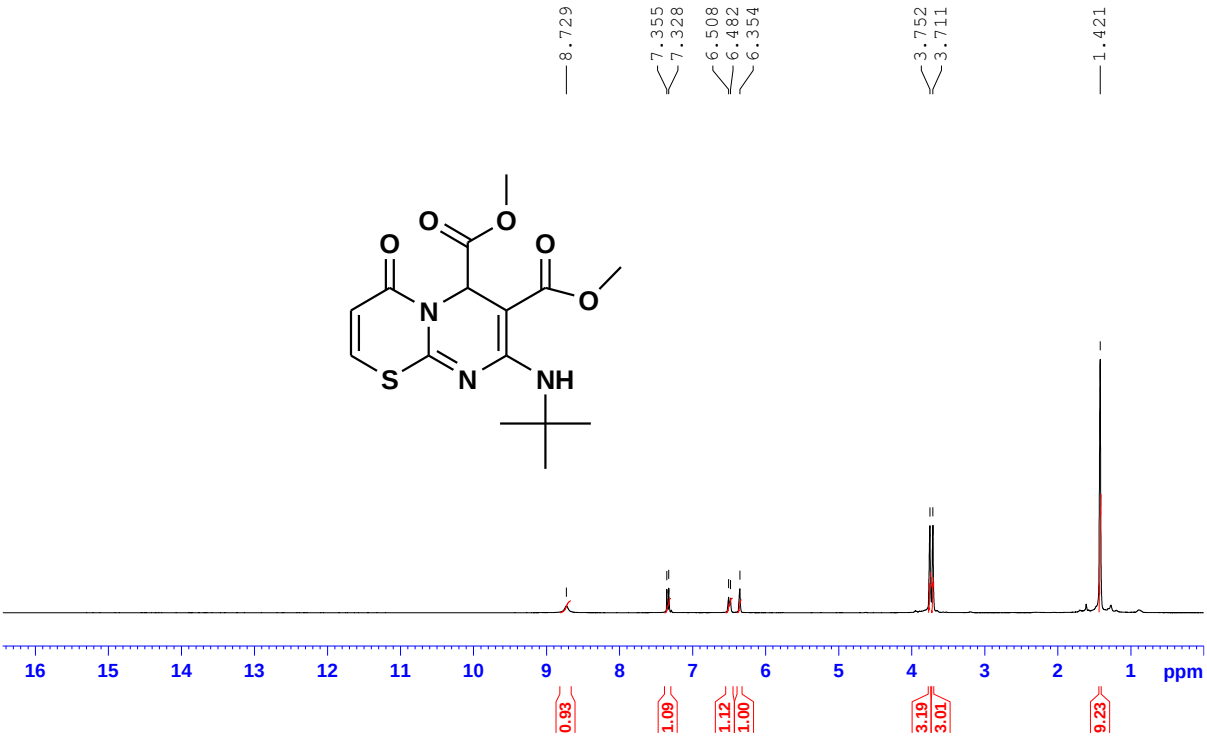



**3.23a**

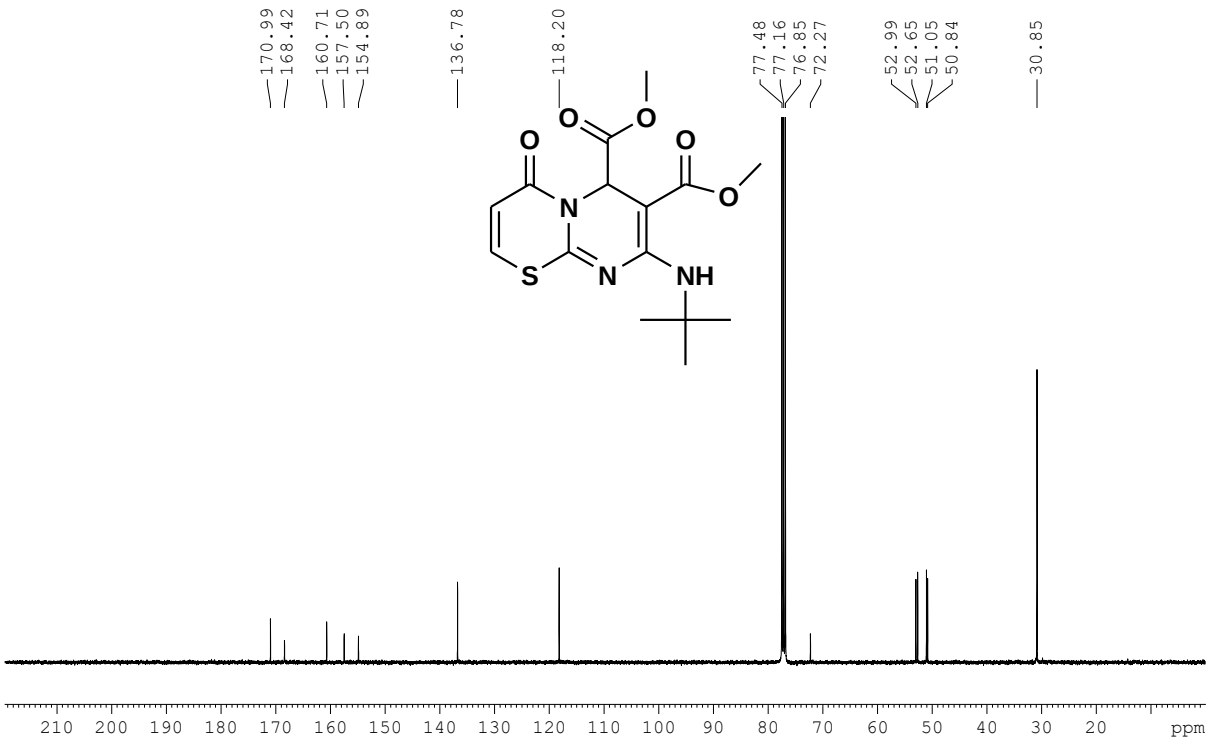



**CHAPTER 4: selected spectroscopic data**

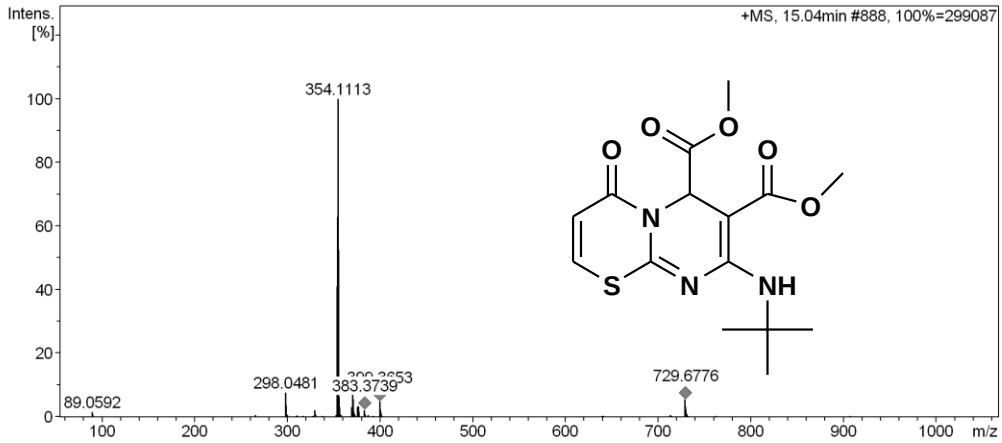
**4.8a**

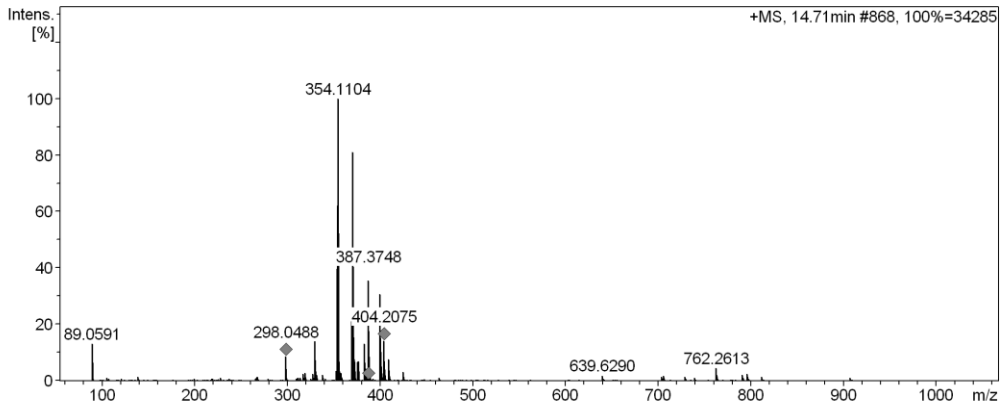



**4.8a**

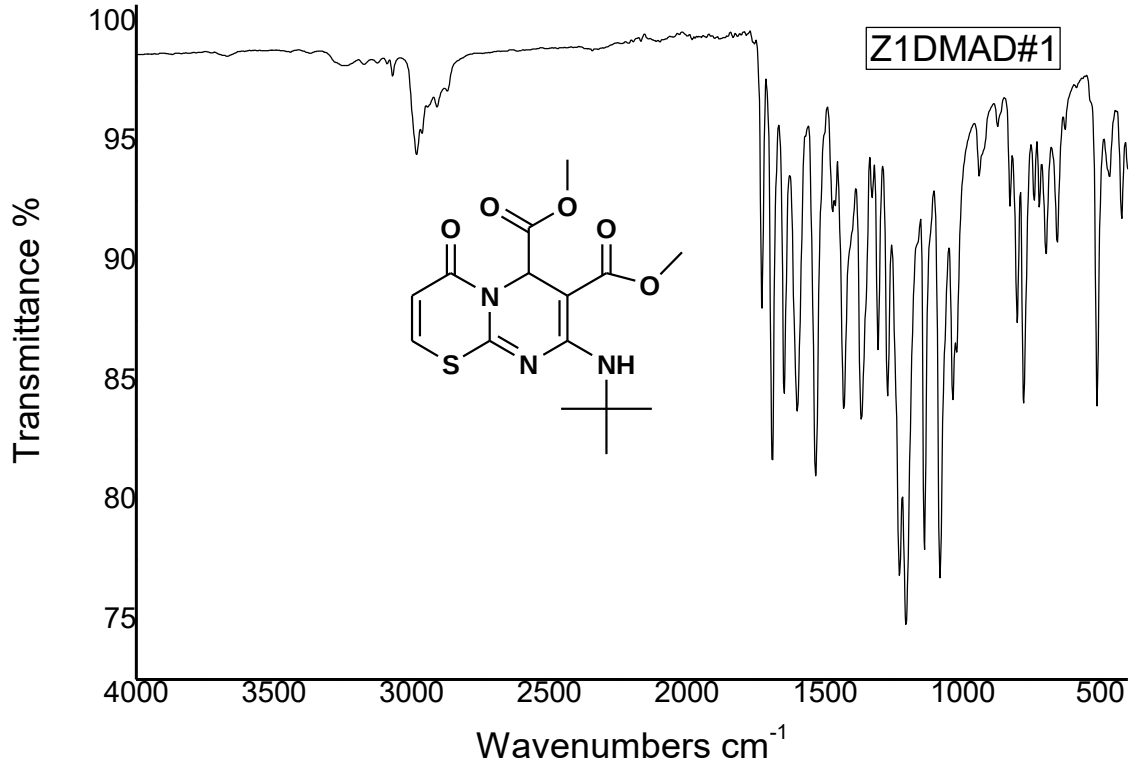

170.99
168.42
160.71
157.50
154.89
136.78
118.20
77.48
77.16
76.85
72.27
52.99
52.65
51.05
50.84
30.85
O
O
O
O
O
N
S
N
NH
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

**4.8a**

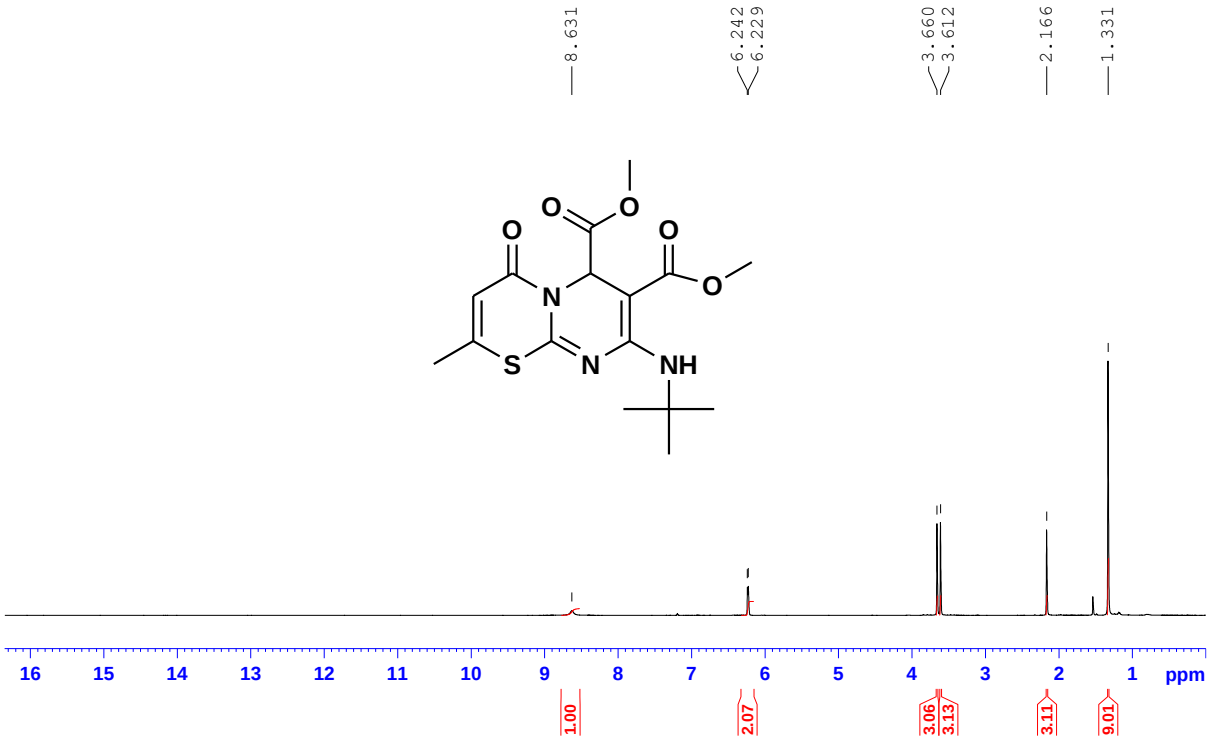

Intens.
[%]
+MS, 15.04min #888, 100%=299087
354.1113
298.0481
383.3739
89.0592
729.6776
O
O
O
O
O
N
S
N
NH
100 200 300 400 500 600 700 800 900 1000 m/z


Intens.
[%]
+MS, 14.71min #868, 100%=34285
354.1104
387.3748
404.2075
89.0591
298.0488
639.6290
762.2613
100 200 300 400 500 600 700 800 900 1000 m/z

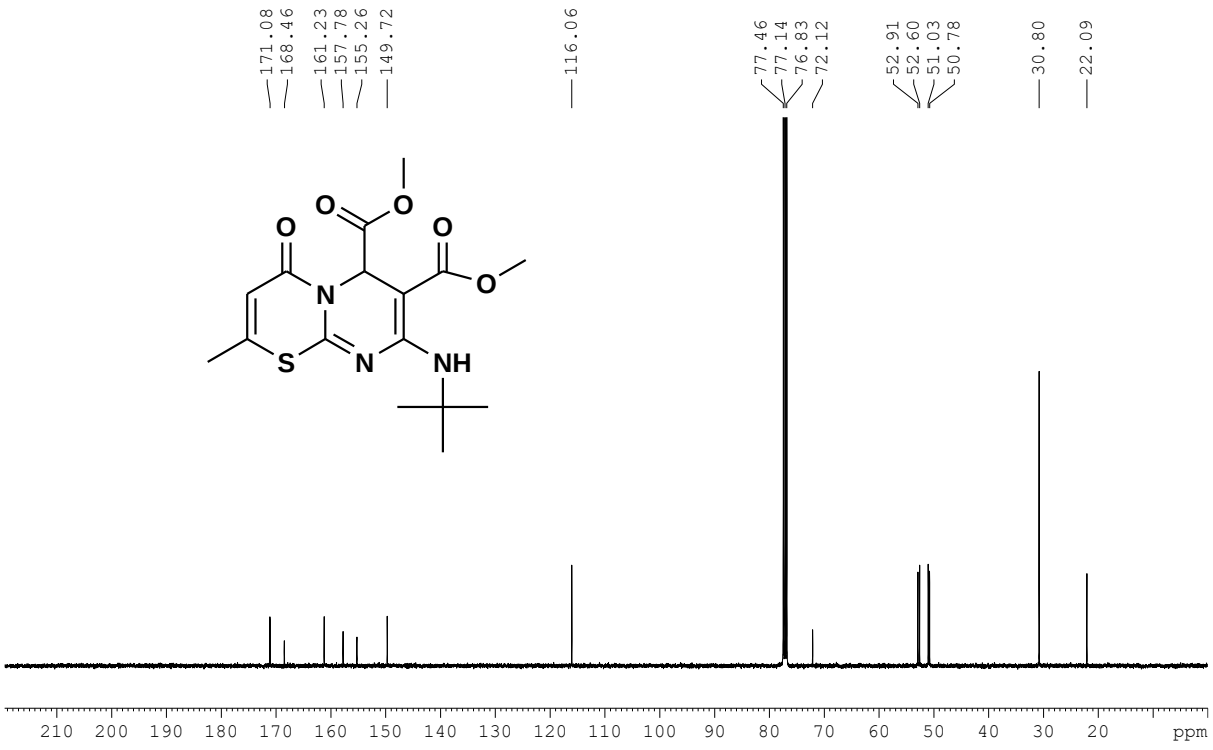
**4.8a**


Z1DMAD#1
Transmittance %
100
95
90
85
80
75
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers cm-1
O
O
O
O
O
N
S
N
NH

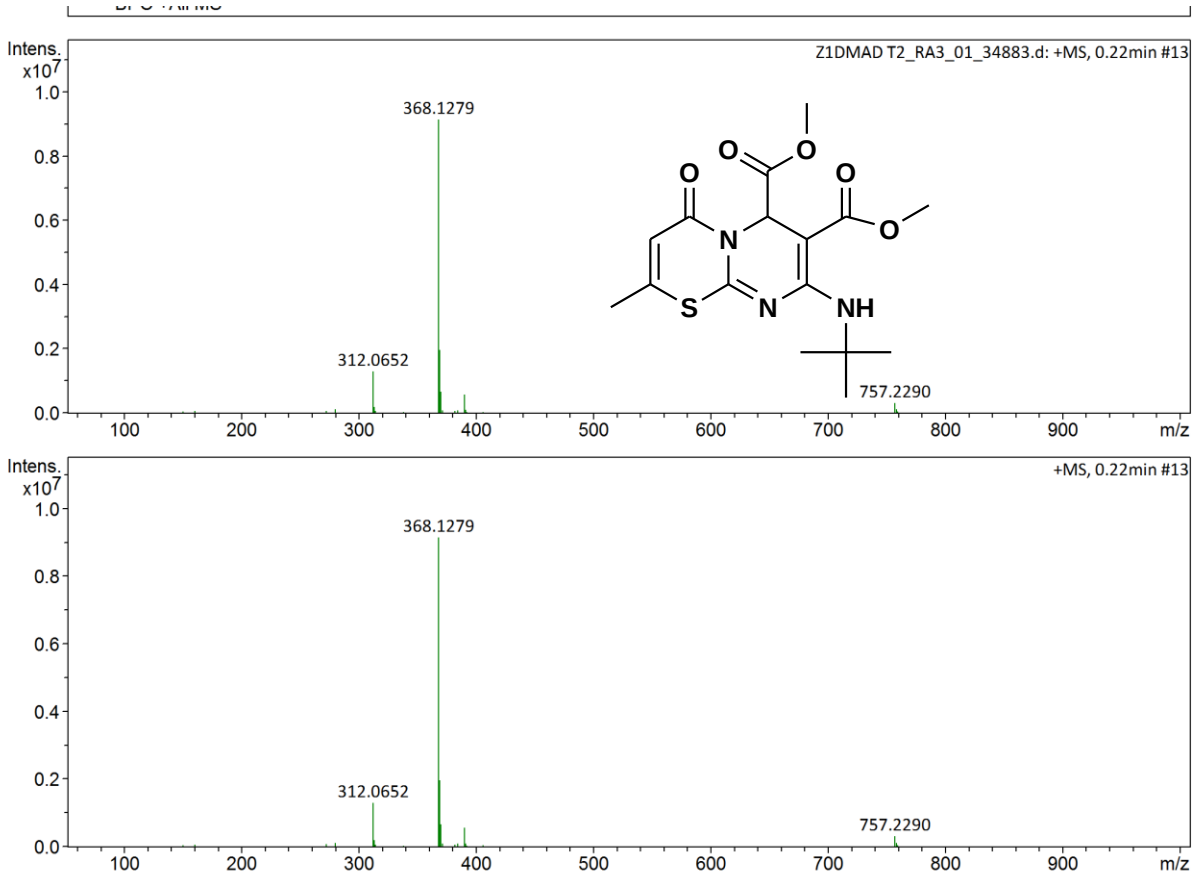
**4.8c**


8.631
6.242
6.229
3.660
3.612
2.166
1.331
O
O
O
O
O
N
S
N
NH
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
2.07
3.06
3.13
3.11
9.01

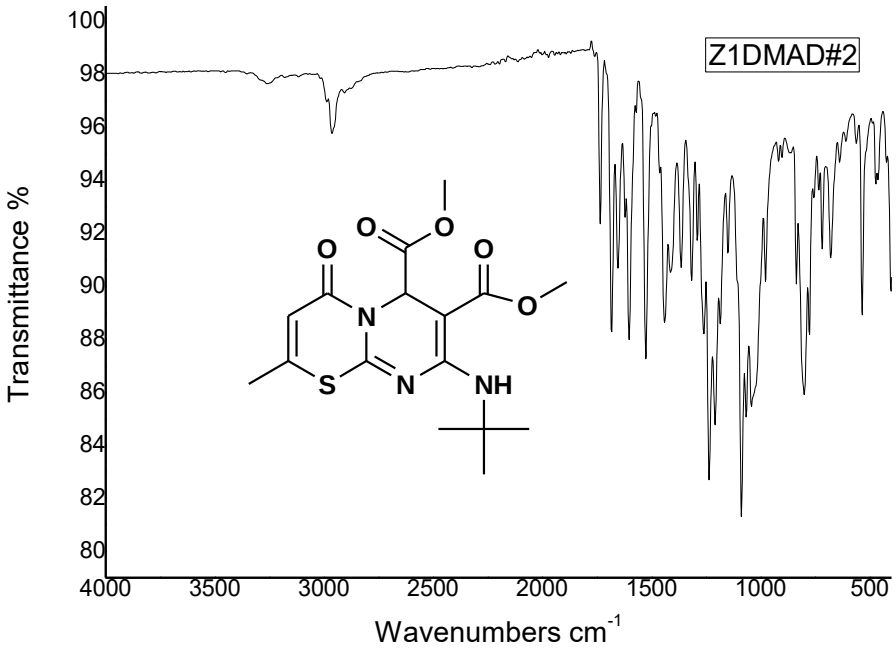
**4.8c**


171.08
168.46
161.23
157.78
155.26
149.72
116.06
77.46
77.14
76.83
72.12
52.91
52.60
51.03
50.78
30.80
22.09
O
O
O
O
N
S
N
NH
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

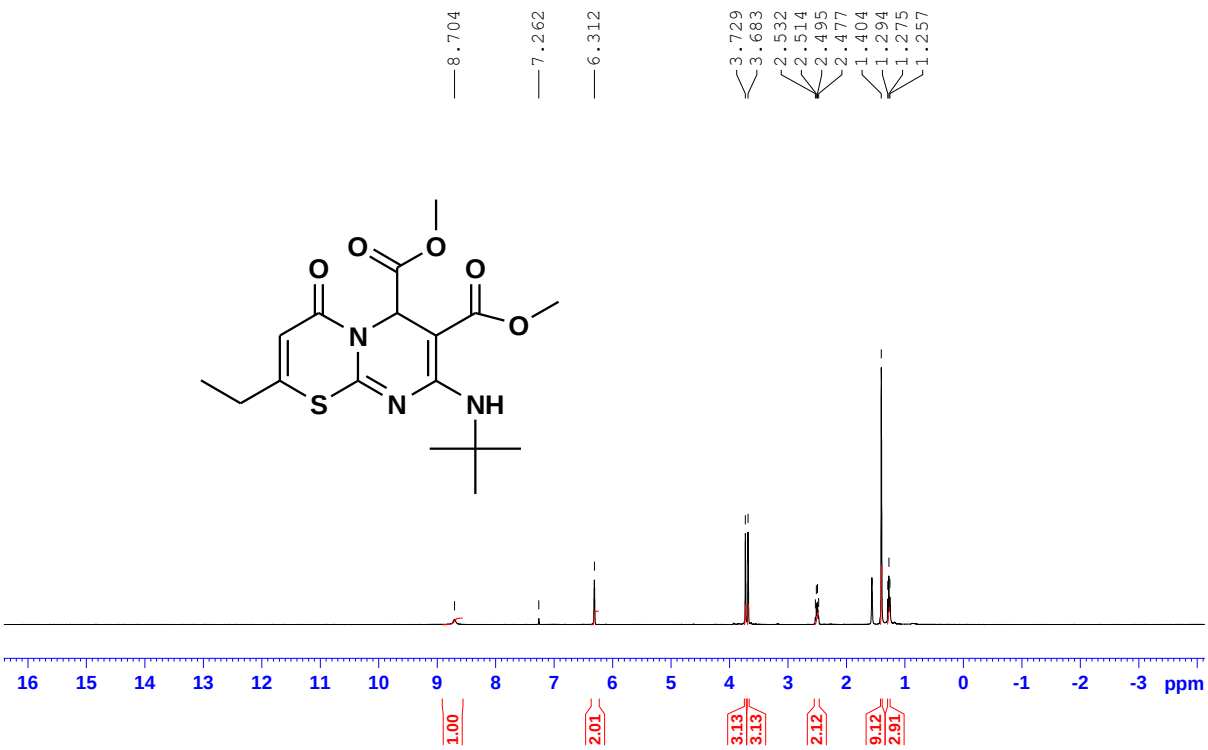
**4.8c**


Intens.
x10^7
1.0
0.8
0.6
0.4
0.2
0.0
Z1DMAD T2_RA3_01_34883.d: +MS, 0.22min #13
368.1279
312.0652
757.2290
O
O
O
O
N
S
N
NH
100 200 300 400 500 600 700 800 900 m/z
Intens.
x10^7
1.0
0.8
0.6
0.4
0.2
0.0
+MS, 0.22min #13
368.1279
312.0652
757.2290
100 200 300 400 500 600 700 800 900 m/z

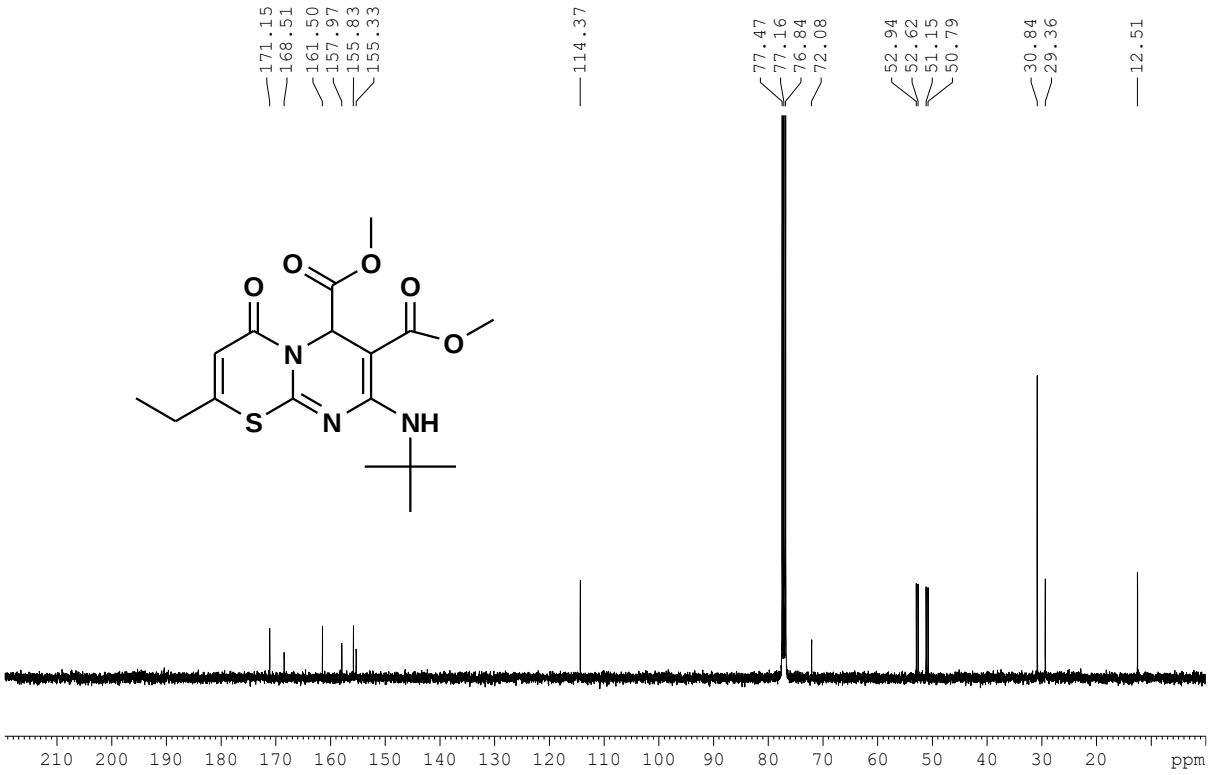
**4.8c**


Z1DMAD#2
Transmittance %
100
98
96
94
92
90
88
86
84
82
80
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers cm-1
O
O
O
O
O
N
S
N
NH

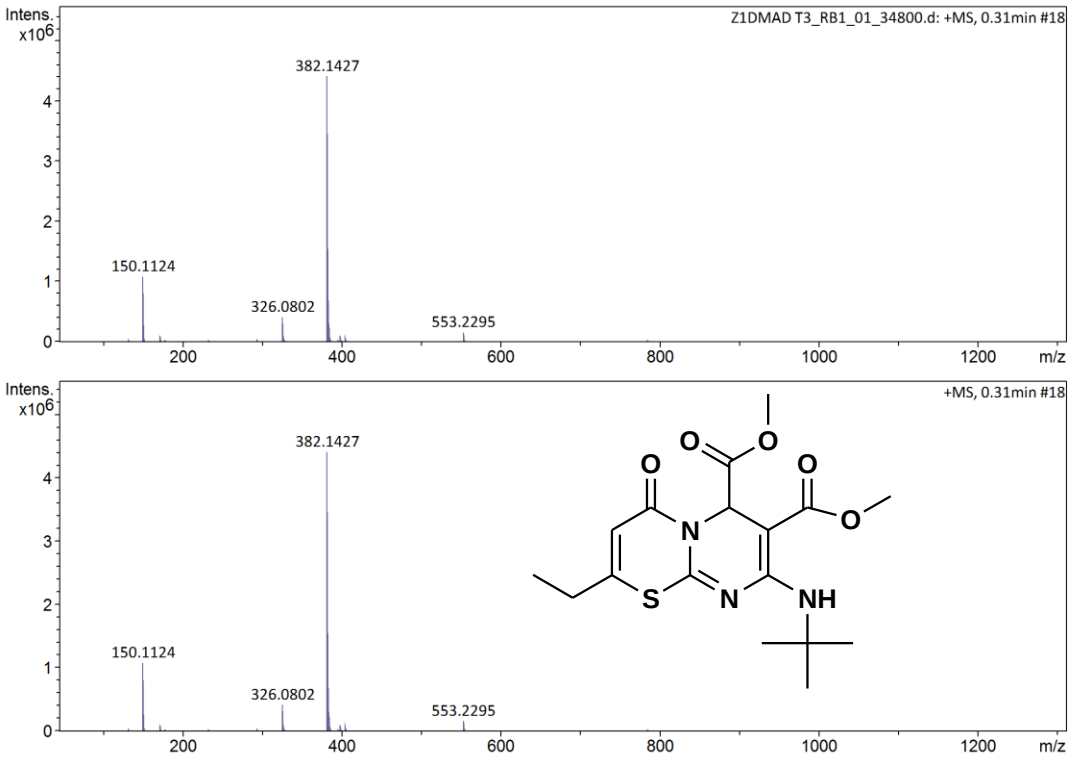
**4.8i**


8.704
7.262
6.312
3.729
3.683
2.532
2.514
2.495
2.477
1.404
1.294
1.275
1.257
O
O
O
O
O
N
S
N
NH
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
ppm
1.00
2.01
3.13
3.13
2.12
9.12
2.91

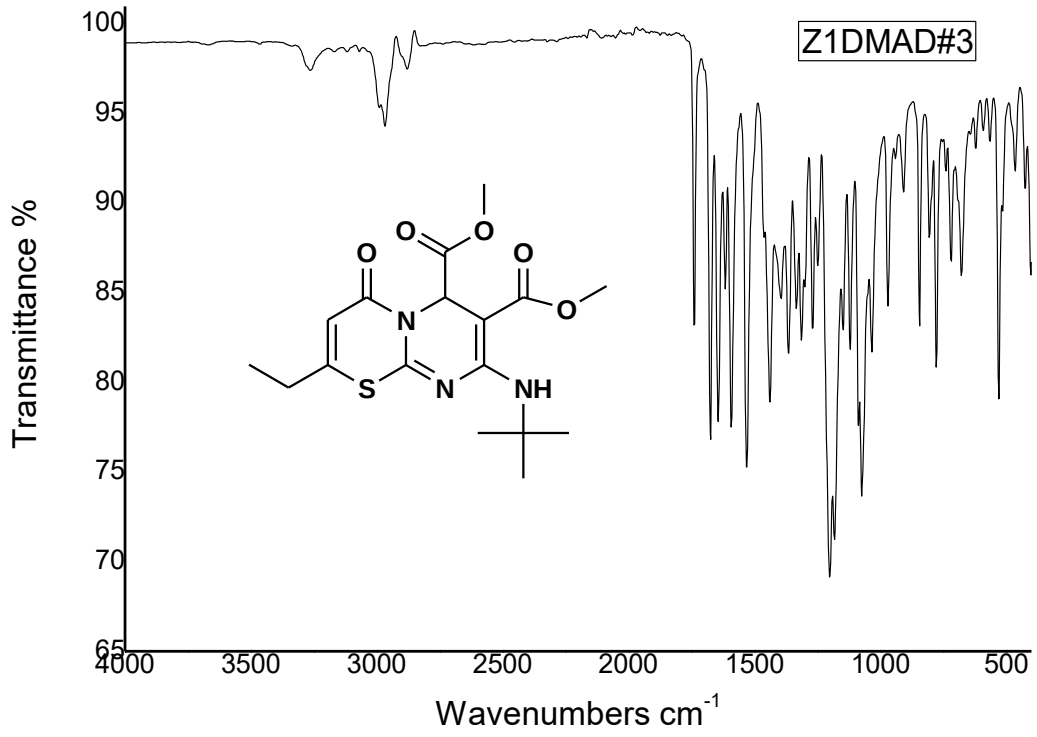
**4.8i**


171.15
168.51
161.50
157.97
155.83
155.33
114.37
77.47
77.16
76.84
72.08
52.94
52.62
51.15
50.79
30.84
29.36
12.51
O
O
O
O
O
N
S
N
NH
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

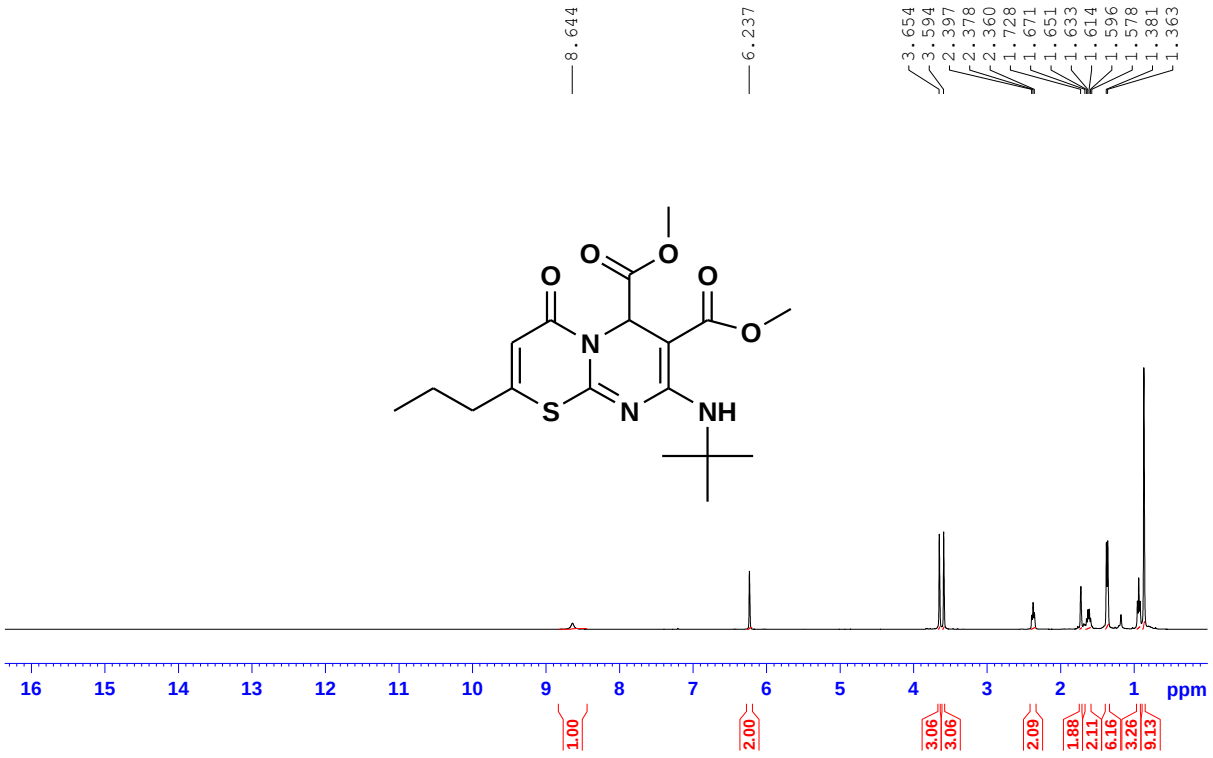
**4.8i**


Intens.
x10^6
Z1DMAD T3_RB1_01_34800.d: +MS, 0.31min #18
382.1427
150.1124
326.0802
553.2295
200 400 600 800 1000 1200 m/z
Intens.
x10^6
+MS, 0.31min #18
382.1427
150.1124
326.0802
553.2295
200 400 600 800 1000 1200 m/z

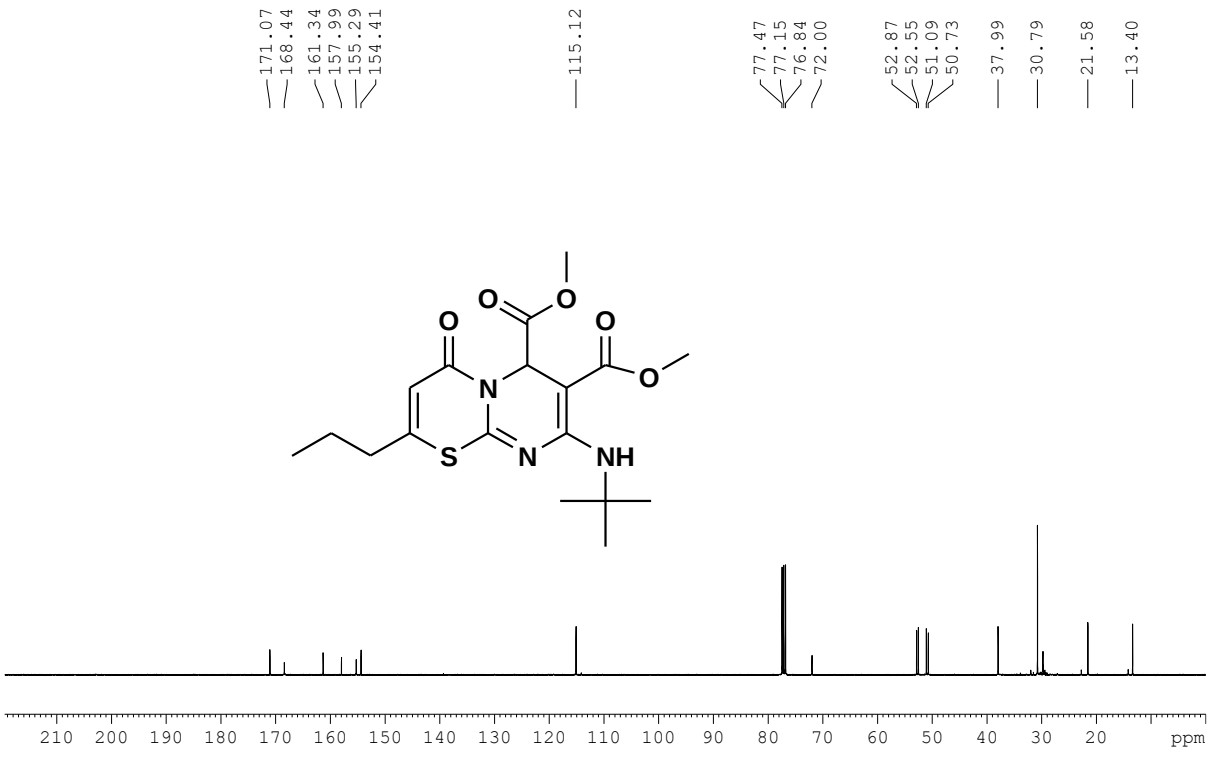
**4.8i**


Z1DMAD#3
Transmittance %
Wavenumbers cm-1
100
95
90
85
80
75
70
65
4000
3500
3000
2500
2000
1500
1000
500
O
O
O
O
O
N
S
N
NH

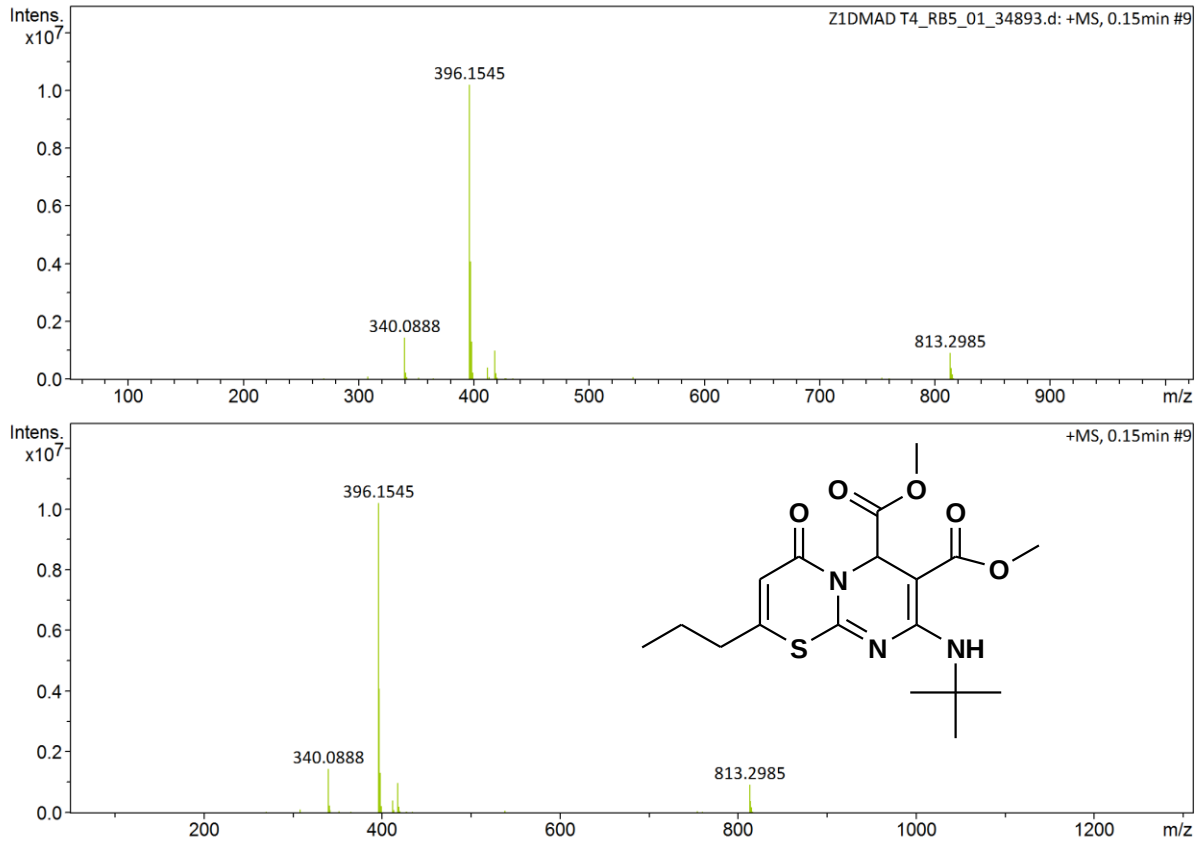
**4.8m**


8.644
6.237
3.654
3.594
2.397
2.378
2.360
1.728
1.671
1.651
1.633
1.614
1.596
1.578
1.381
1.363
O
O
O
O
O
N
S
N
NH
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
2.00
3.06
3.06
2.09
1.88
2.11
6.16
3.26
9.13

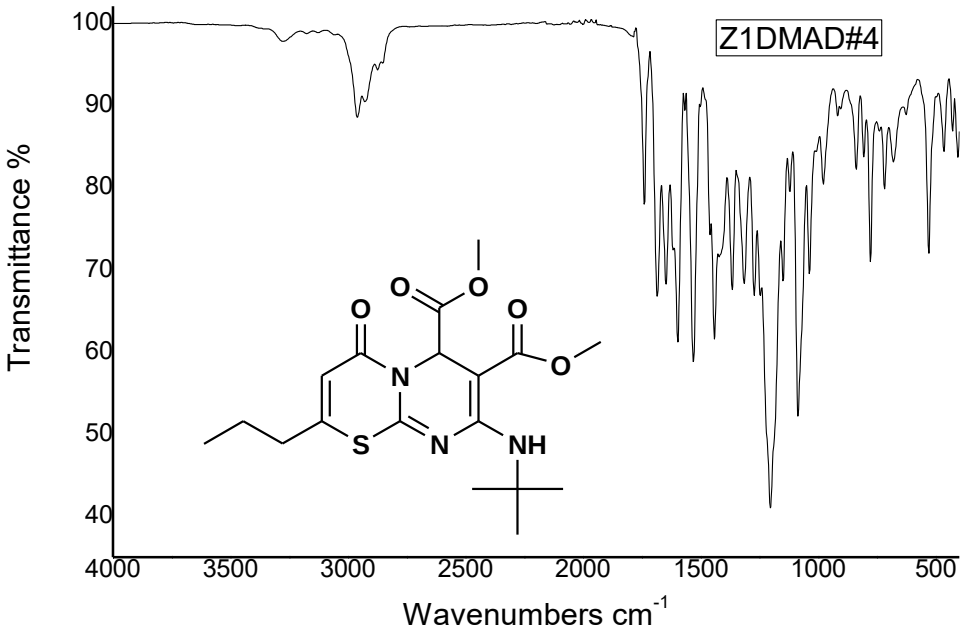
**4.8m**


171.07
168.44
161.34
157.99
155.29
154.41
115.12
77.47
77.15
76.84
72.00
52.87
52.55
51.09
50.73
37.99
30.79
21.58
13.40
O
O
O
O
N
S
N
NH
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

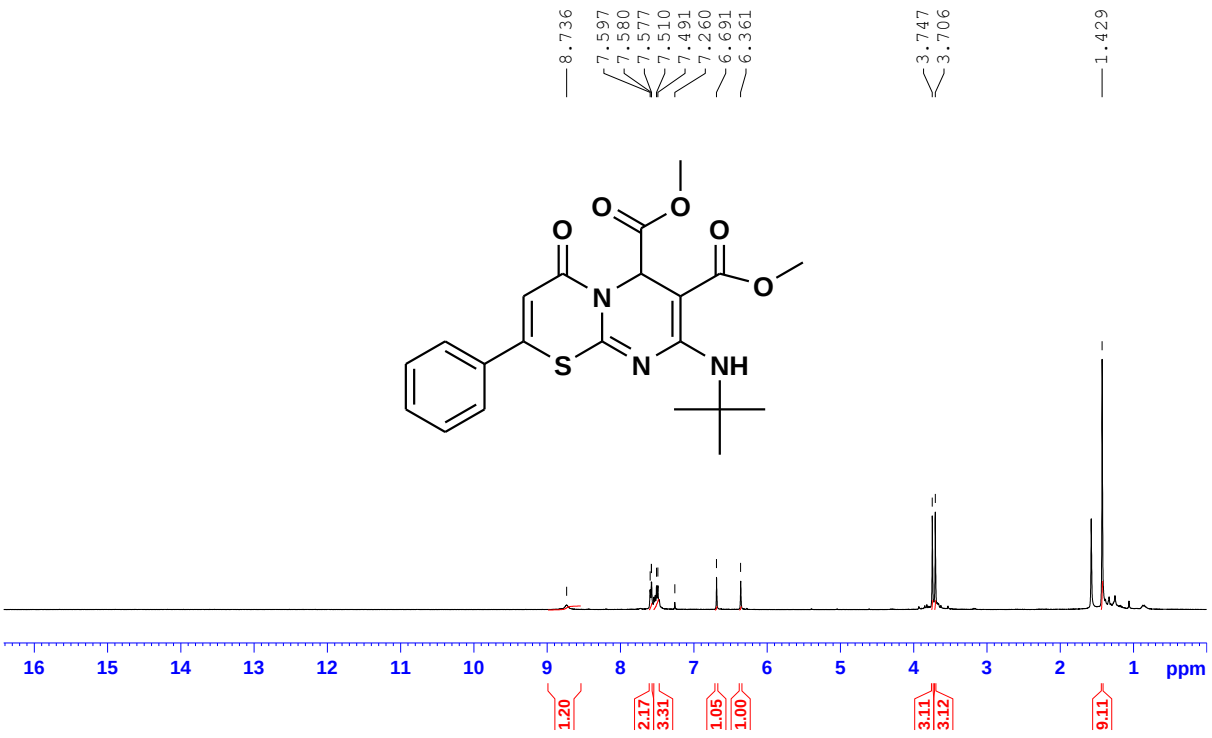
**4.8m**


Intens.
x10^7
Z1DMAD T4_RB5_01_34893.d: +MS, 0.15min #9
396.1545
340.0888
813.2985
100 200 300 400 500 600 700 800 900 m/z
Intens.
x10^7
+MS, 0.15min #9
396.1545
340.0888
813.2985
200 400 600 800 1000 1200 m/z

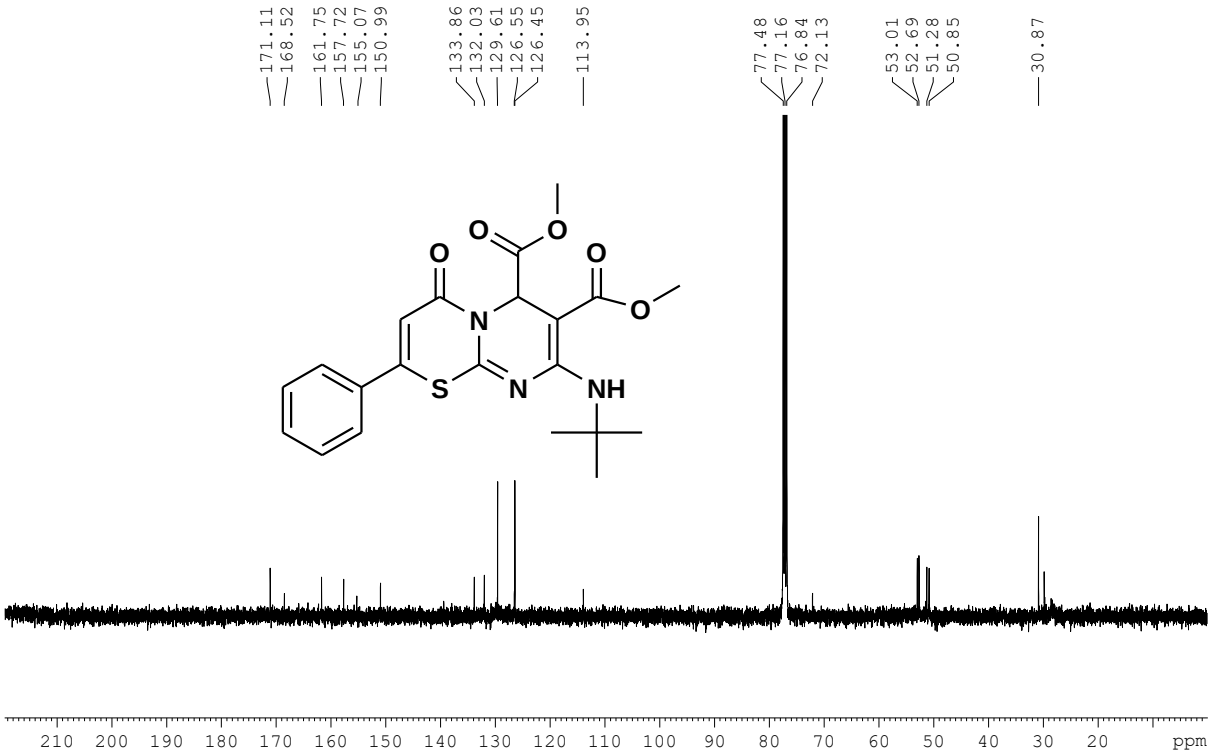
**4.8m**


Z1DMAD#4
Transmittance %
100
90
80
70
60
50
40
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers cm-1
O
O
O
O
O
N
S
N
NH

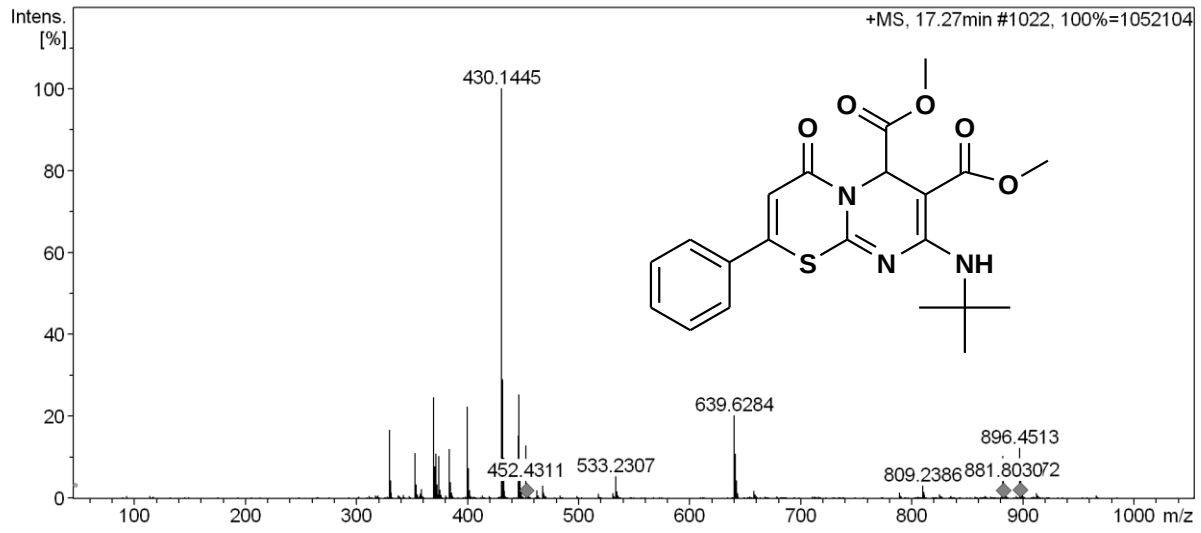
**4.8q**


8.736
7.597
7.580
7.577
7.510
7.491
7.260
6.691
6.361
3.747
3.706
1.429
O
O
O
O
O
N
S
N
NH
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.20
2.17
3.31
1.05
1.00
3.11
3.12
9.11

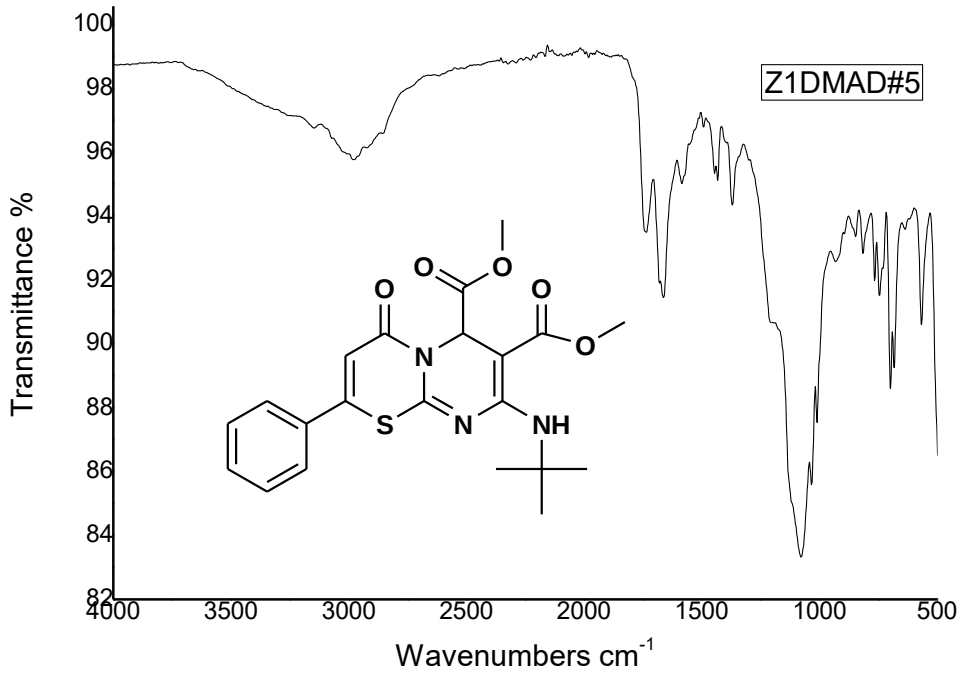
**4.8q**


171.11
168.52
161.75
157.72
155.07
150.99
133.86
132.03
129.61
126.55
126.45
113.95
77.48
77.16
76.84
72.13
53.01
52.69
51.28
50.85
30.87
O
O
O
O
O
N
S
N
NH
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

**4.8q**

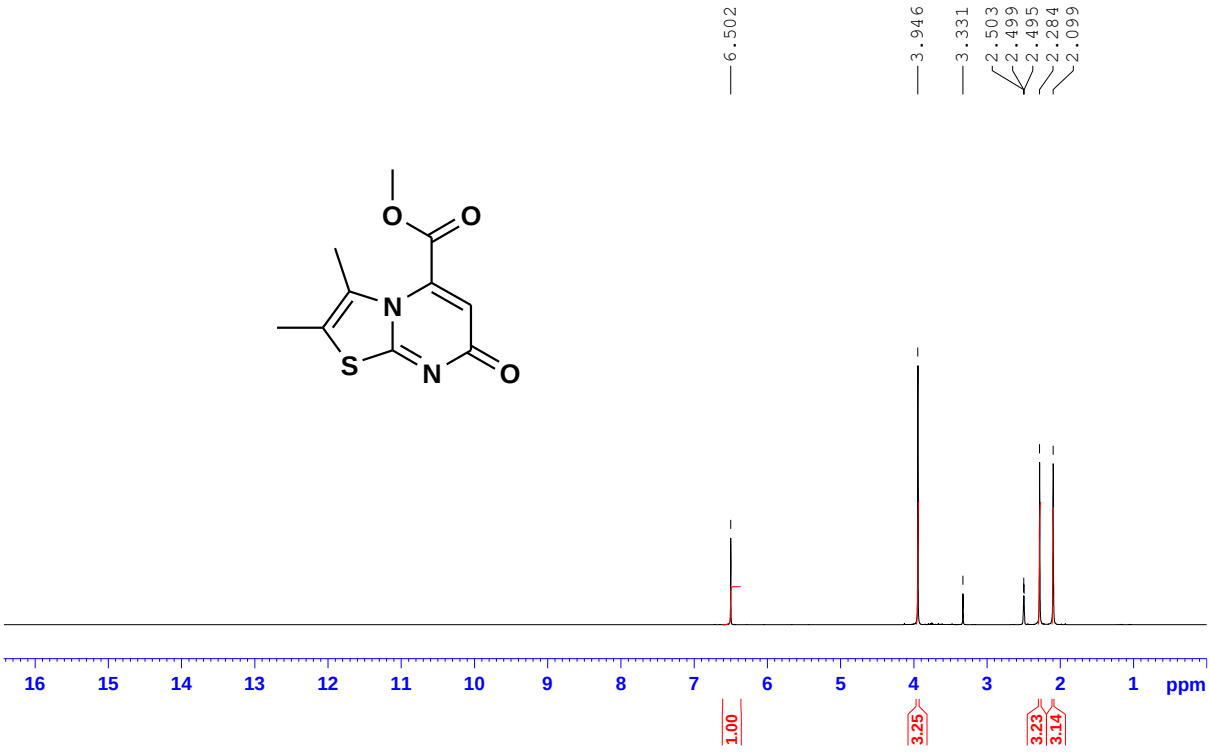

Intens.
[%]
+MS, 17.27min #1022, 100%=1052104
430.1445
639.6284
896.4513
452.4311
533.2307
809.2386
881.8030
O
O
O
O
O
N
S
N
NH
100
80
60
40
20
0
100 200 300 400 500 600 700 800 900 1000 m/z

**4.8q**

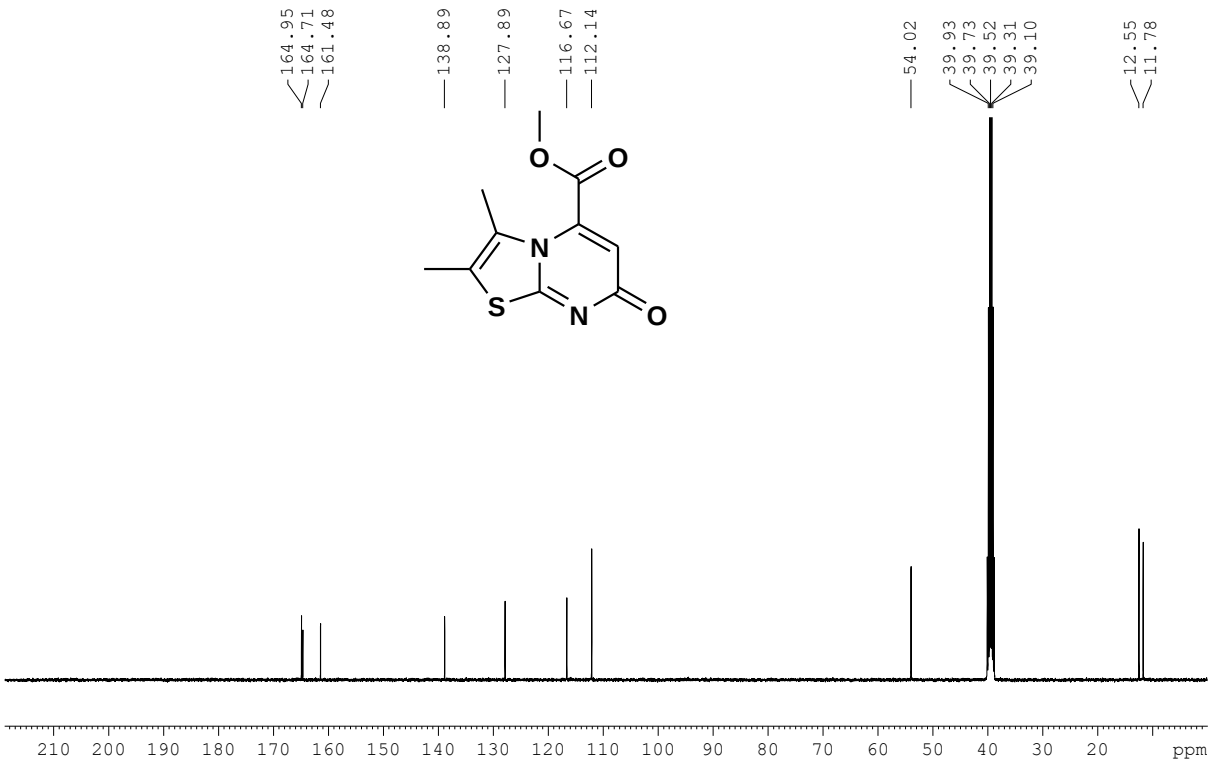



**CHAPTER 5: selected spectroscopic data**

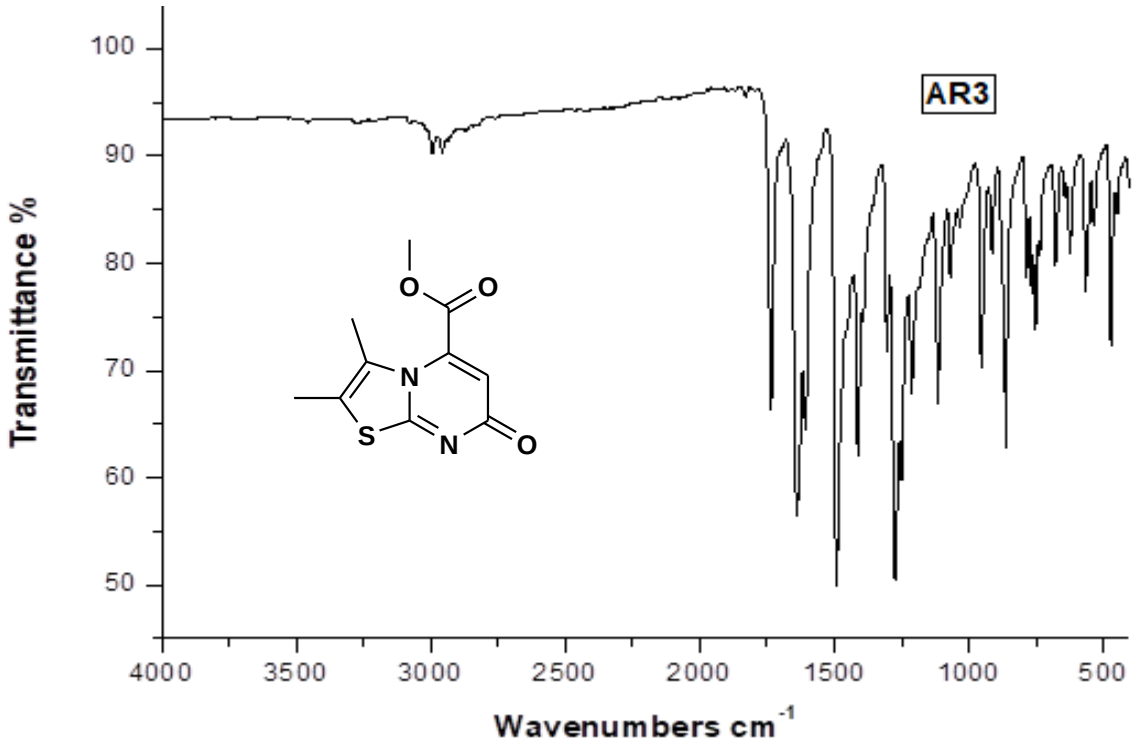
**5.17c**



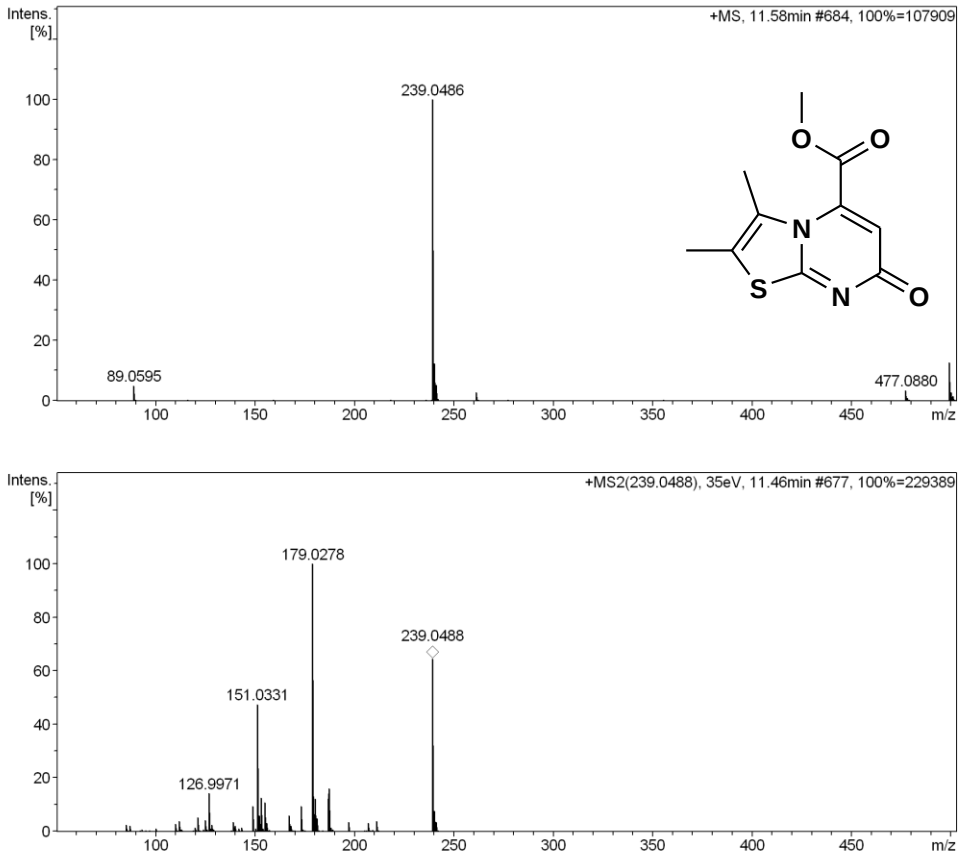

**5.17c**


164.95
164.71
161.48
138.89
127.89
116.67
112.14
54.02
39.93
39.73
39.52
39.31
39.10
12.55
11.78
O
O
N
S
N
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

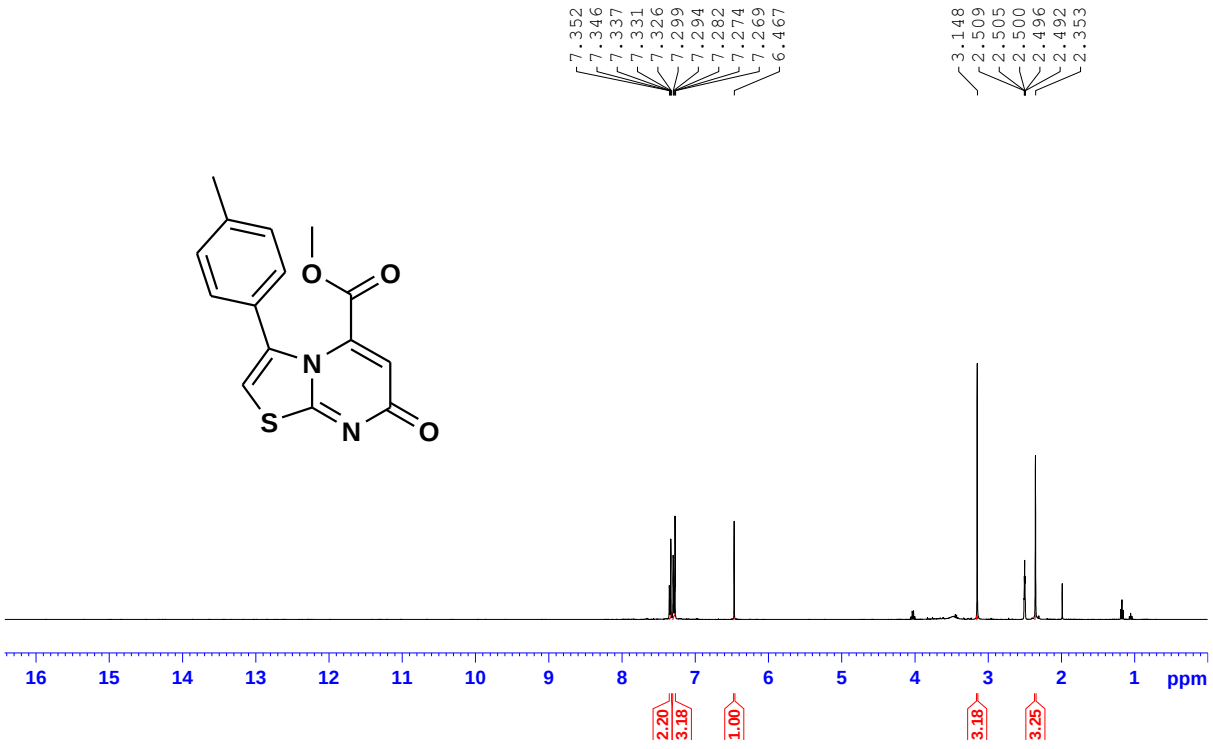
**5.17c**


AR3
Transmittance %
100
90
80
70
60
50
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers cm-1
O
O
N
S
N
O

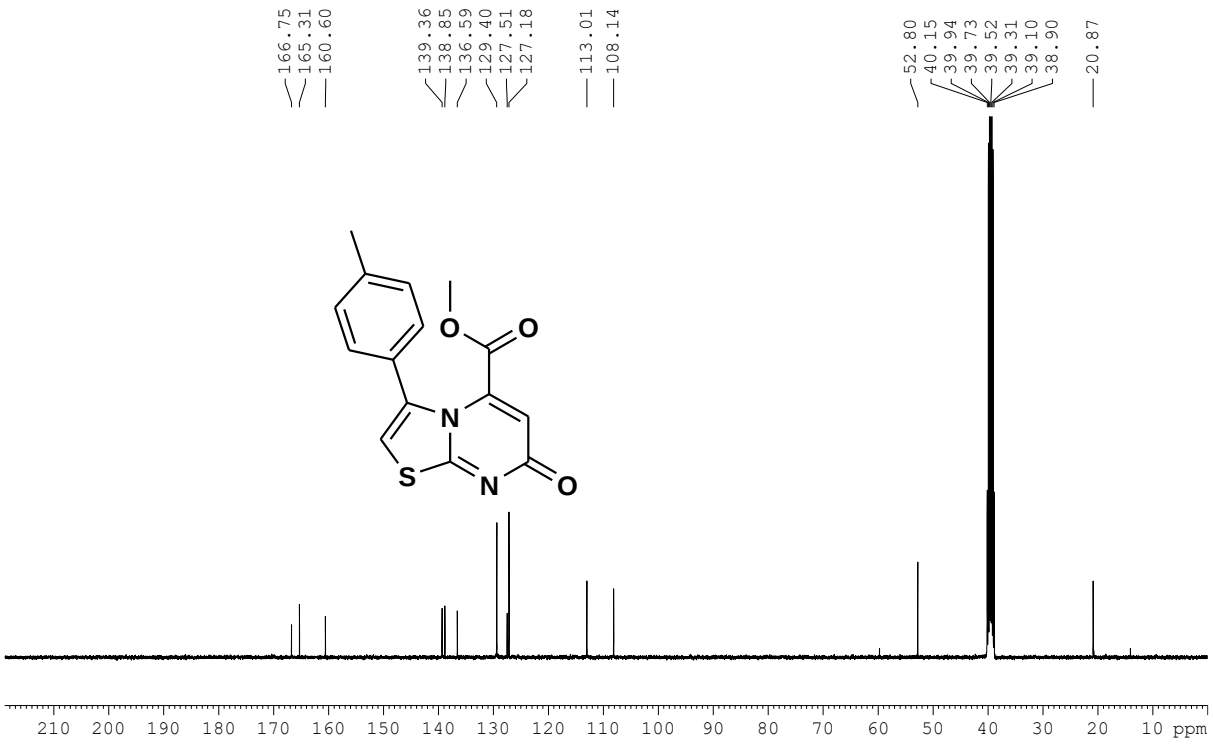
**5.17c**


Intens.
[%]
+MS, 11.58min #684, 100%=107909
239.0486
89.0595
477.0880
m/z
+MS2(239.0488), 35eV, 11.46min #677, 100%=229389
179.0278
239.0488
151.0331
126.9971

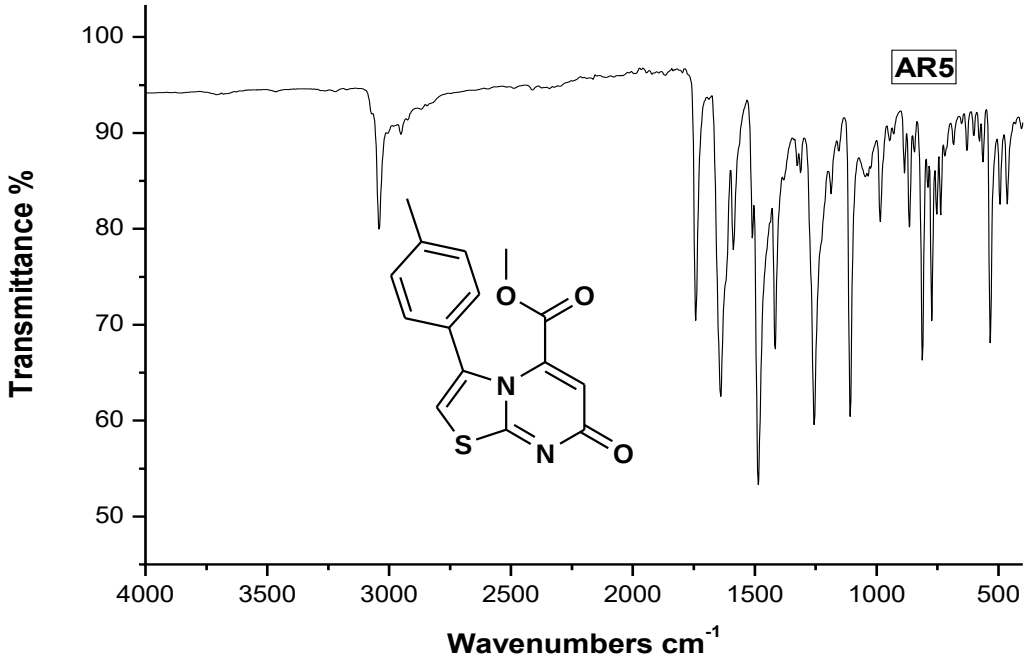
**5.17e**


7.352
7.346
7.337
7.331
7.326
7.299
7.294
7.282
7.274
7.269
6.467
3.148
2.509
2.505
2.500
2.496
2.492
2.353
ppm
2.20
3.18
1.00
3.18
3.25

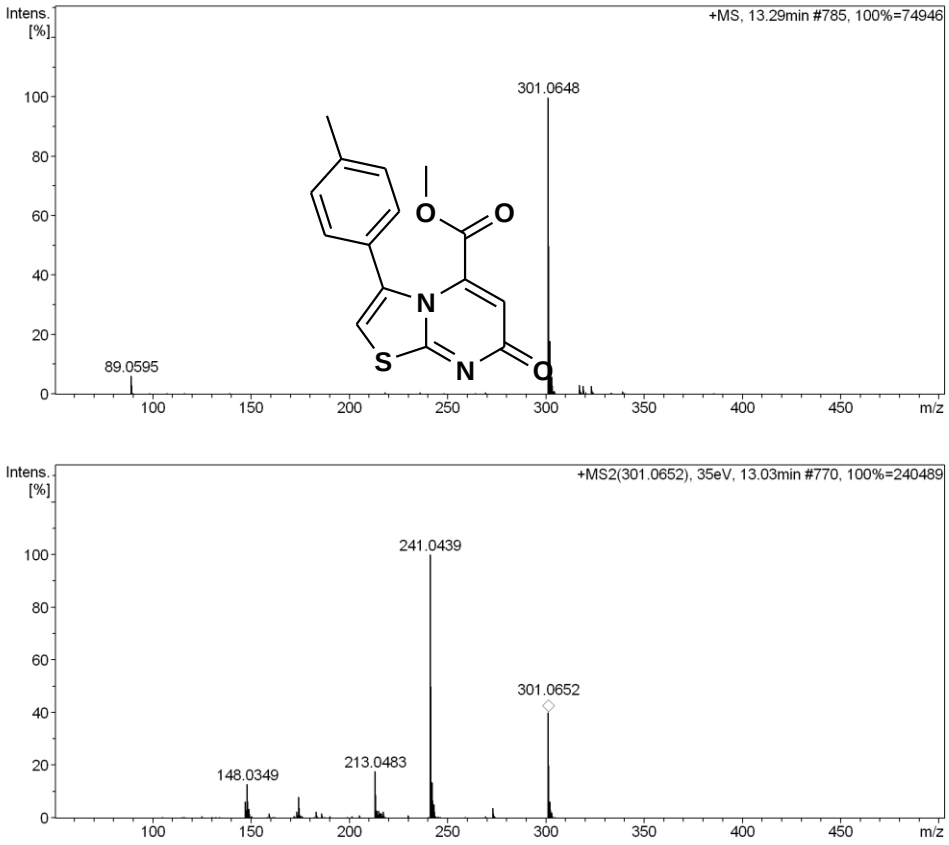
**5.17e**


166.75
165.31
160.60
139.36
138.85
136.59
129.40
127.51
127.18
113.01
108.14
52.80
40.15
39.94
39.73
39.52
39.31
39.10
38.90
20.87
O
O
N
S
N
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

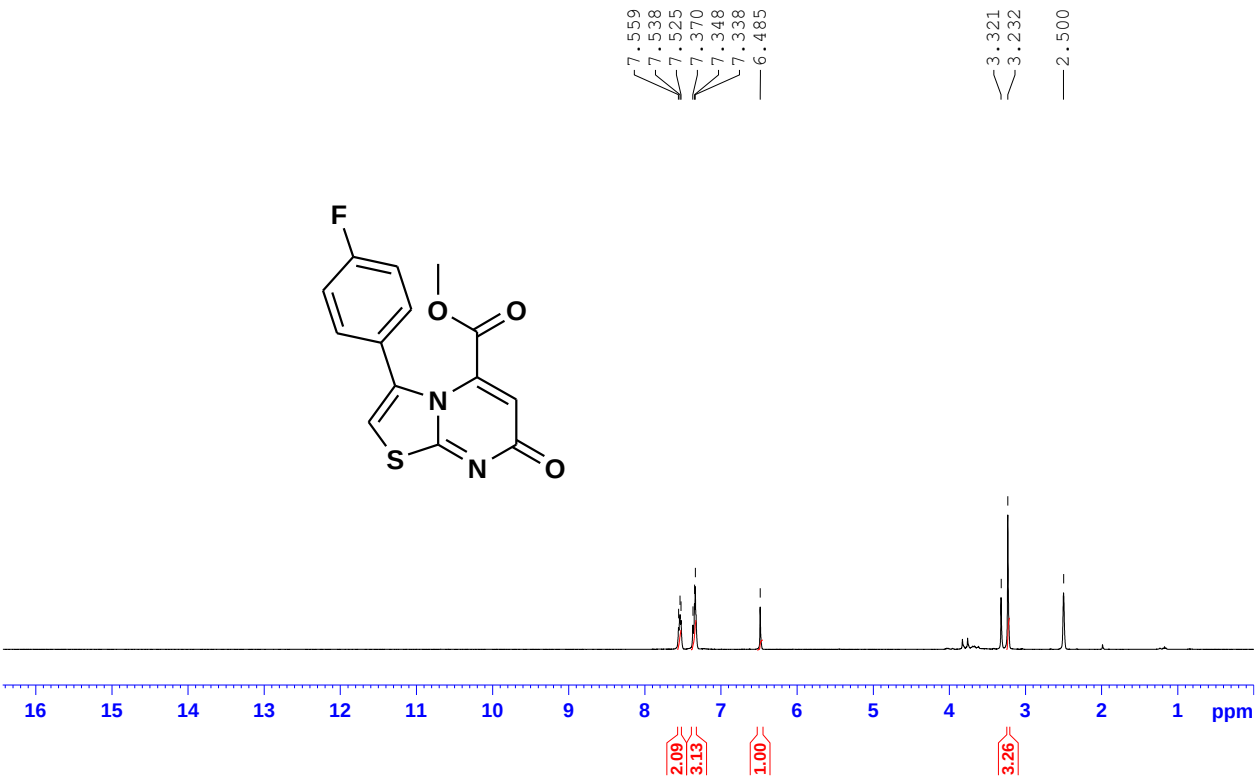
**5.17e**


AR5
Transmittance %
100
90
80
70
60
50
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers cm-1
O
O
N
S
N
O

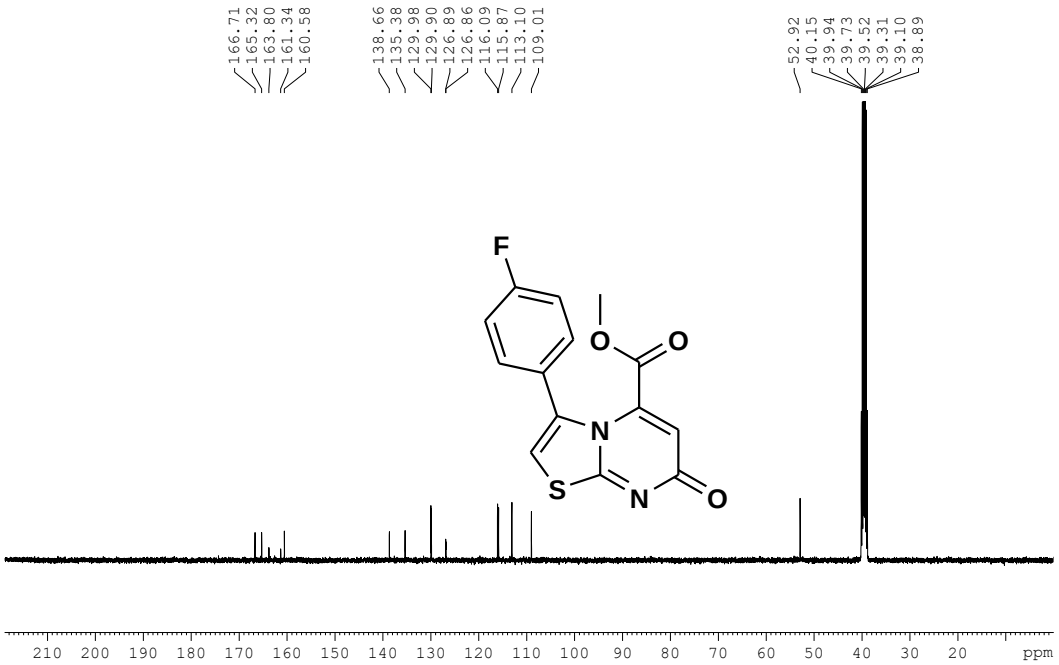
**5.17e**


Intens.
[%]
+MS, 13.29min #785, 100%=74946
301.0648
89.0595
+MS2(301.0652), 35eV, 13.03min #770, 100%=240489
241.0439
301.0652
213.0483
148.0349
m/z

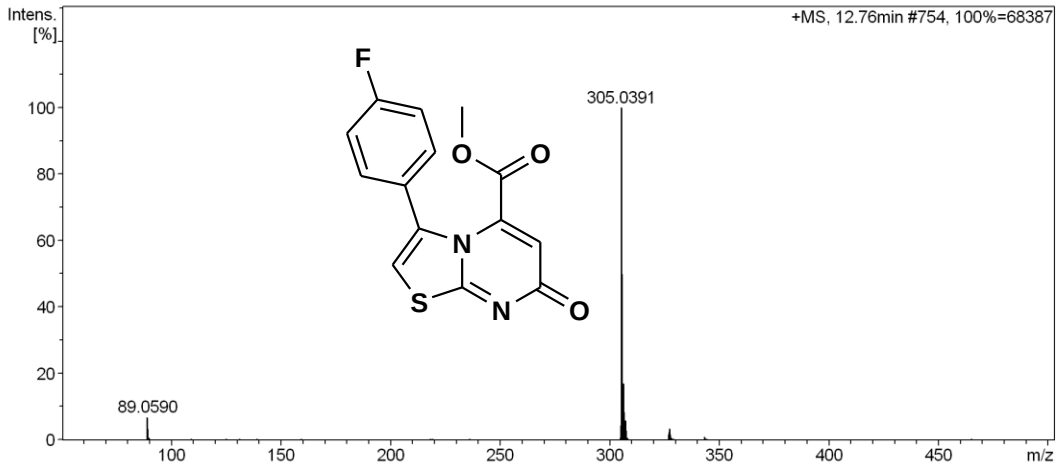
**5.17f**

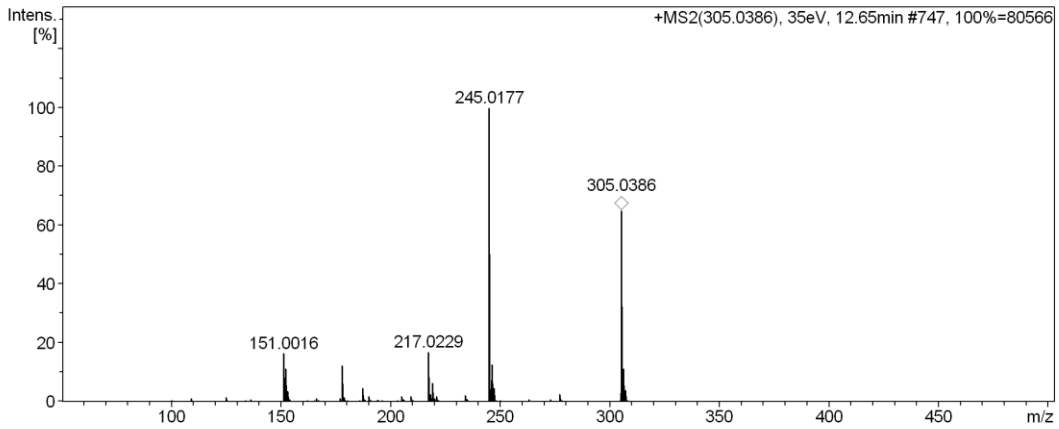

7.559
7.538
7.525
7.370
7.348
7.338
6.485
3.321
3.232
2.500
F
ppm
2.09
3.13
1.00
3.26

**5.17f**

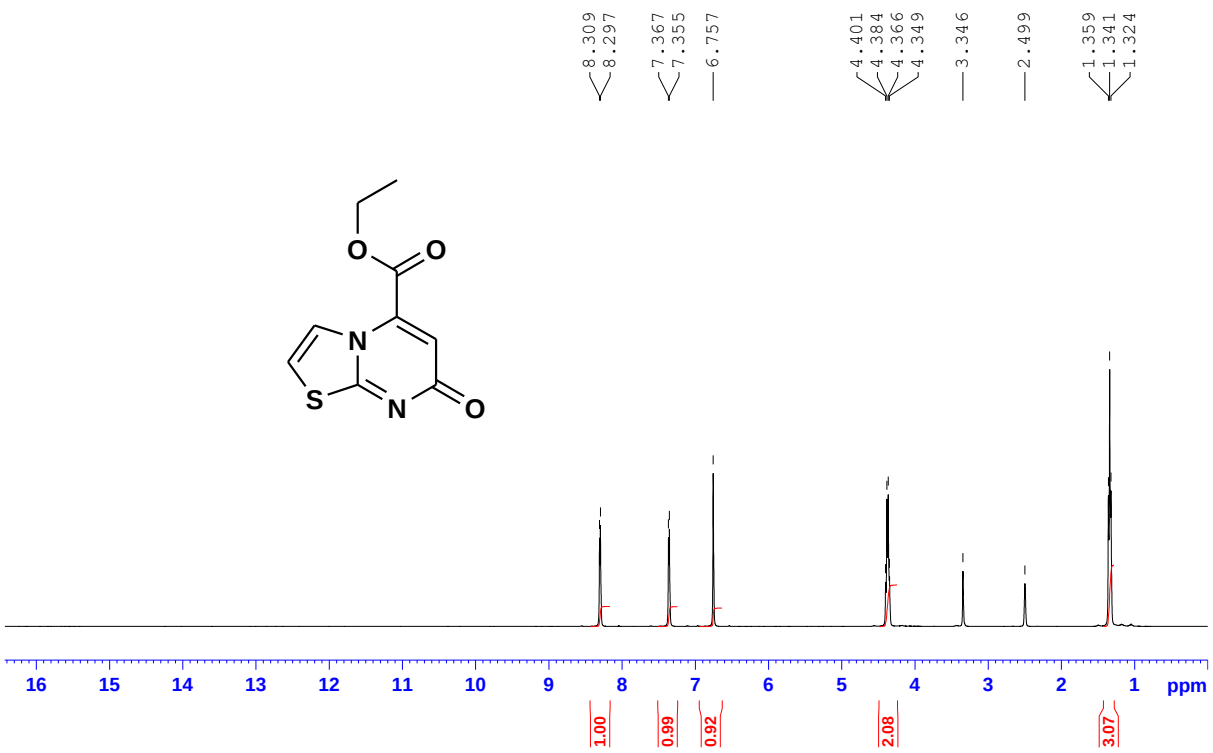

166.71
165.32
163.80
161.34
160.58
138.66
135.38
129.98
129.90
126.89
126.86
116.09
115.87
113.10
109.01
52.92
40.15
39.94
39.73
39.52
39.31
39.10
38.89
F
O
O
N
S
N
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

**5.17f**

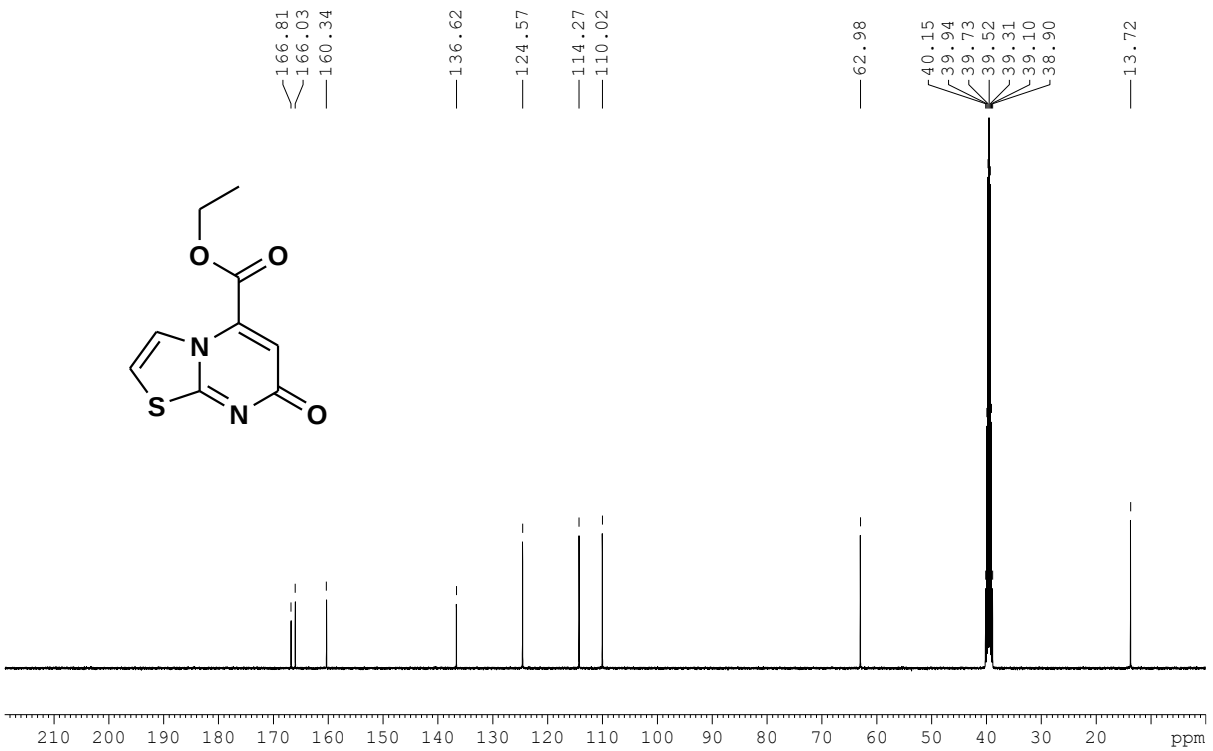

Intens.
[%]
+MS, 12.76min #754, 100%=68387
305.0391
89.0590
F
O
O
N
S
N
O
100
80
60
40
20
0
100 150 200 250 300 350 400 450 m/z


Intens.
[%]
+MS2(305.0386), 35eV, 12.65min #747, 100%=80566
245.0177
305.0386
151.0016
217.0229
100
80
60
40
20
0
100 150 200 250 300 350 400 450 m/z

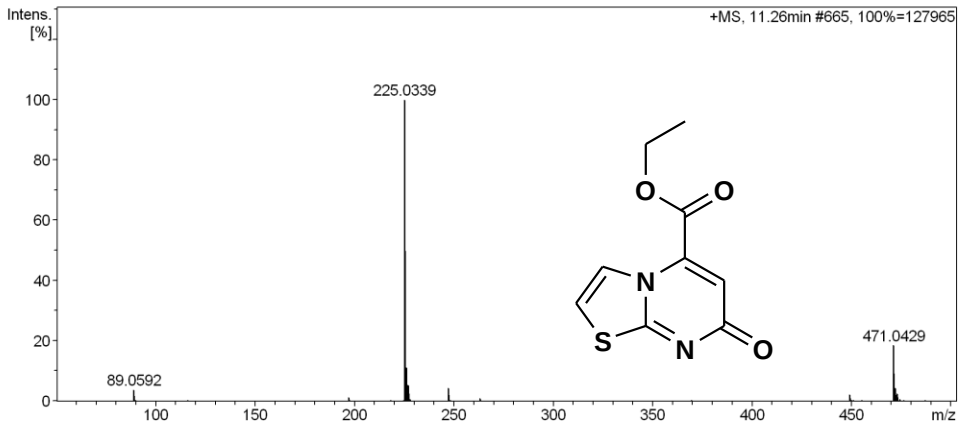
**5.17h**

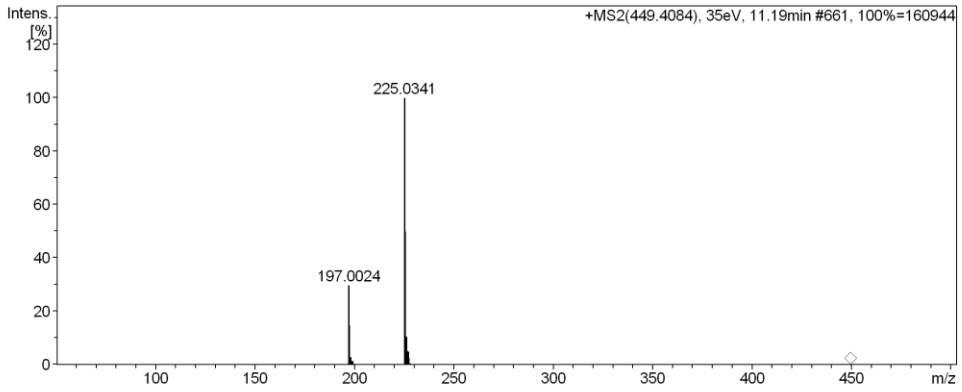



**5.17h**

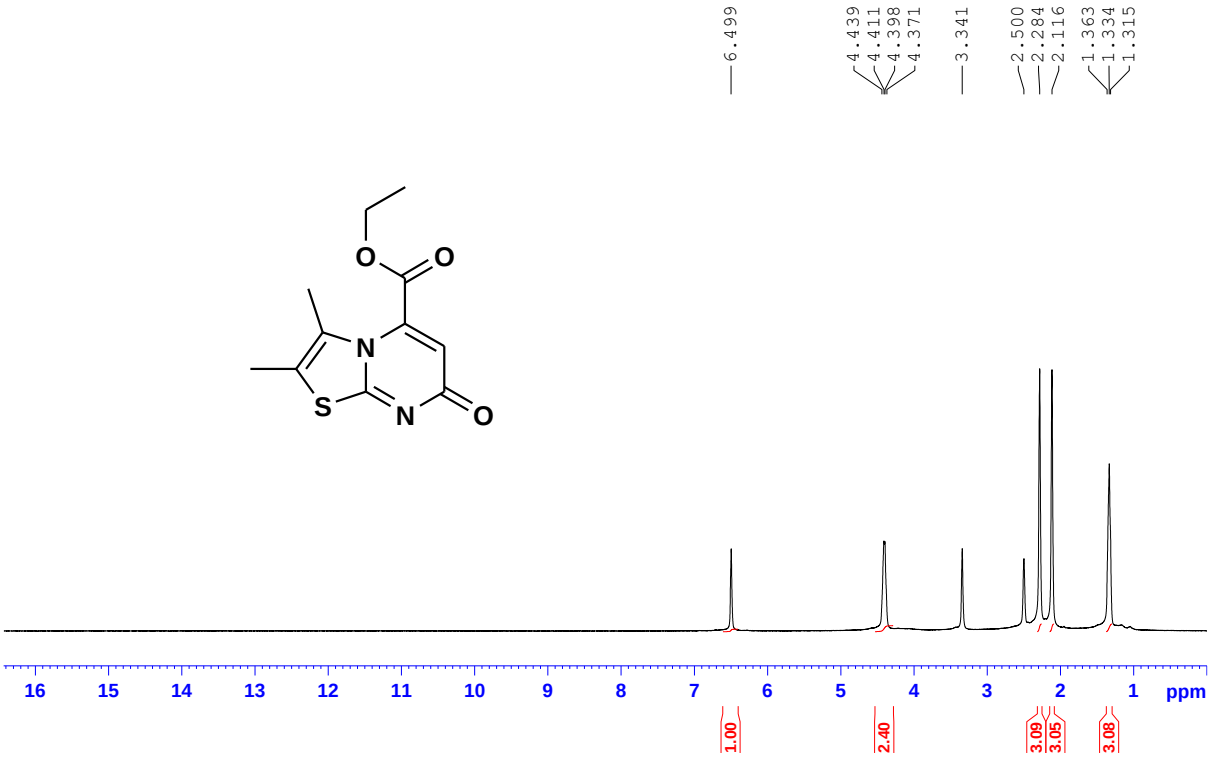



**5.17h**

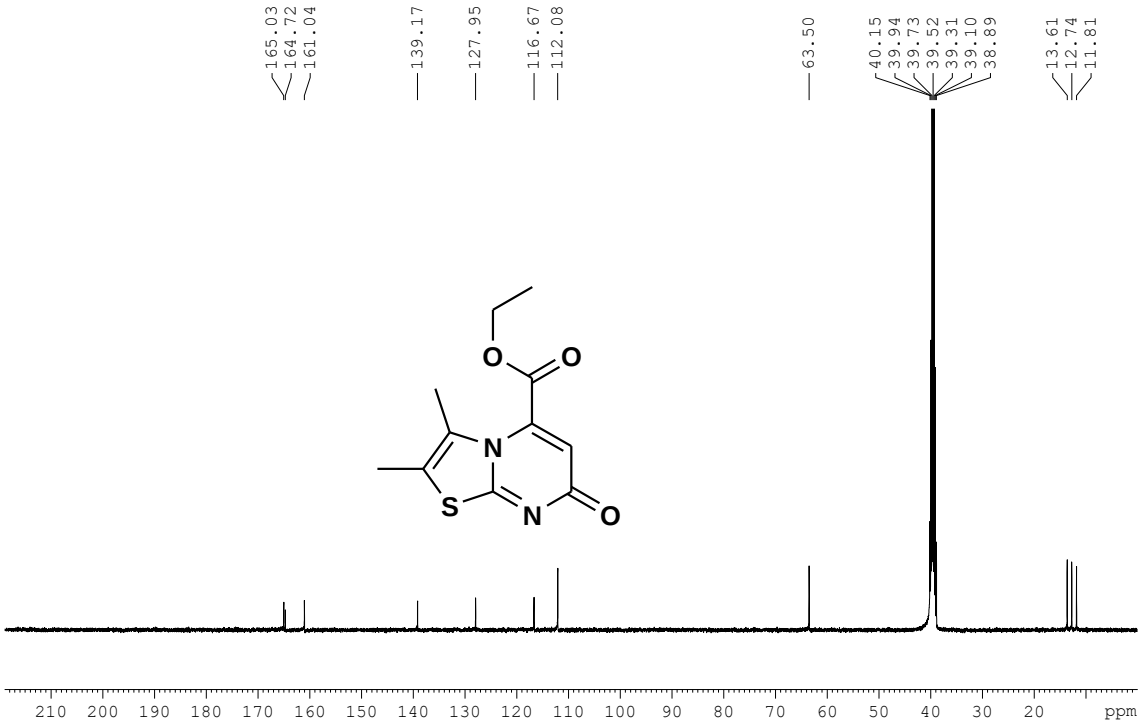

Intens.
[%]
+MS, 11.26min #665, 100%=127965
225.0339
471.0429
89.0592
m/z


Intens.
[%]
+MS2(449.4084), 35eV, 11.19min #661, 100%=160944
225.0341
197.0024
m/z

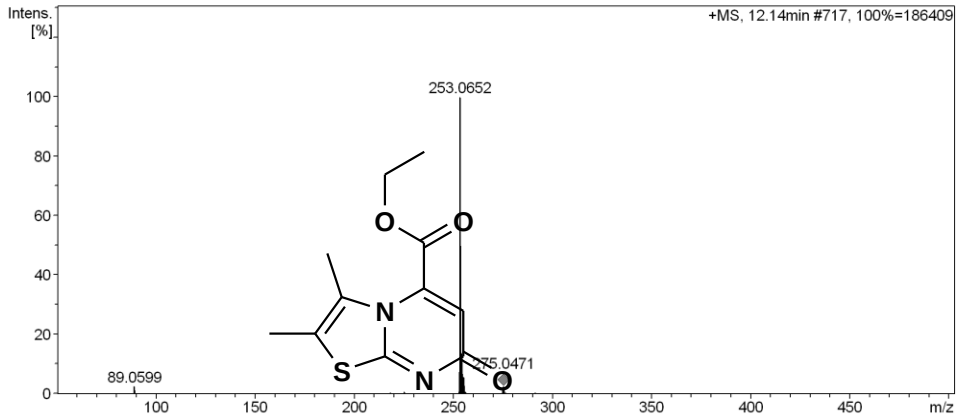
**5.17j**

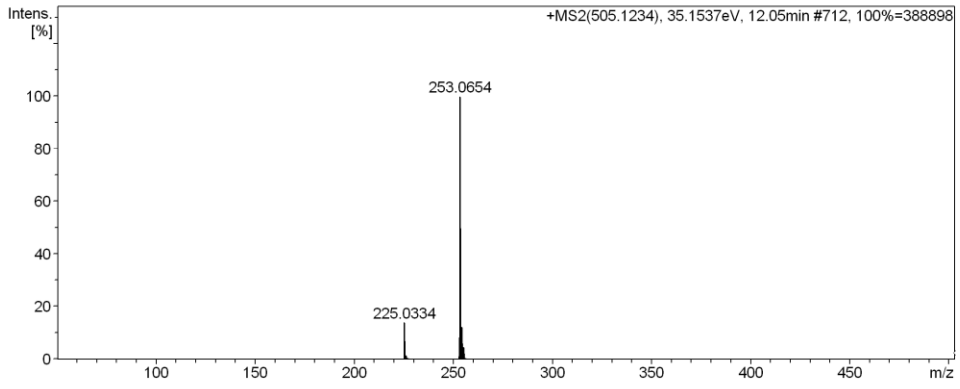

6.499
4.439
4.411
4.398
4.371
3.341
2.500
2.284
2.116
1.363
1.334
1.315
ppm
1.00
2.40
3.09
3.05
3.08

**5.17j**

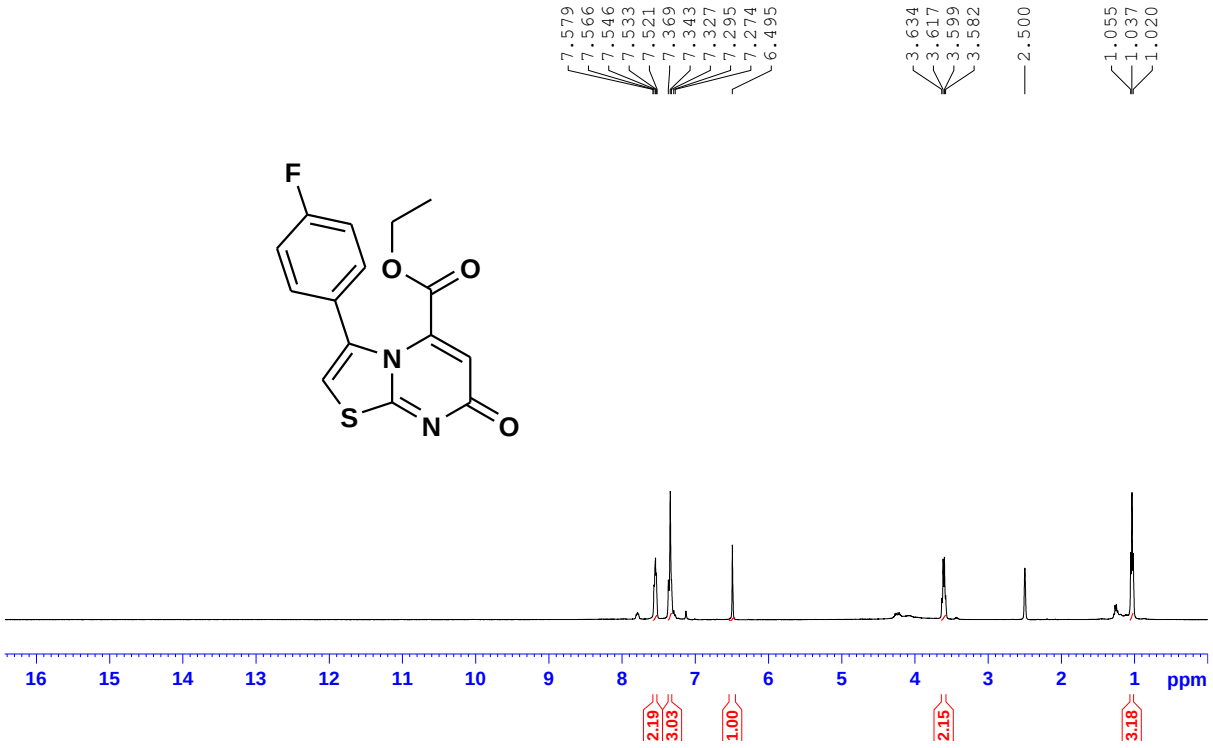

165.03
164.72
161.04
139.17
127.95
116.67
112.08
63.50
40.15
39.94
39.73
39.52
39.31
39.10
38.89
13.61
12.74
11.81
O
O
N
S
N
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

**5.17j**

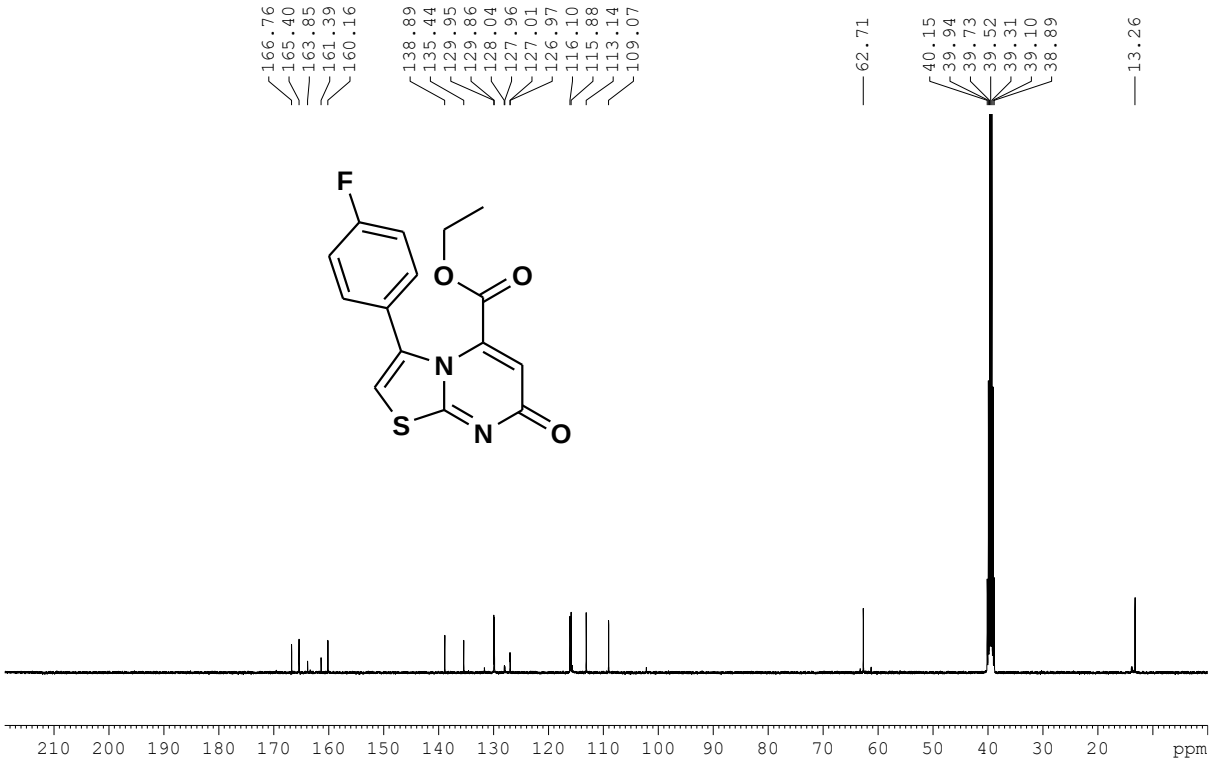

Intens.
[%]
+MS, 12.14min #717, 100%=186409
253.0652
89.0599
275.0471
O
O
N
S
N
O
100
80
60
40
20
0
100 150 200 250 300 350 400 450 m/z


Intens.
[%]
+MS2(505.1234), 35.1537eV, 12.05min #712, 100%=388898
253.0654
225.0334
100
80
60
40
20
0
100 150 200 250 300 350 400 450 m/z

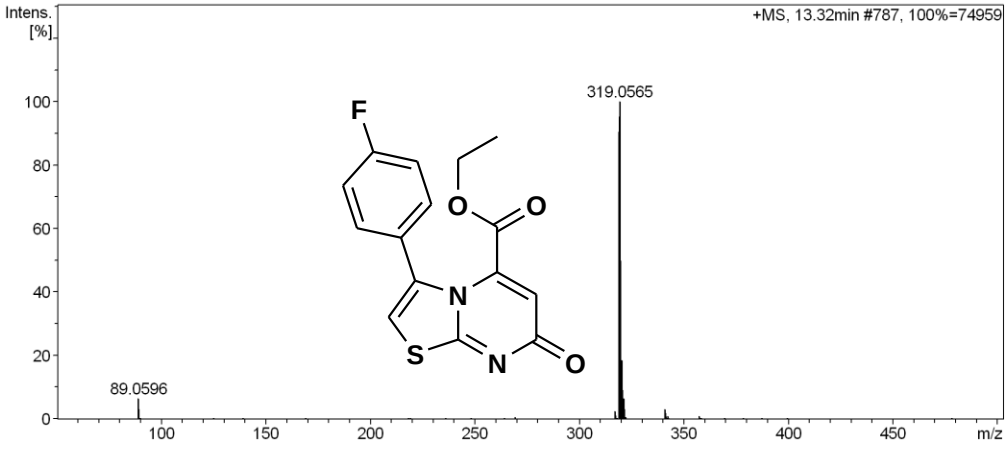
**5.17m**

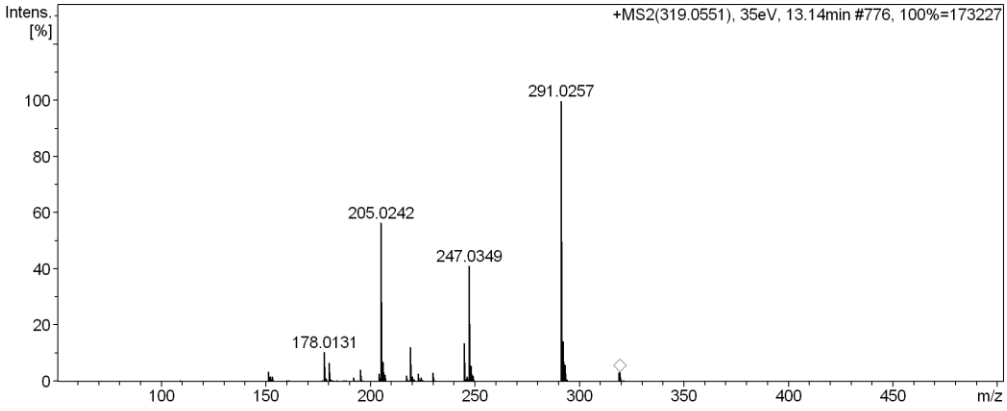



**5.17m**

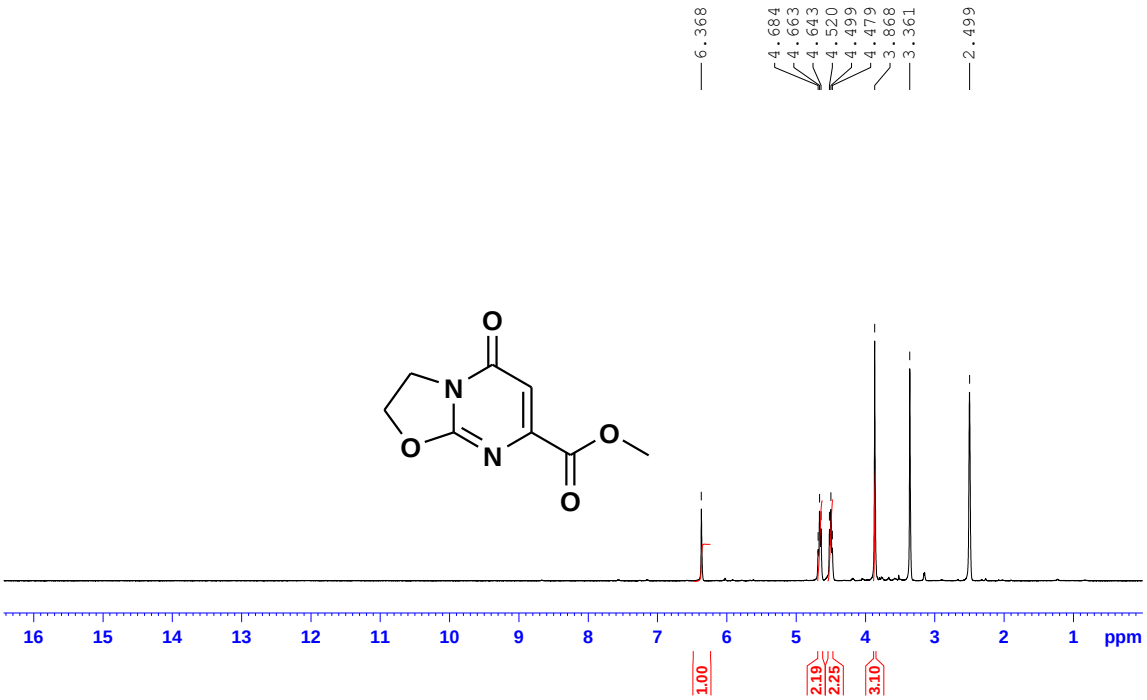



**5.17m**

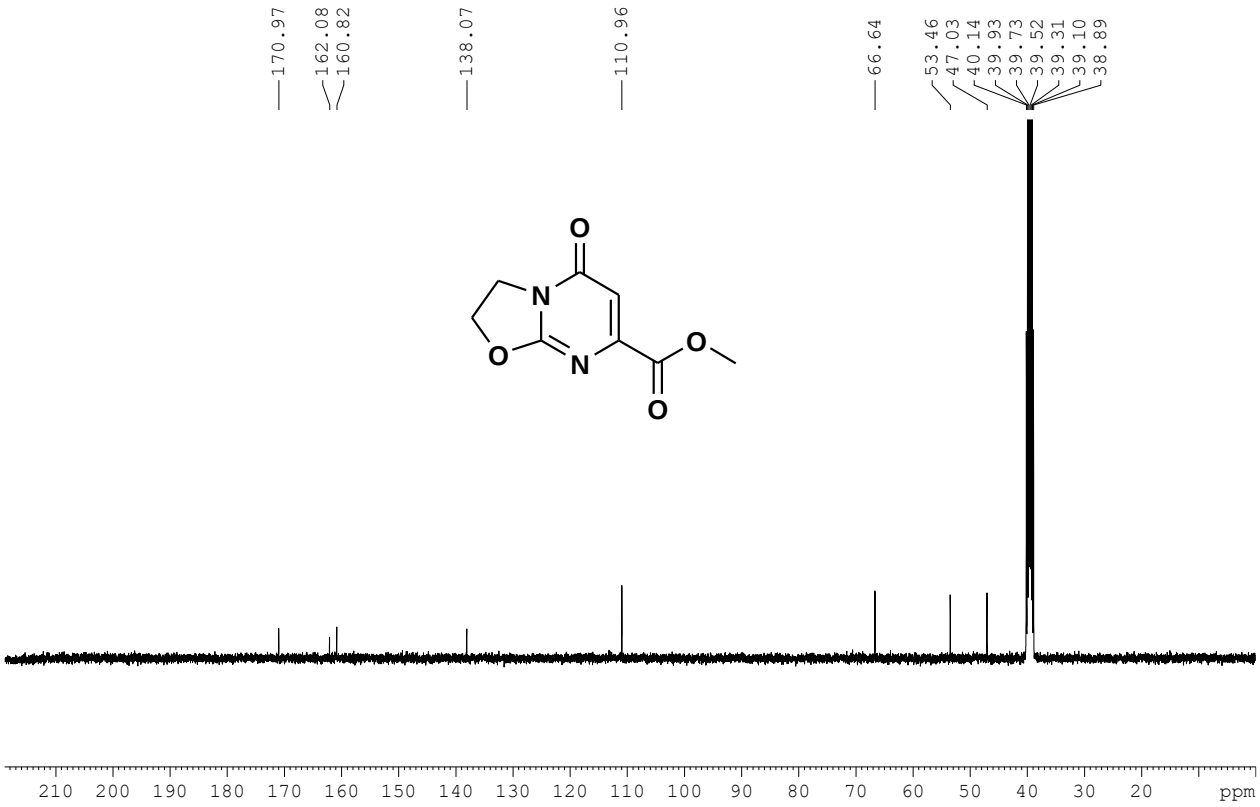

Intens.
[%]
+MS, 13.32min #787, 100%=74959
319.0565
89.0596
F
O
O
N
S
N
O
100
80
60
40
20
0
100
150
200
250
300
350
400
450
m/z


Intens.
[%]
+MS2(319.0551), 35eV, 13.14min #776, 100%=173227
291.0257
205.0242
247.0349
178.0131
100
80
60
40
20
0
100
150
200
250
300
350
400
450
m/z

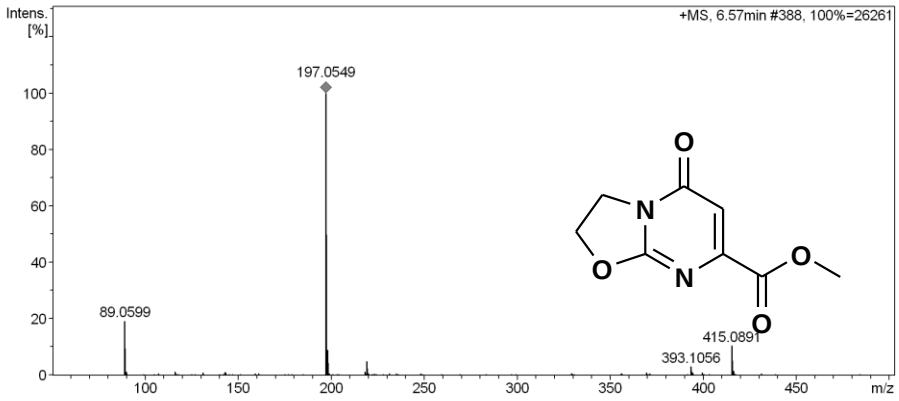
**5.18a**

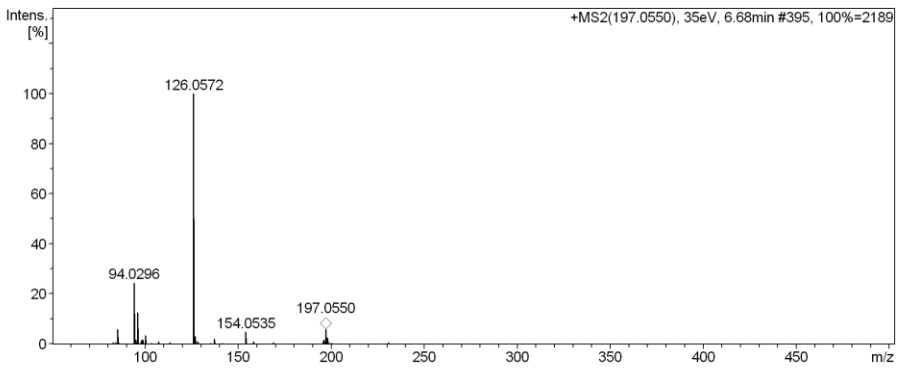

6.368
4.684
4.663
4.643
4.520
4.499
4.479
3.868
3.361
2.499
O
N
O
N
O
O
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
2.19
2.25
3.10

**5.18a**

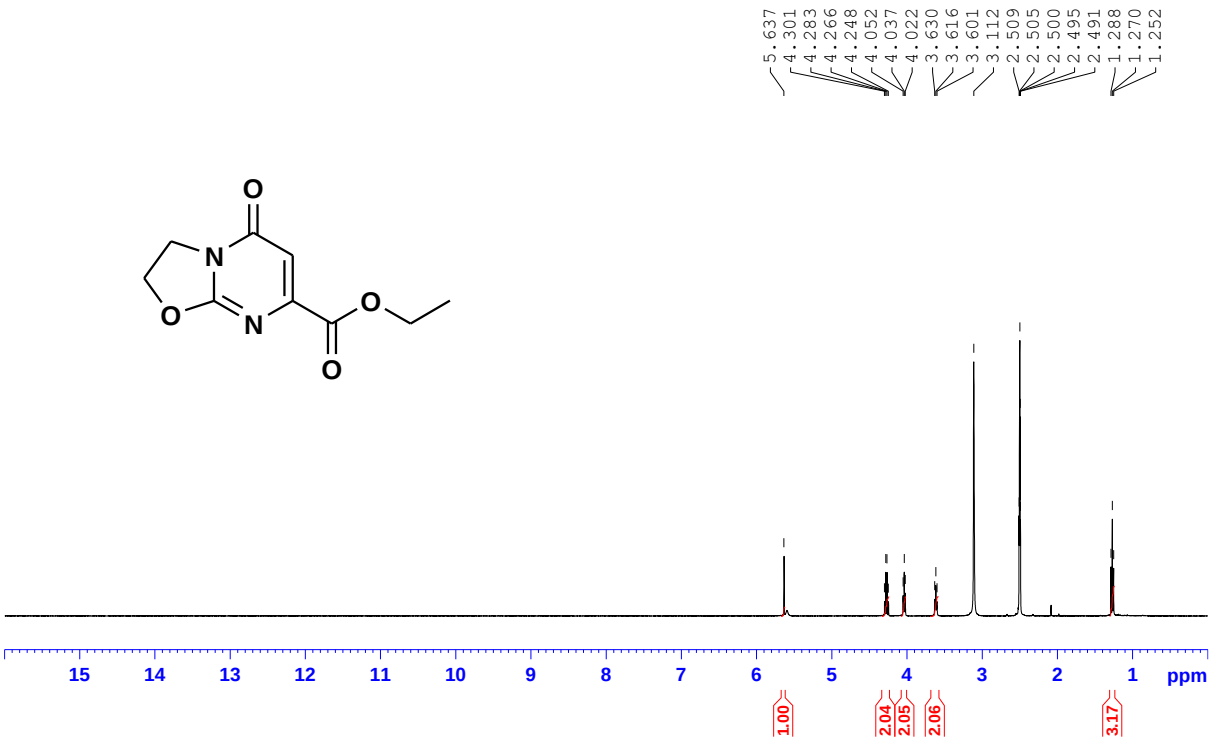

170.97
162.08
160.82
138.07
110.96
66.64
53.46
47.03
40.14
39.93
39.73
39.52
39.31
39.10
38.89
O
N
O
N
O
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

**5.18a**

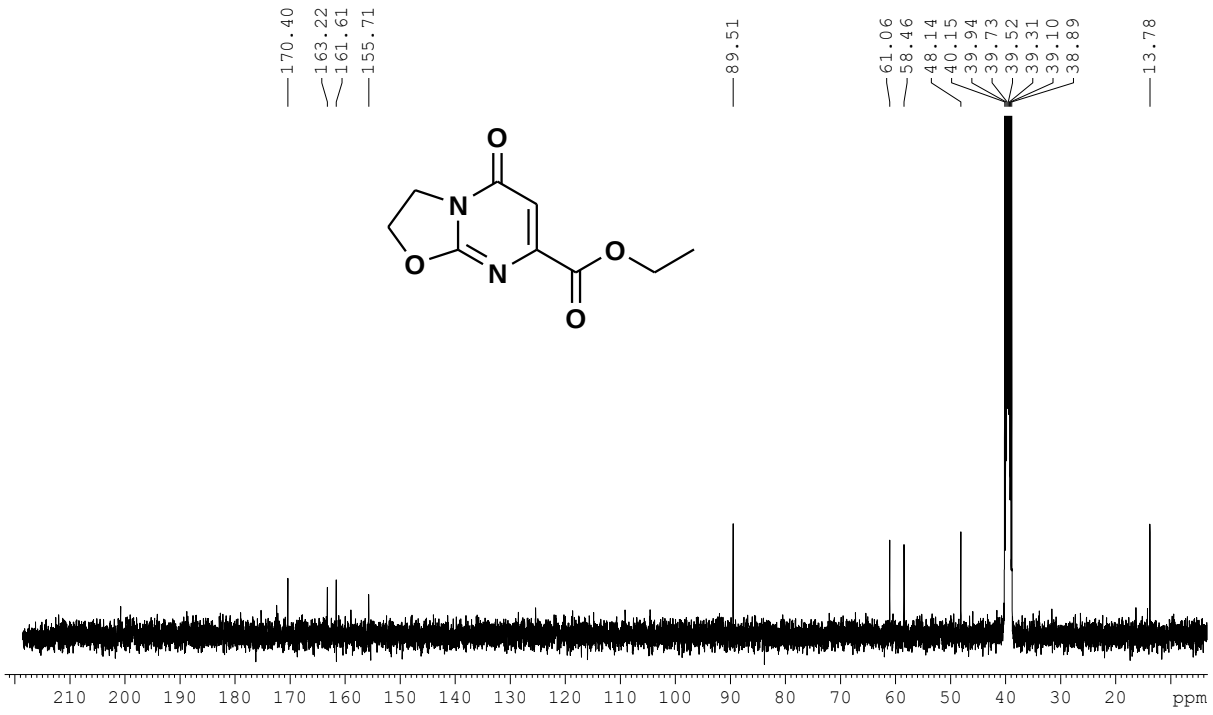

Intens.
[%]
+MS, 6.57min #388, 100%=26261
197.0549
89.0599
415.0891
393.1056
O
N
O
N
O
O
100 150 200 250 300 350 400 450 m/z


Intens.
[%]
+MS2(197.0550), 35eV, 6.68min #395, 100%=2189
126.0572
94.0296
154.0535
197.0550
100 150 200 250 300 350 400 450 m/z

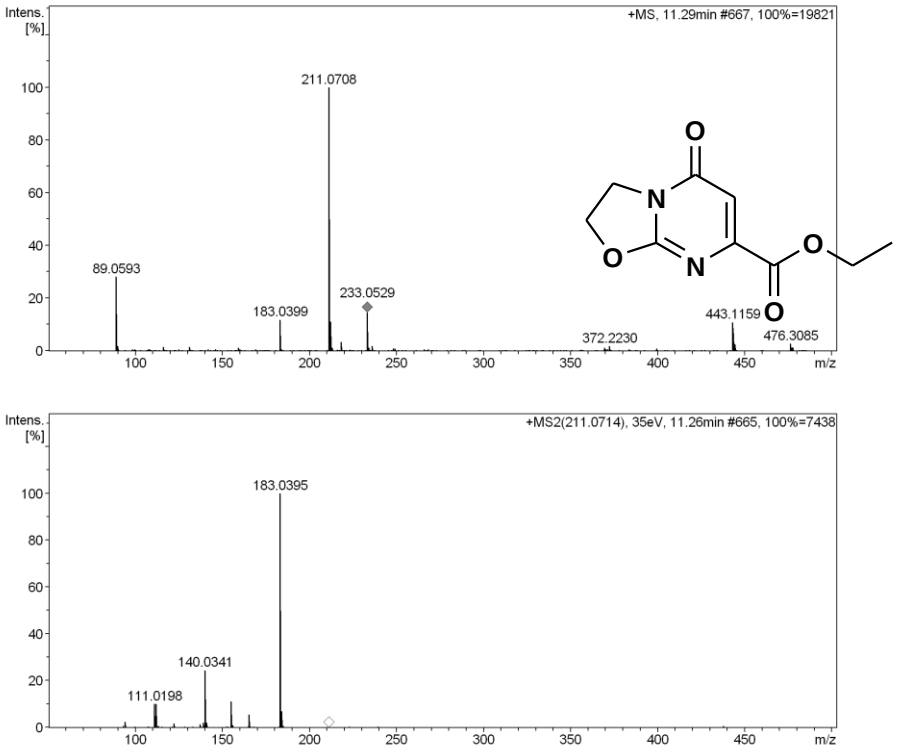
**5.18b**


5.637
4.301
4.283
4.266
4.248
4.052
4.037
4.022
3.630
3.616
3.601
3.112
2.509
2.505
2.500
2.495
2.491
1.288
1.270
1.252
O
N
O
N
O
O
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
1.00
2.04
2.05
2.06
3.17

**5.18b**


170.40
163.22
161.61
155.71
89.51
61.06
58.46
48.14
40.15
39.94
39.73
39.52
39.31
39.10
38.89
13.78
O
N
O
N
O
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

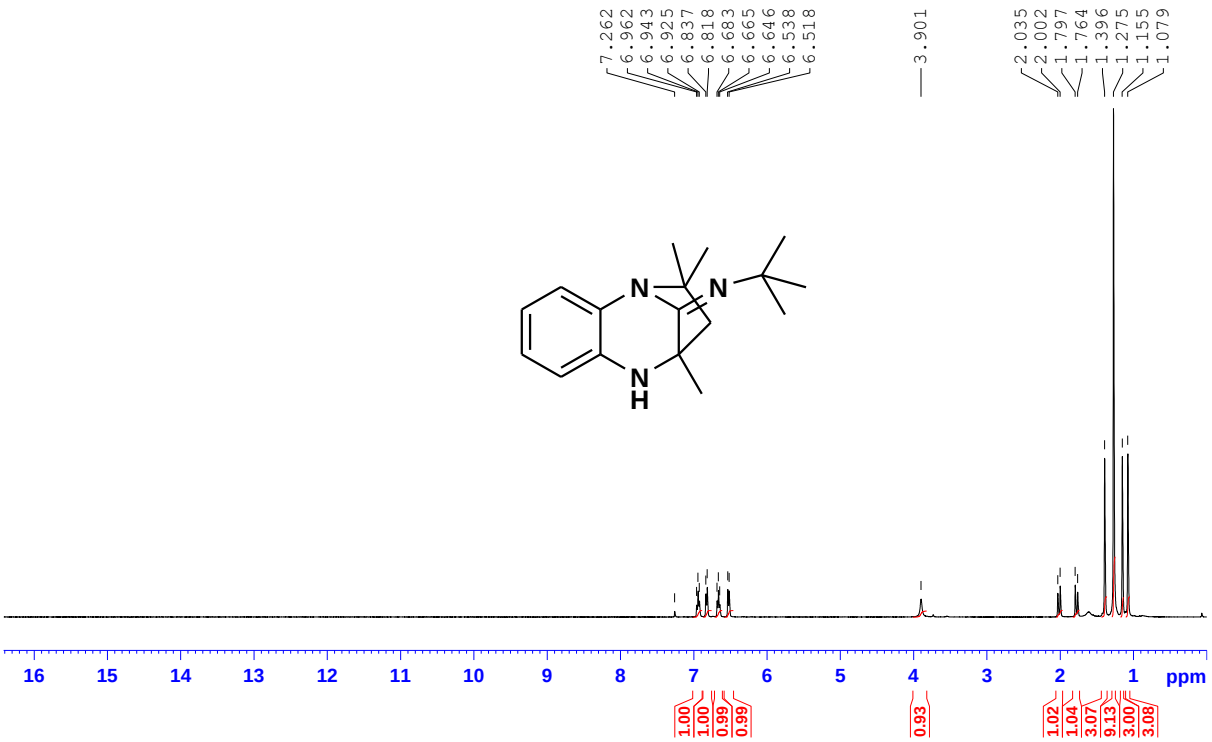
**5.18b**



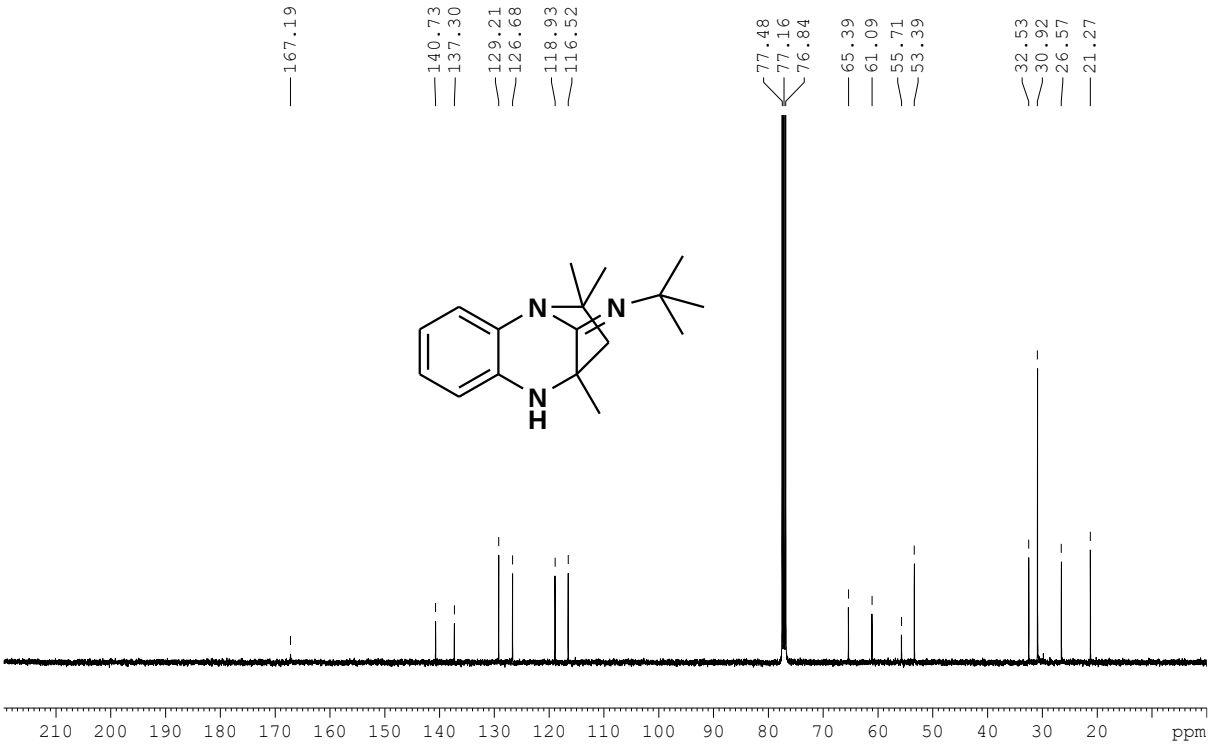

**CHAPTER 6: selected spectroscopic data**

*2-Methyl-N-(2,2,4-trimethyl-2,3,4,5-tetrahydro-1,4-methanobenzo[b][1,4]diazepin-10-ylidene)propan-2-amine derivatives* ***6.8***

**6.8a**

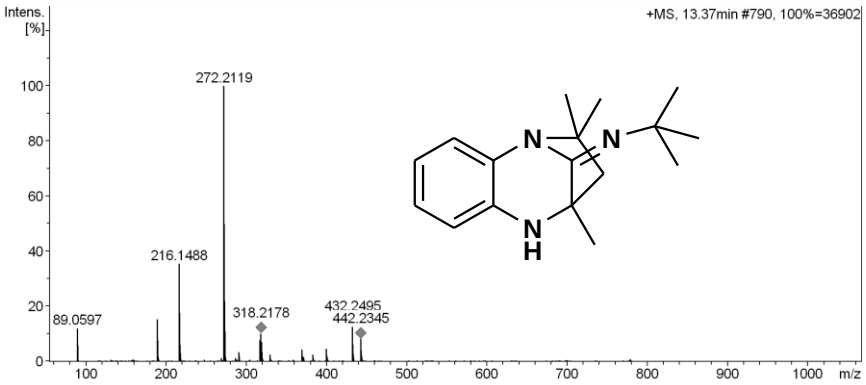

7.262
6.962
6.943
6.925
6.837
6.818
6.683
6.665
6.646
6.538
6.518
3.901
2.035
2.002
1.797
1.764
1.396
1.275
1.155
1.079
N
N
N
H
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
1.00
1.00
0.99
0.99
0.93
1.02
1.04
3.07
9.13
3.00
3.08

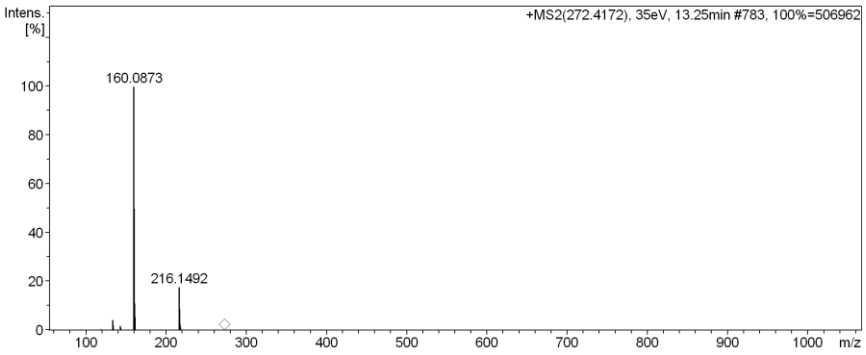
**6.8a**


167.19
140.73
137.30
129.21
126.68
118.93
116.52
77.48
77.16
76.84
65.39
61.09
55.71
53.39
32.53
30.92
26.57
21.27
N
N
N
H
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

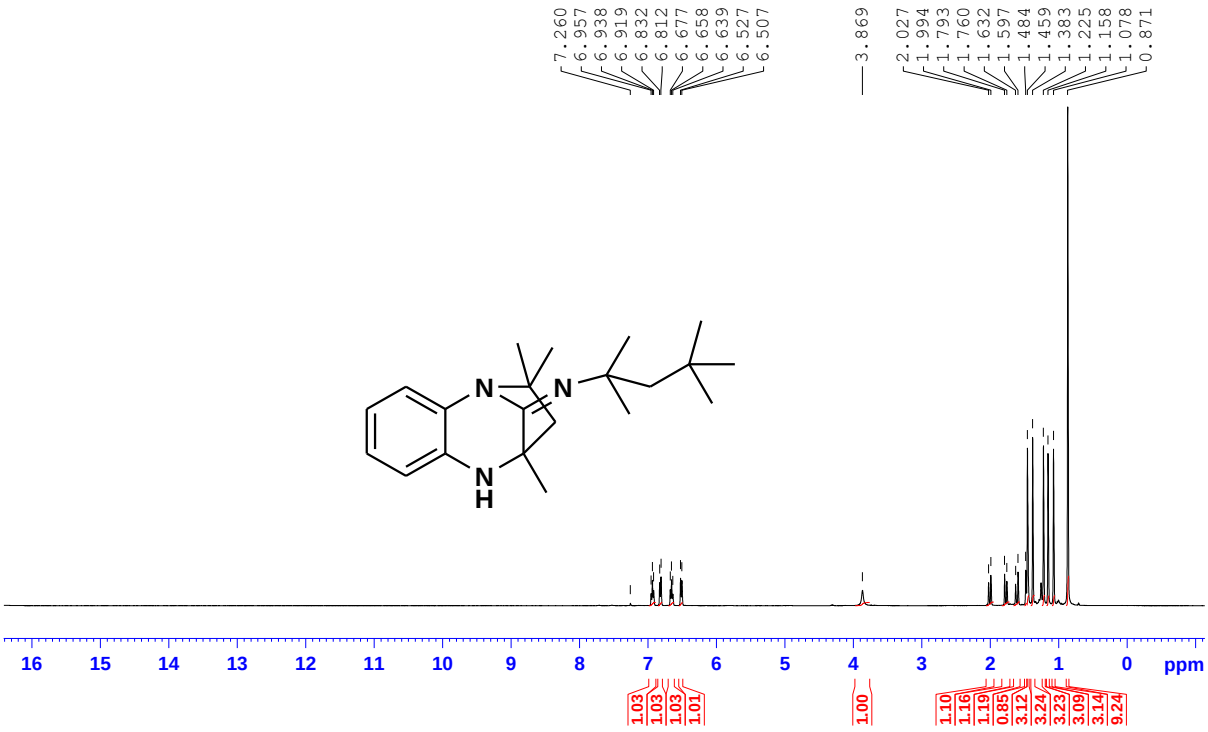
**6.8a**

S

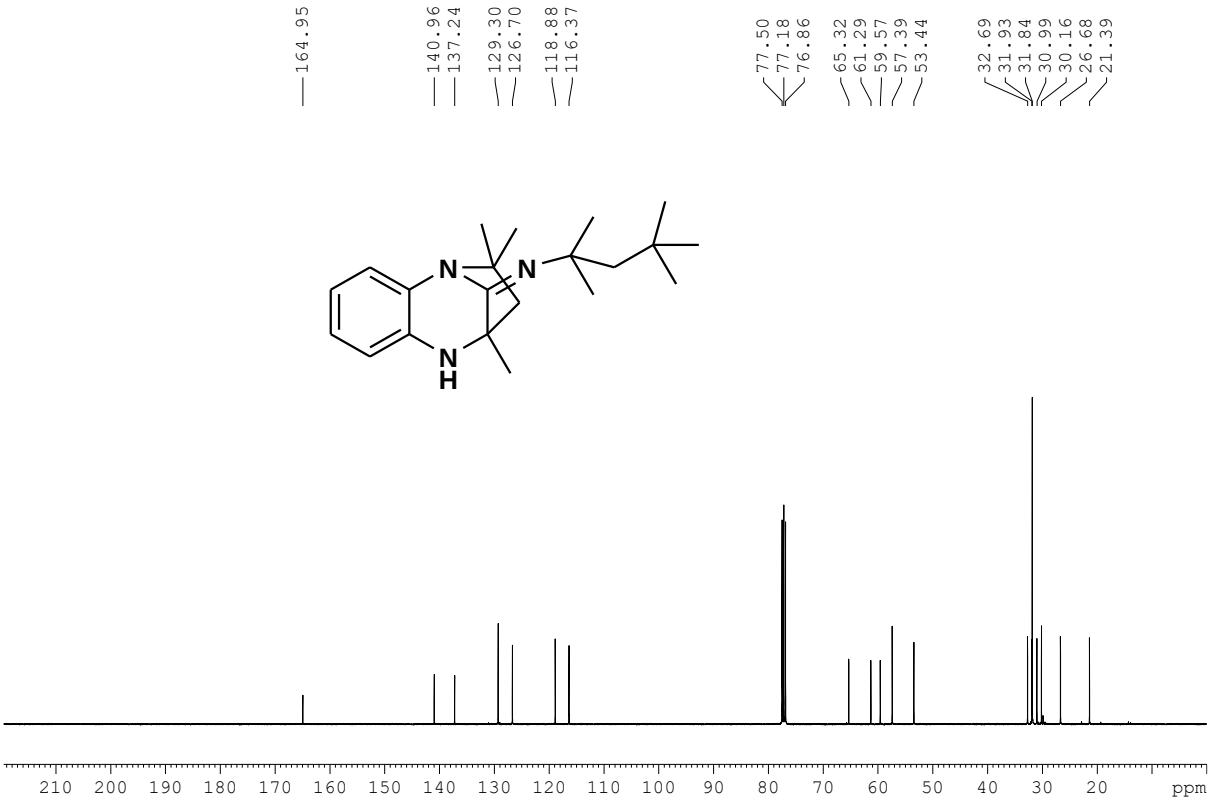

Intens.
[%]
+MS, 13.37min #790, 100%=36902
272.2119
216.1488
89.0597
318.2178
432.2495
442.2345
N
N
N
H
100
200
300
400
500
600
700
800
900
1000
m/z


Intens.
[%]
+MS2(272.4172), 35eV, 13.25min #783, 100%=506962
160.0873
216.1492
100
200
300
400
500
600
700
800
900
1000
m/z

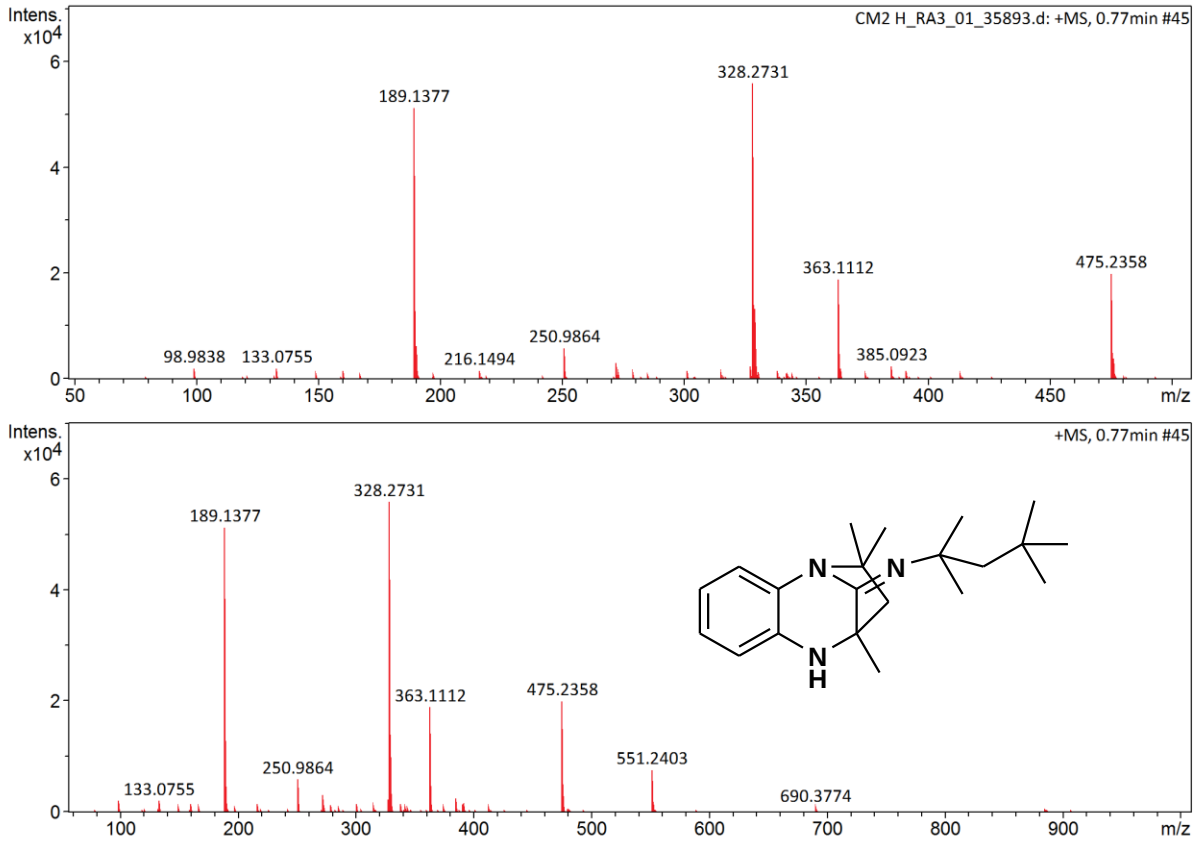
**6.8b**


7.260
6.957
6.938
6.919
6.832
6.812
6.677
6.658
6.639
6.527
6.507
3.869
2.027
1.994
1.793
1.760
1.632
1.597
1.484
1.459
1.383
1.225
1.158
1.078
0.871
N
N
N
H
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.03
1.03
1.03
1.01
1.00
1.10
1.16
1.19
0.85
3.12
3.24
3.23
3.09
3.14
9.24

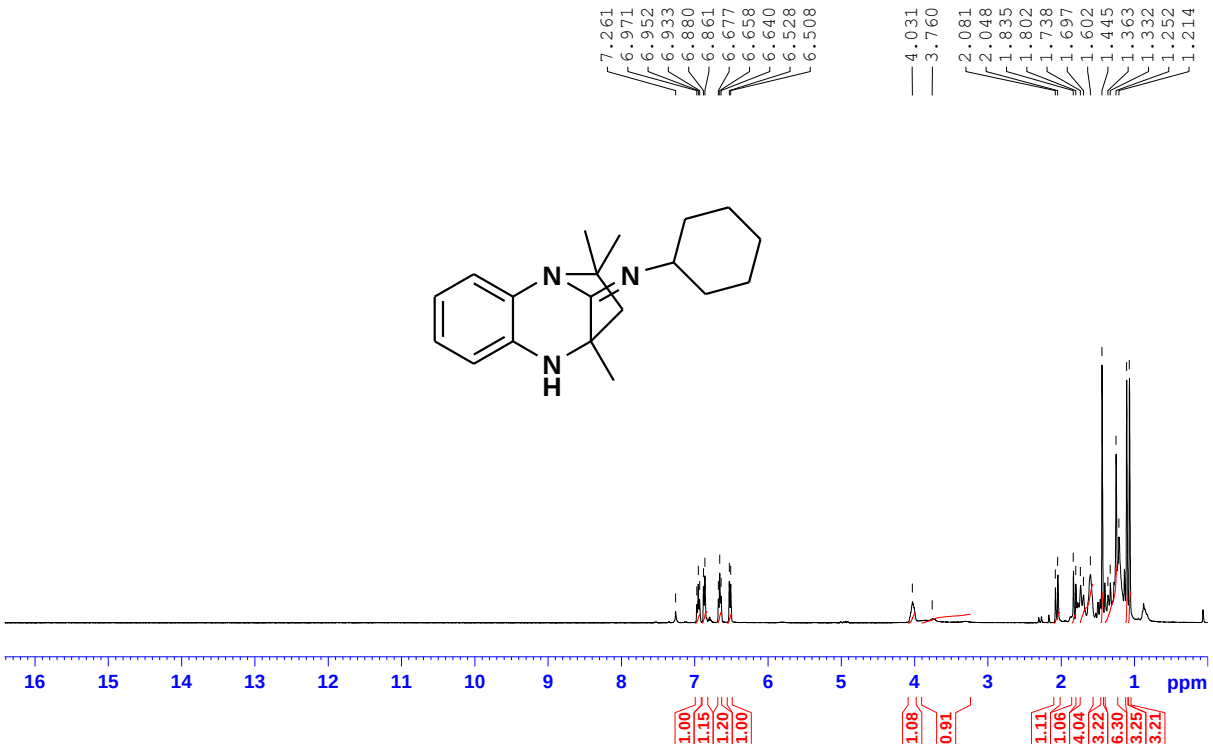
**6.8b**


164.95
140.96
137.24
129.30
126.70
118.88
116.37
77.50
77.18
76.86
65.32
61.29
59.57
57.39
53.44
32.69
31.93
31.84
30.99
30.16
26.68
21.39
N
N
N
H
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

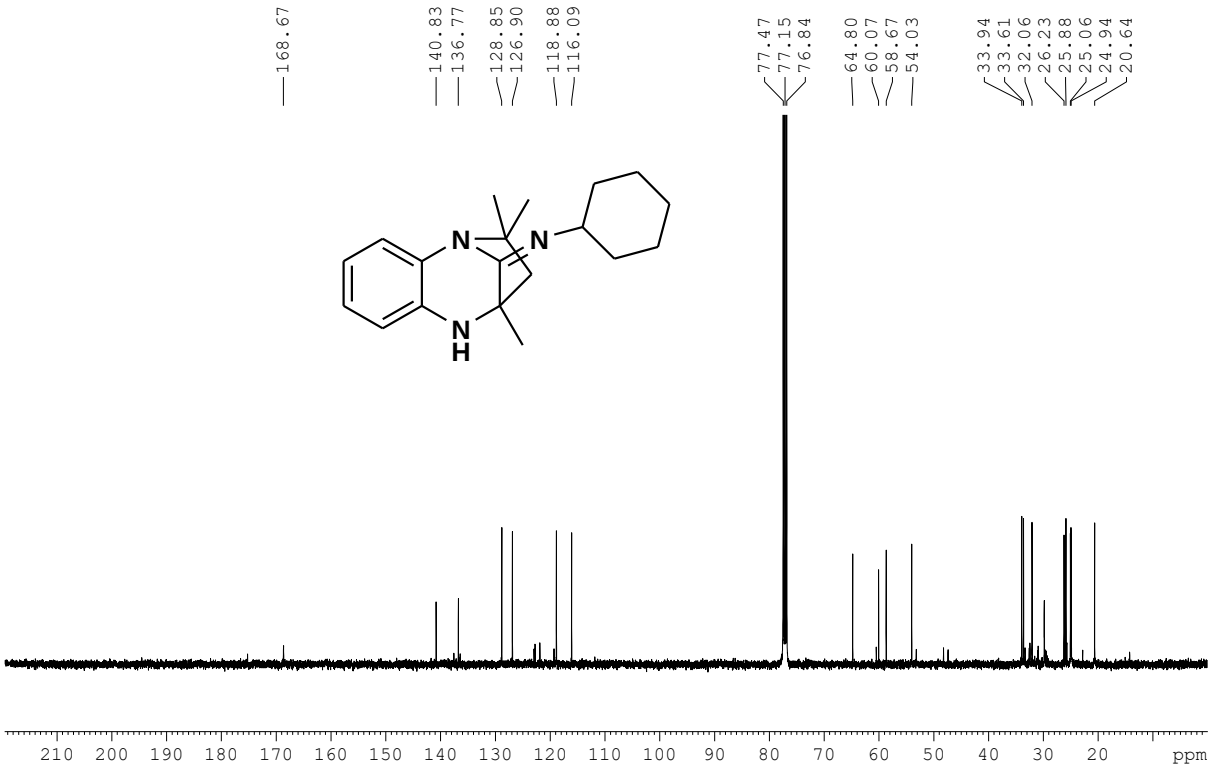
## 6.8b


Intens.
x10^4
CM2 H_RA3_01_35893.d: +MS, 0.77min #45
328.2731
189.1377
363.1112
475.2358
250.9864
98.9838
133.0755
216.1494
385.0923
m/z
+MS, 0.77min #45
328.2731
189.1377
363.1112
475.2358
551.2403
250.9864
133.0755
690.3774
N
N
N
H

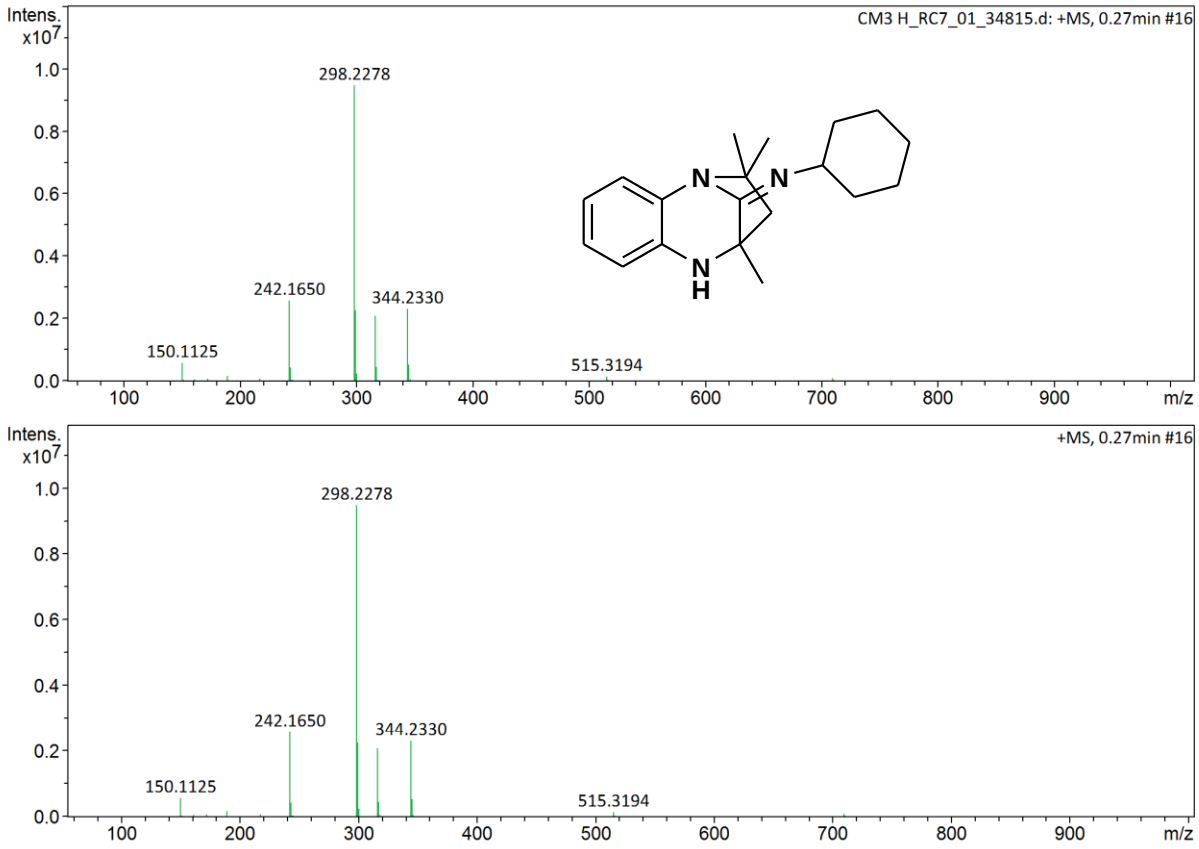
**6.8c**


7.261
6.971
6.952
6.933
6.880
6.861
6.677
6.658
6.640
6.528
6.508
4.031
3.760
2.081
2.048
1.835
1.802
1.738
1.697
1.602
1.445
1.363
1.332
1.252
1.214
N
N
N
H
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
1.00 1.15 1.20 1.00
1.08 0.91
1.11 1.06 4.04 3.22 6.30 3.25 3.21

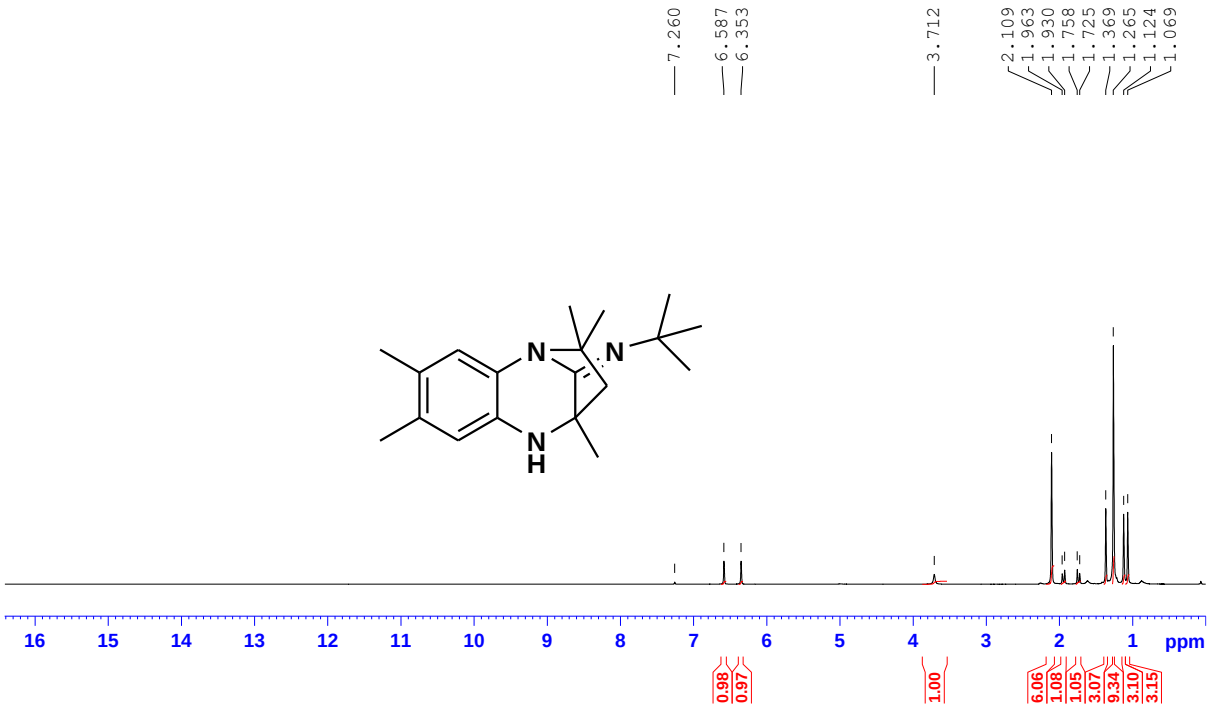
**6.8c**


168.67
140.83
136.77
128.85
126.90
118.88
116.09
77.47
77.15
76.84
64.80
60.07
58.67
54.03
33.94
33.61
32.06
26.23
25.88
25.06
24.94
20.64
N
N
N
H
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

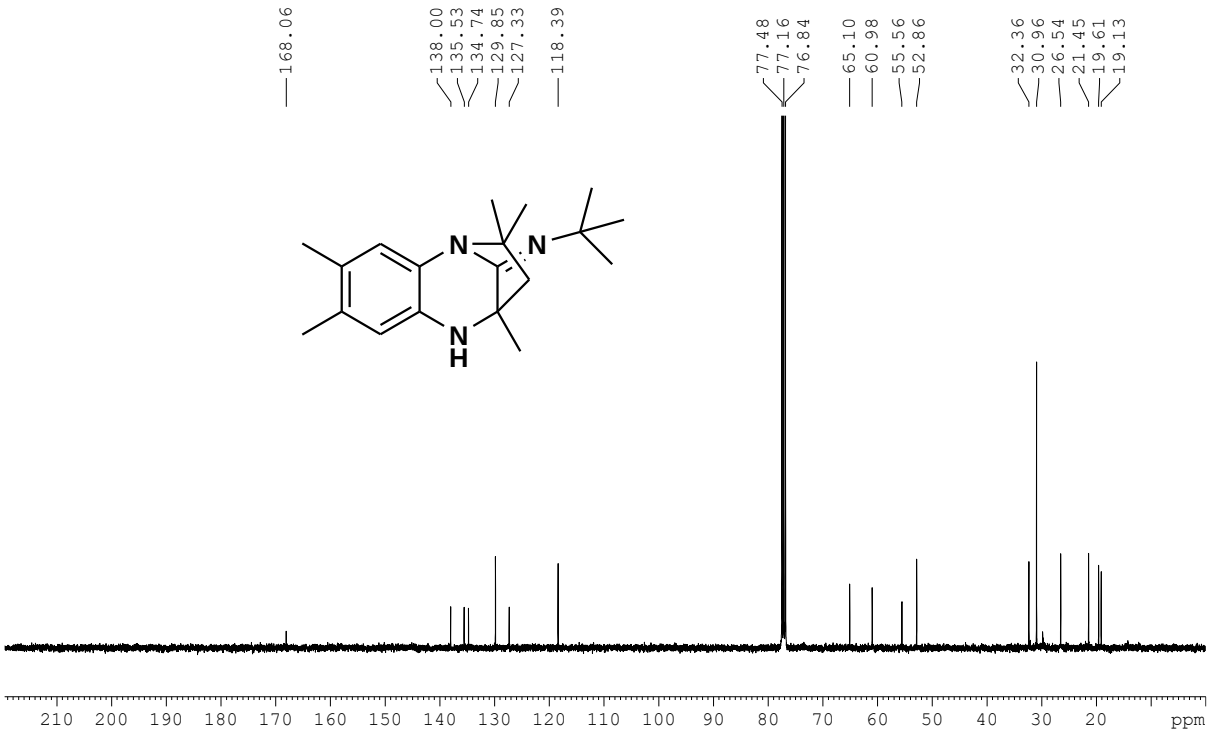
**6.8c**


CM3 H_RC7_01_34815.d: +MS, 0.27min #16
Intens.
x10^7
298.2278
242.1650
344.2330
150.1125
515.3194
m/z
+MS, 0.27min #16
Intens.
x10^7
298.2278
242.1650
344.2330
150.1125
515.3194
m/z

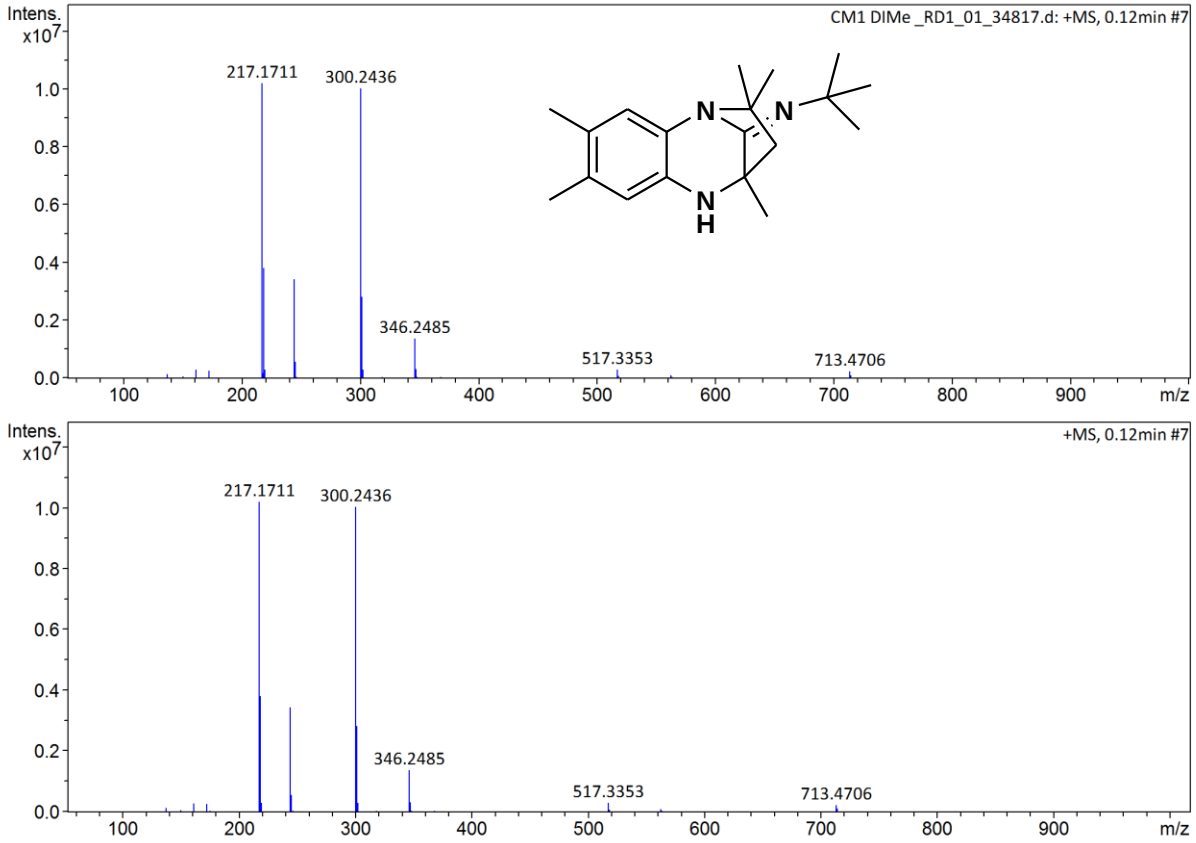
**6.8e**


7.260
6.587
6.353
3.712
2.109
1.963
1.930
1.758
1.725
1.369
1.265
1.124
1.069
ppm
0.98
0.97
1.00
6.06
1.08
1.05
3.07
9.34
3.10
3.15

**6.8e**

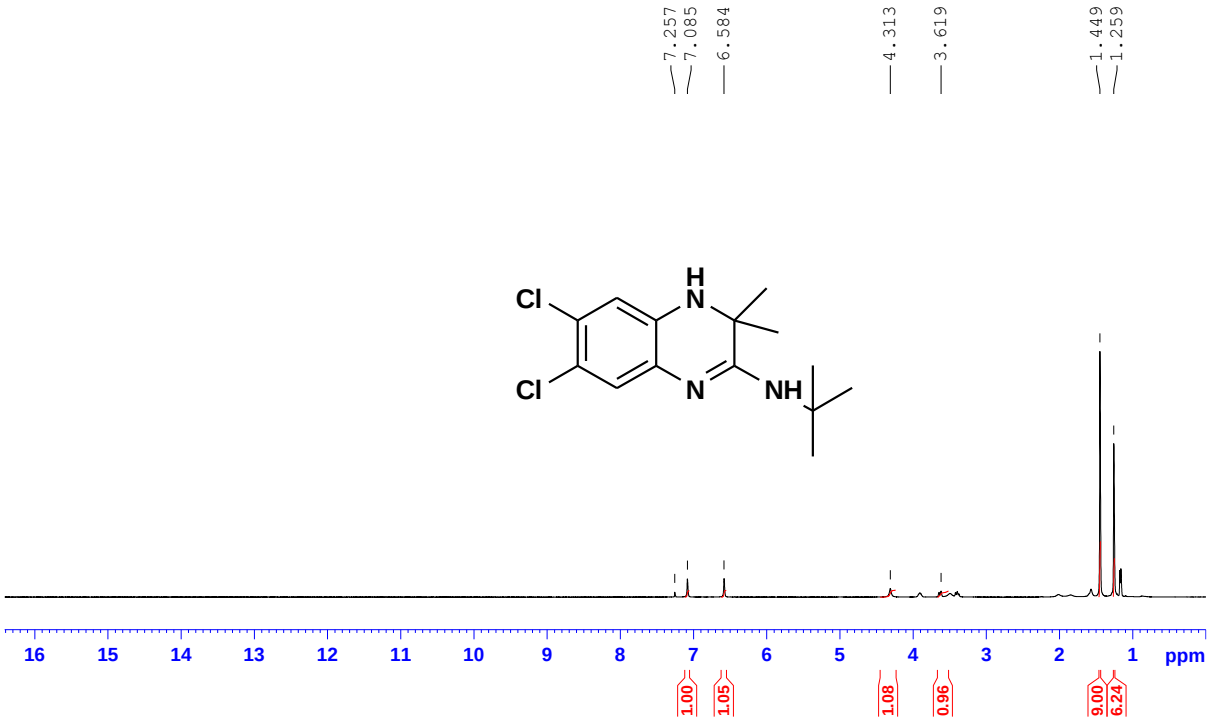

168.06
138.00
135.53
134.74
129.85
127.33
118.39
77.48
77.16
76.84
65.10
60.98
55.56
52.86
32.36
30.96
26.54
21.45
19.61
19.13
N
N
N
H
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

**6.8e**

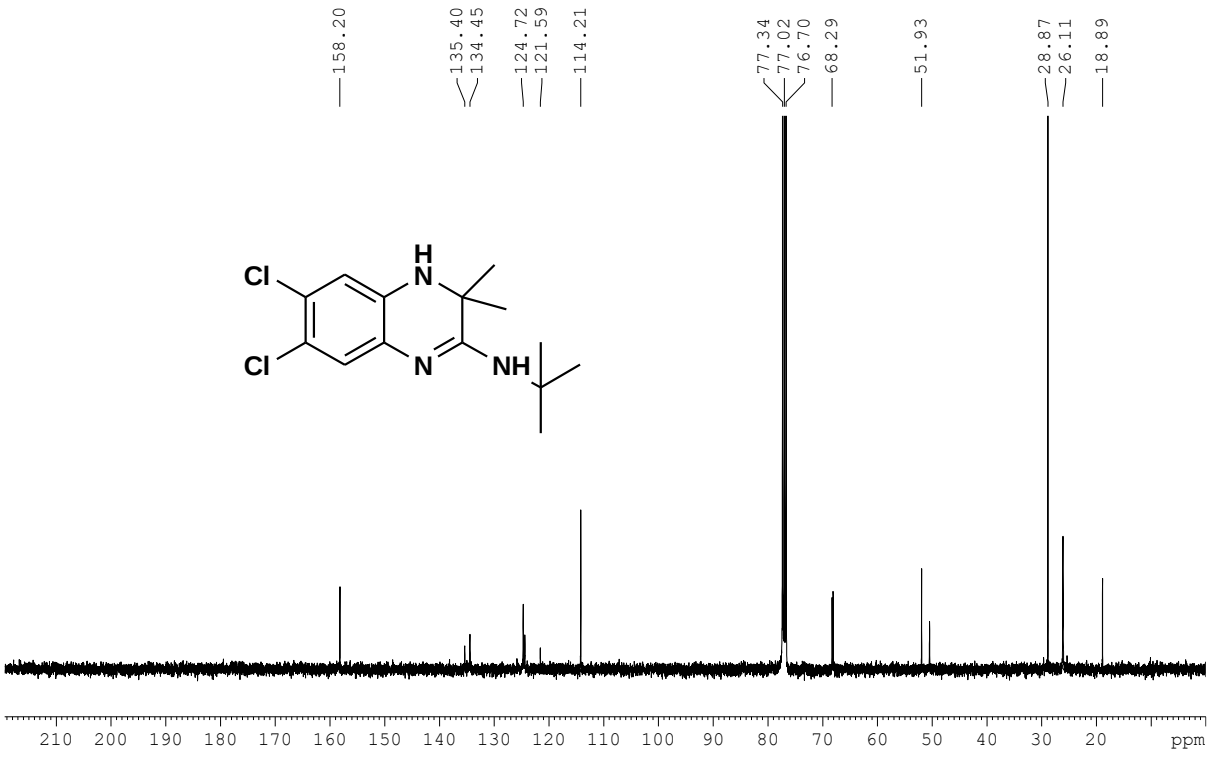

Intens.
x10^7
CM1 DIMe _RD1_01_34817.d: +MS, 0.12min #7
217.1711
300.2436
346.2485
517.3353
713.4706
N
N
N
H
100 200 300 400 500 600 700 800 900 m/z
Intens.
x10^7
+MS, 0.12min #7
217.1711
300.2436
346.2485
517.3353
713.4706
100 200 300 400 500 600 700 800 900 m/z

*(E)-N-(3,3-dimethyl-3,4-dihydroquinoxalin-2(1H)-ylidene)-2-methylpropan-2-amine derivatives* ***6.14***

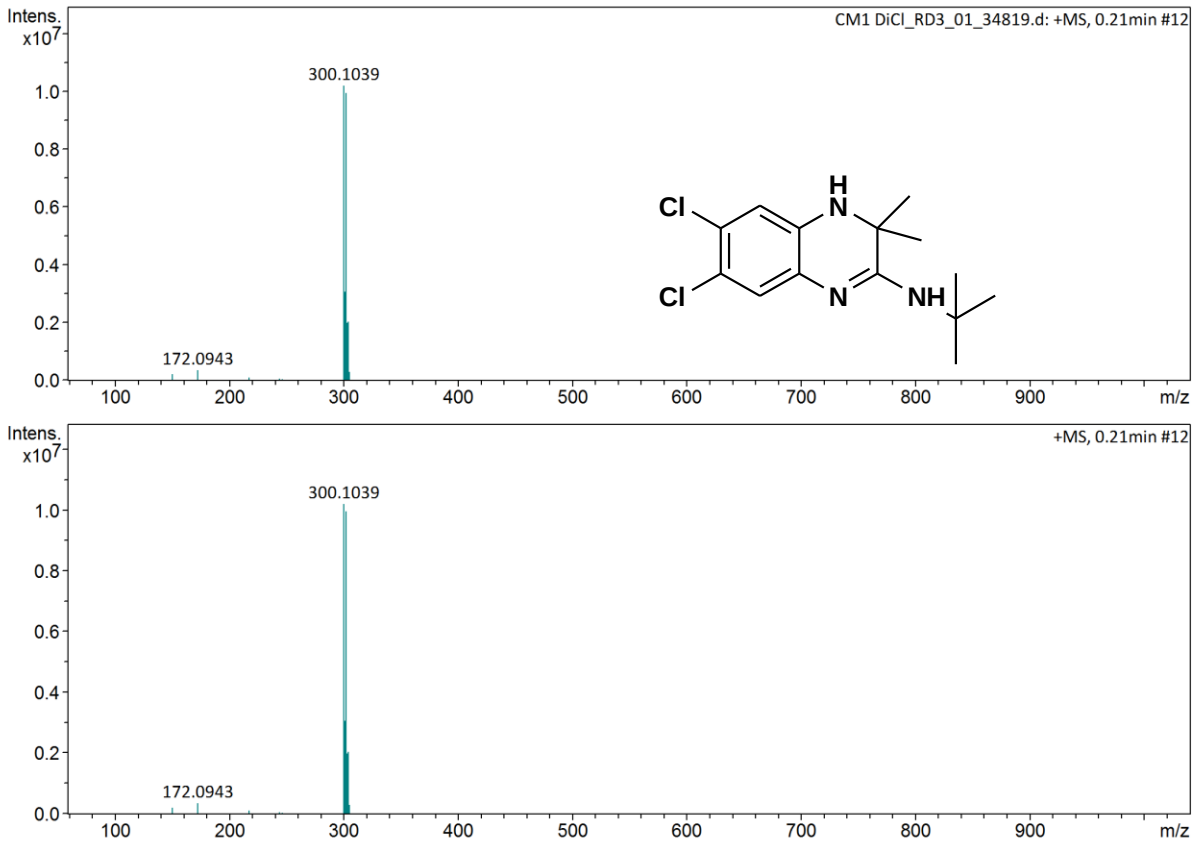
**6.14f**



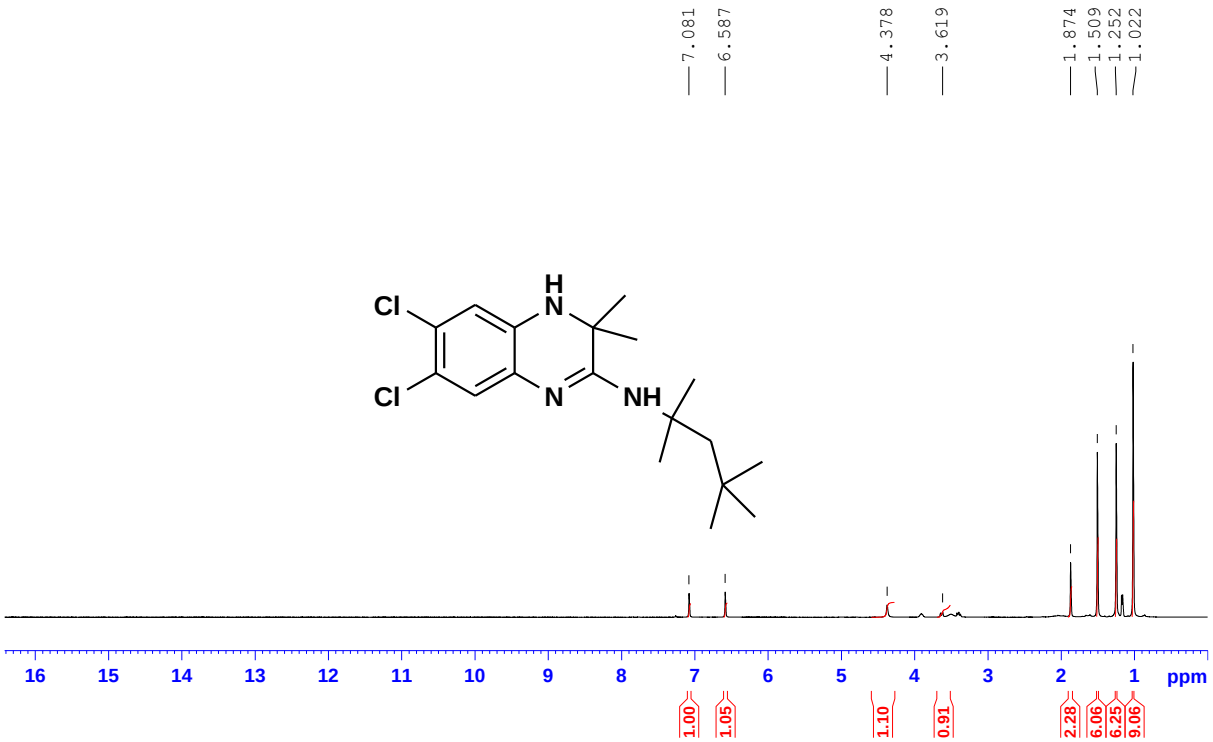

**6.14f**



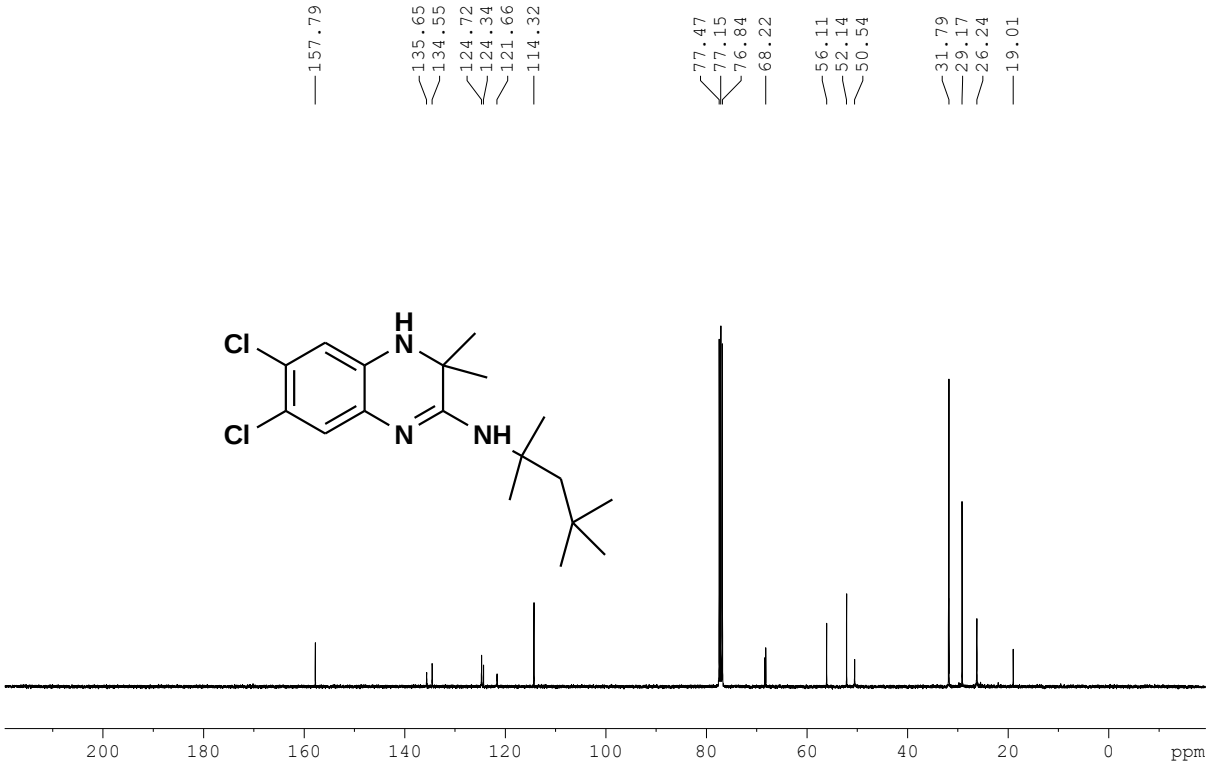

**6.14f**


Intens.
x10^7
CM1 DiCl_RD3_01_34819.d: +MS, 0.21min #12
300.1039
172.0943
Cl
Cl
H
N
N
NH
m/z
+MS, 0.21min #12

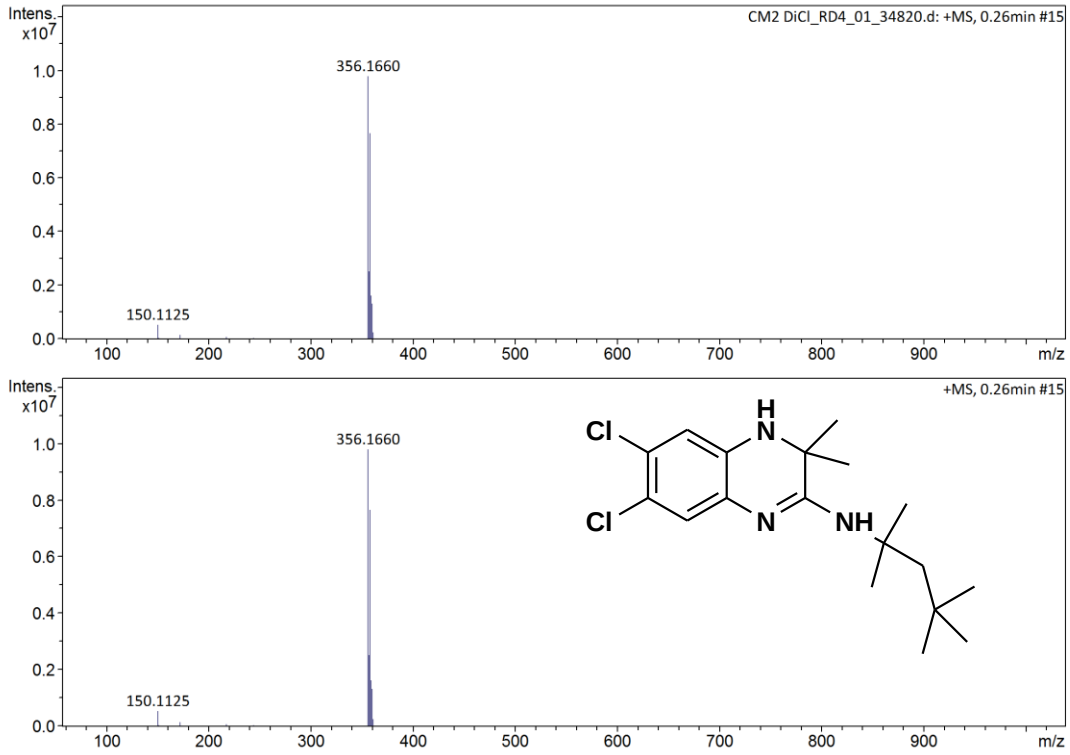
**6.14g**


7.081
6.587
4.378
3.619
1.874
1.509
1.252
1.022
Cl
Cl
H
N
N
NH
ppm
1.00
1.05
1.10
0.91
2.28
6.06
6.25
9.06

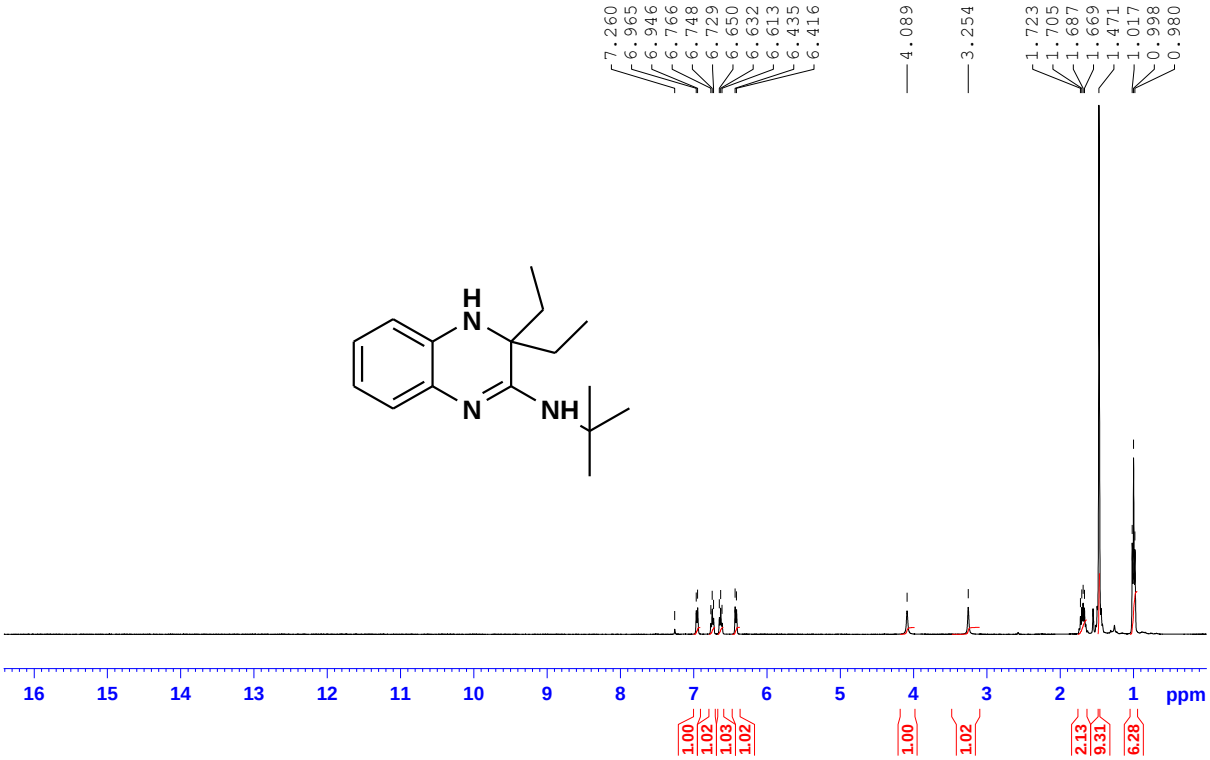
**6.14g**


157.79
135.65
134.55
124.72
124.34
121.66
114.32
77.47
77.15
76.84
68.22
56.11
52.14
50.54
31.79
29.17
26.24
19.01
Cl
Cl
H
N
N
NH
200
180
160
140
120
100
80
60
40
20
0
ppm

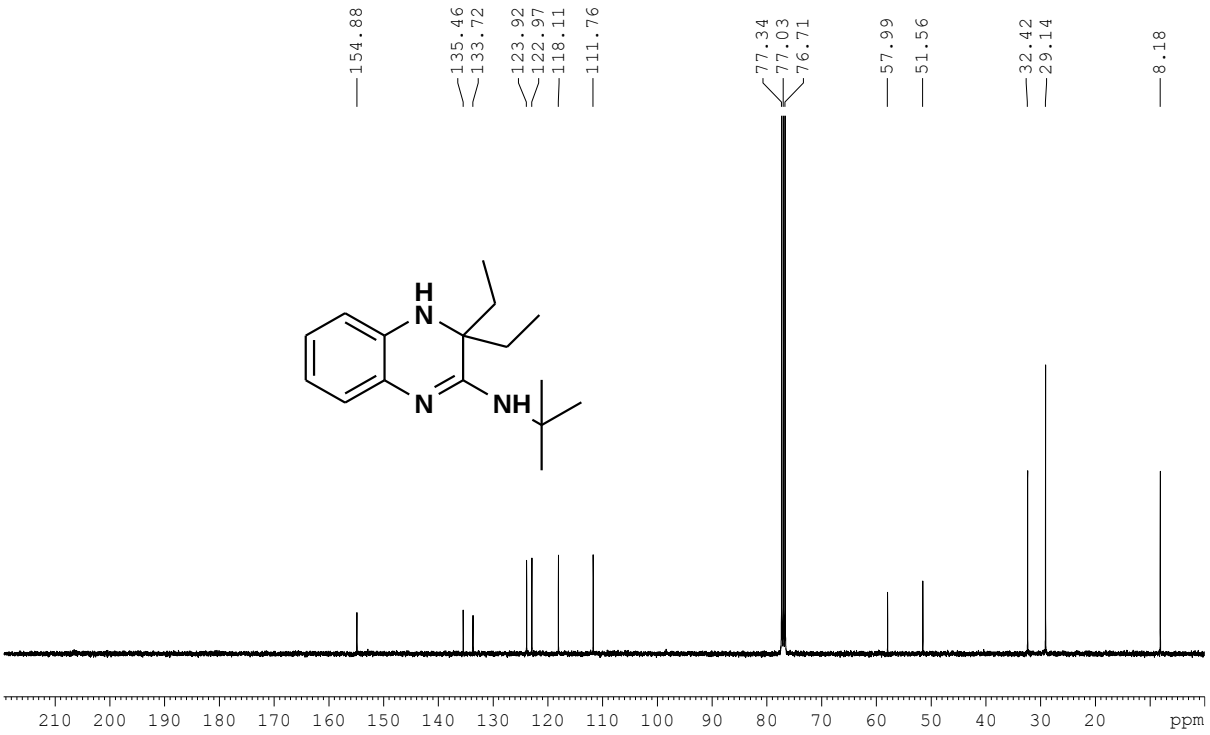
**6.14g**


Intens.
x10^7
CM2 DiCl_RD4_01_34820.d: +MS, 0.26min #15
356.1660
150.1125
m/z
+MS, 0.26min #15
Cl
Cl
H
N
N
NH

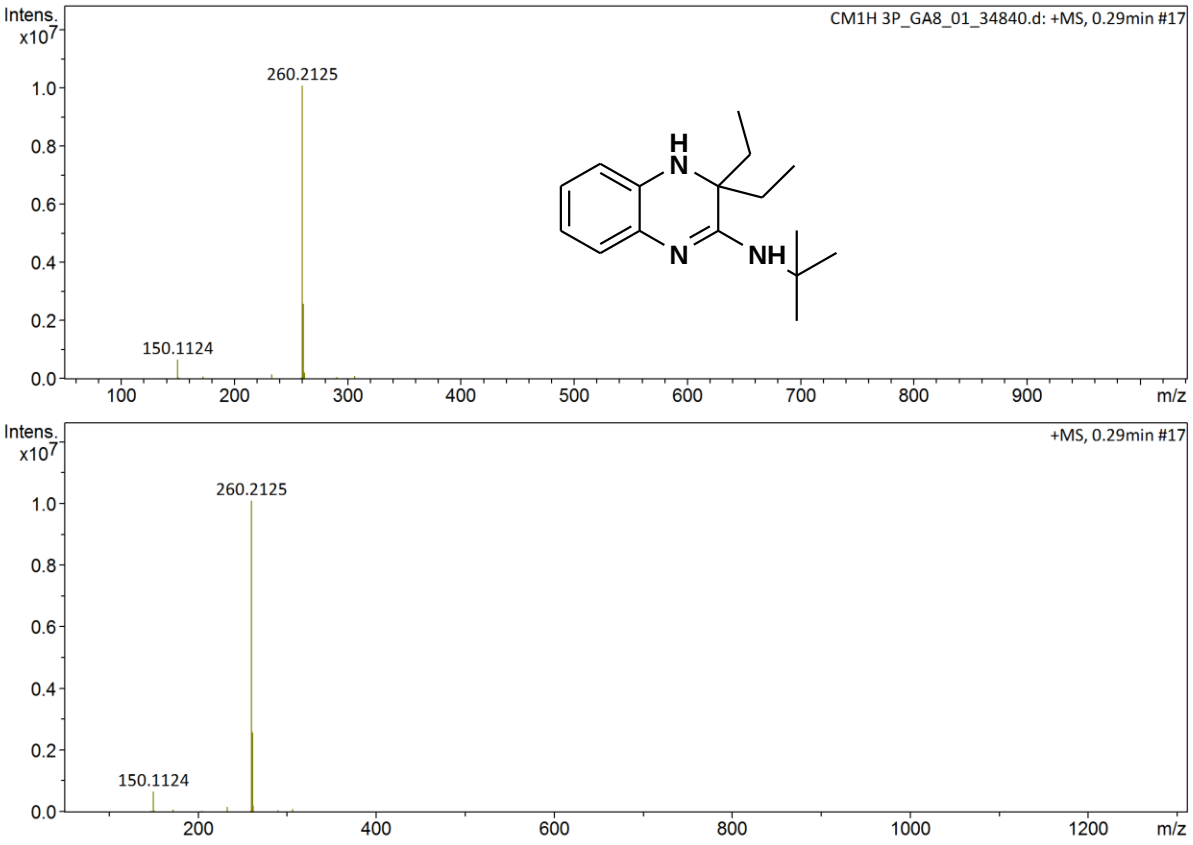
**6.14h**


7.260
6.965
6.946
6.766
6.748
6.729
6.650
6.632
6.613
6.435
6.416
4.089
3.254
1.723
1.705
1.687
1.669
1.471
1.017
0.998
0.980
H
N
N
NH
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
1.00 1.02 1.03 1.02 1.00 1.02 2.13 9.31 6.28

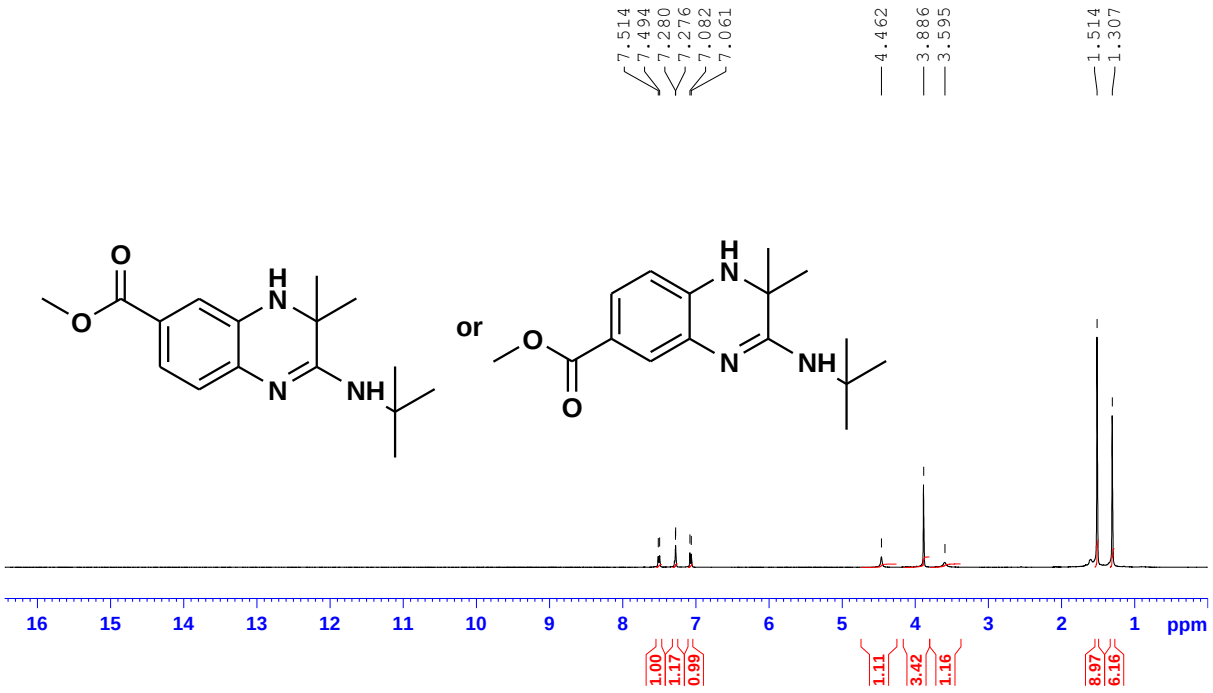
**6.14h**


154.88
135.46
133.72
123.92
122.97
118.11
111.76
77.34
77.03
76.71
57.99
51.56
32.42
29.14
8.18
H
N
N
NH
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

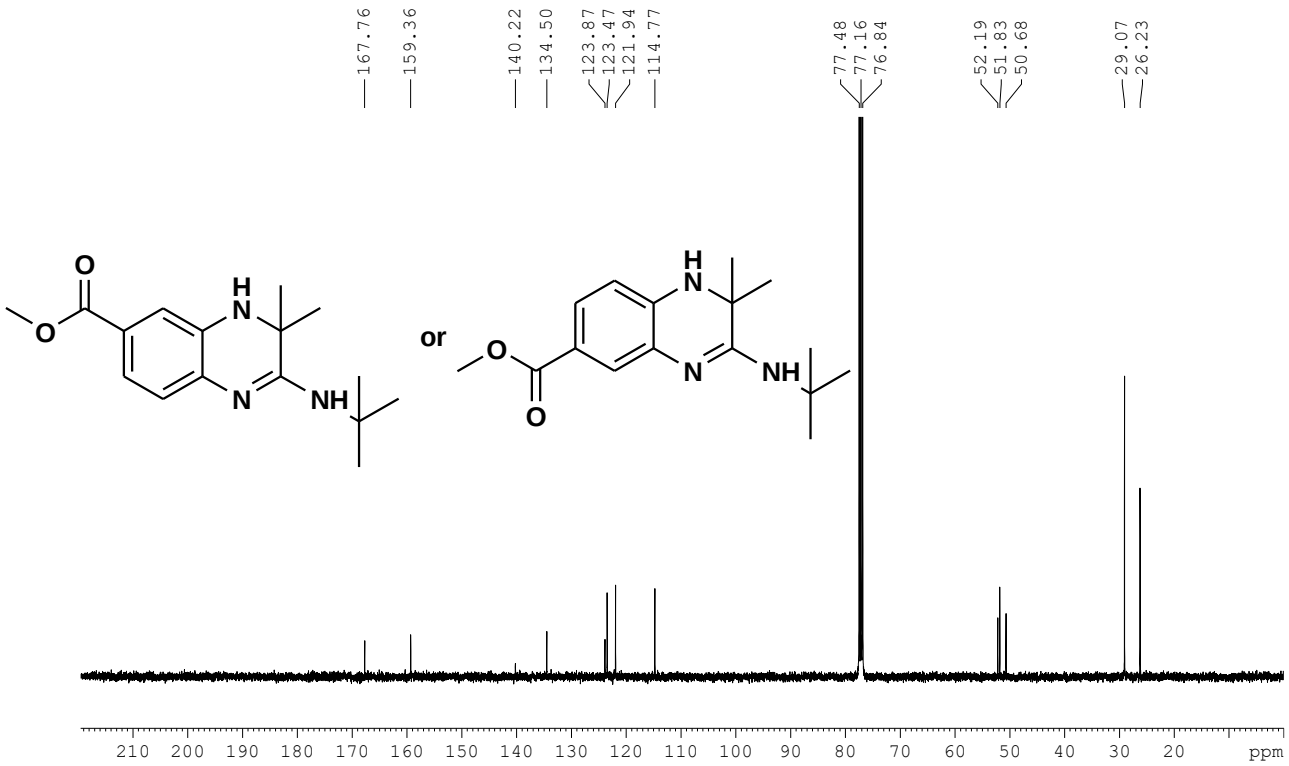
**6.14h**


Intens.
x10^7
CM1H 3P_GA8_01_34840.d: +MS, 0.29min #17
260.2125
150.1124
m/z
+MS, 0.29min #17

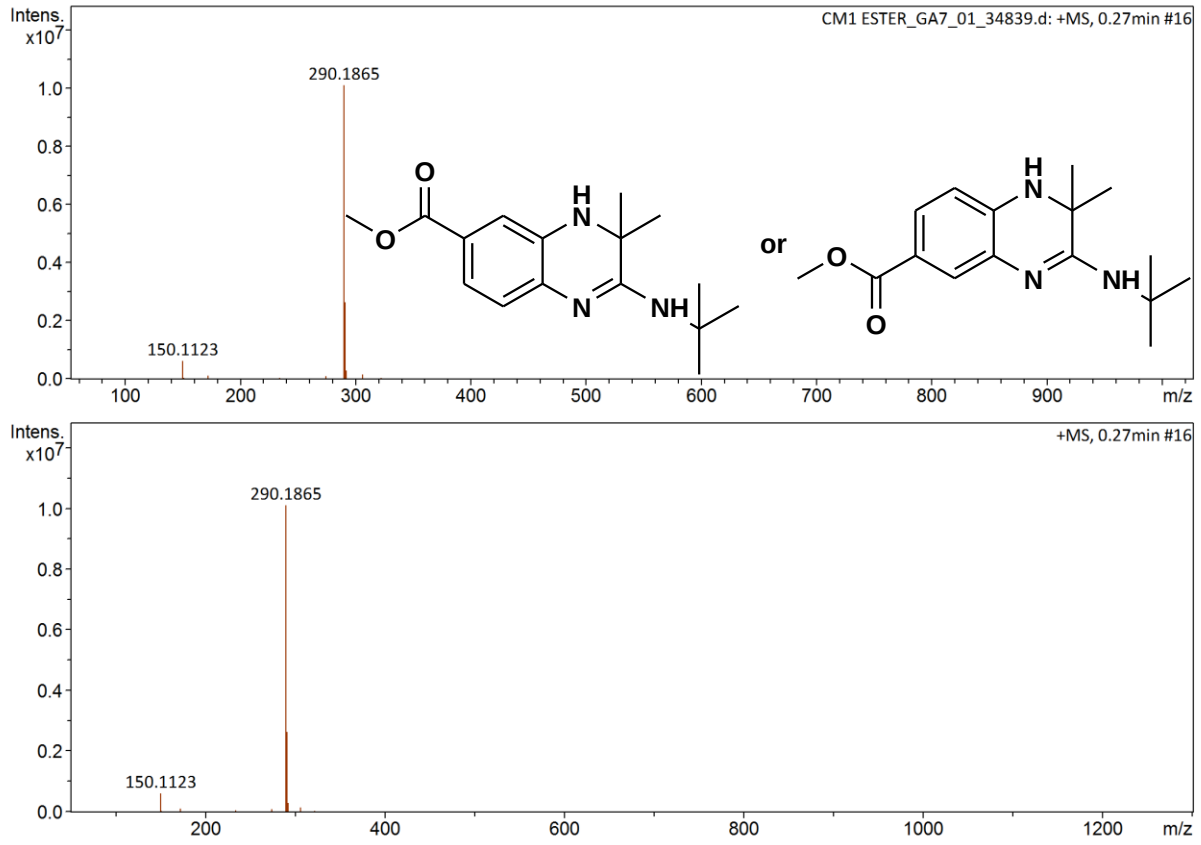
**6.14a**


7.514
7.494
7.280
7.276
7.082
7.061
4.462
3.886
3.595
1.514
1.307
or
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
1.00
1.17
0.99
1.11
3.42
1.16
8.97
6.16

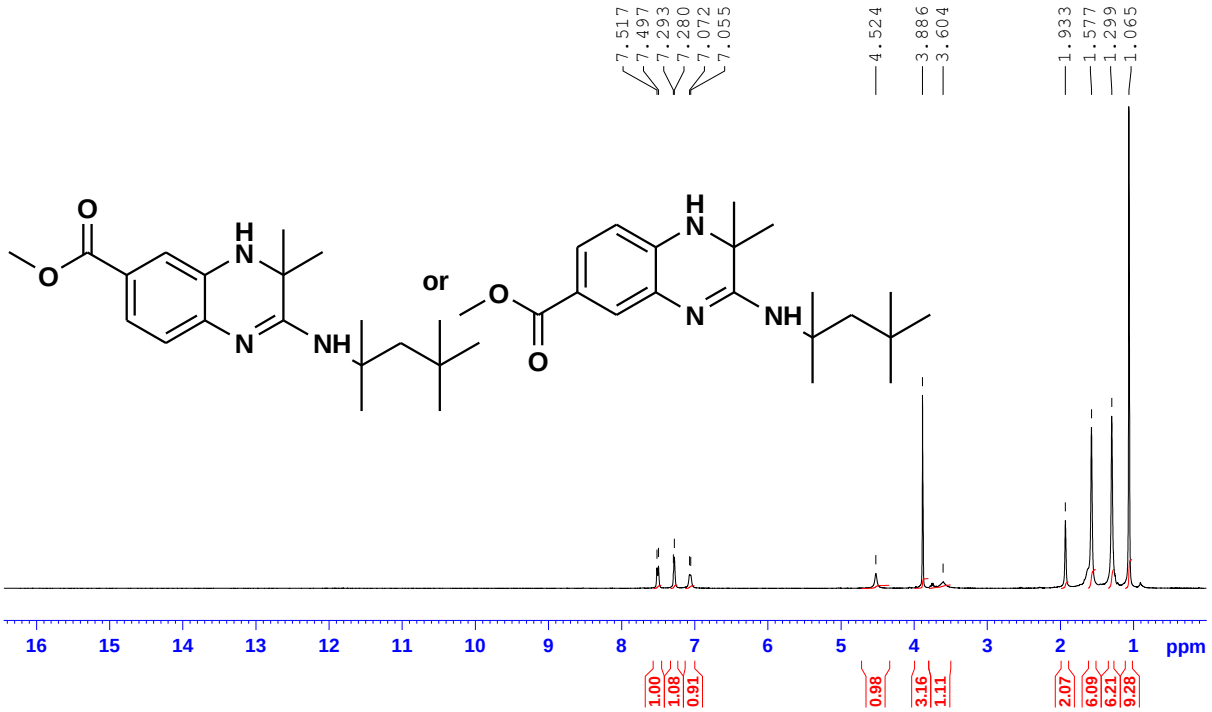
**6.14a**


167.76
159.36
140.22
134.50
123.87
123.47
121.94
114.77
77.48
77.16
76.84
52.19
51.83
50.68
29.07
26.23
or
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

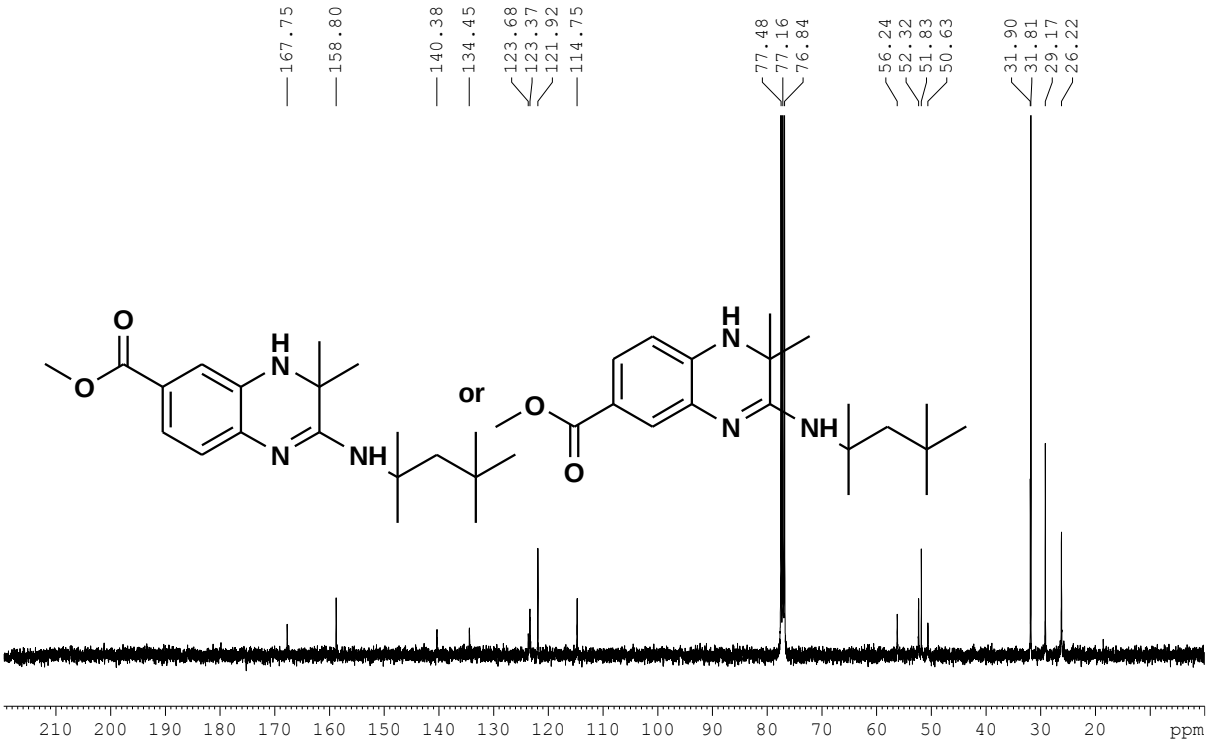
**6.14a**

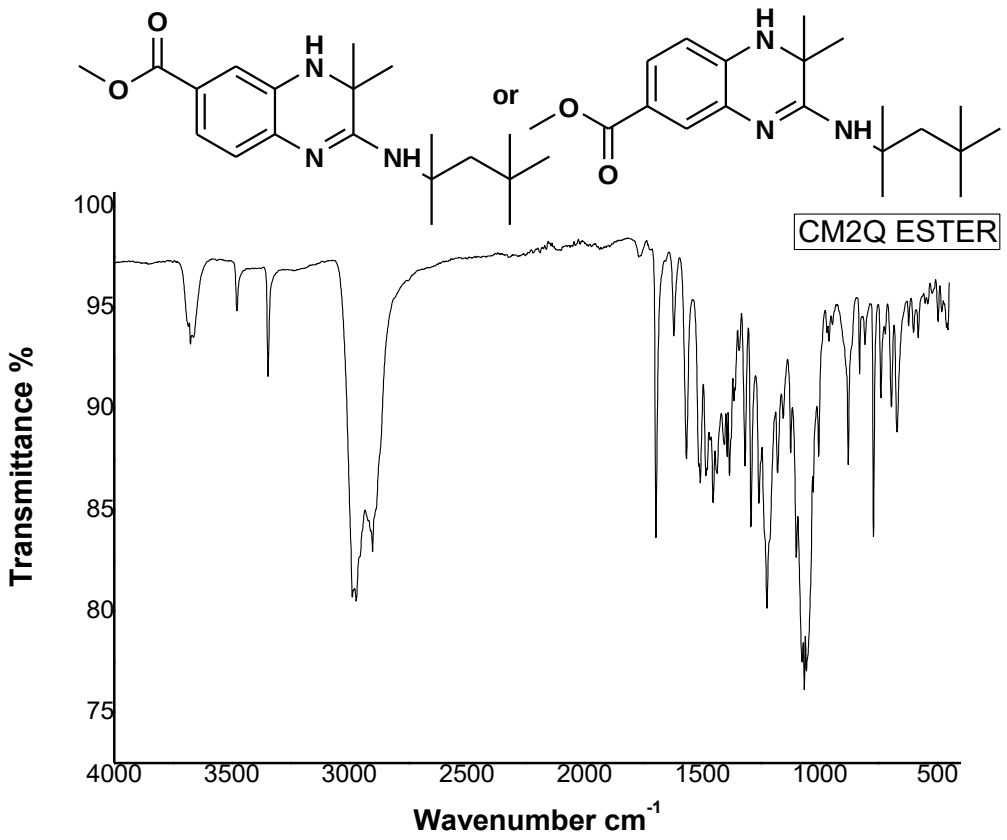

Intens.
x10^7
CM1 ESTER_GA7_01_34839.d: +MS, 0.27min #16
290.1865
150.1123
or
100 200 300 400 500 600 700 800 900 m/z
Intens.
x10^7
+MS, 0.27min #16
290.1865
150.1123
200 400 600 800 1000 1200 m/z

**6.14b**


7.517
7.497
7.293
7.280
7.072
7.055
4.524
3.886
3.604
1.933
1.577
1.299
1.065
or
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
1.00 1.08 0.91 0.98 3.16 1.11 2.07 6.09 6.21 9.28

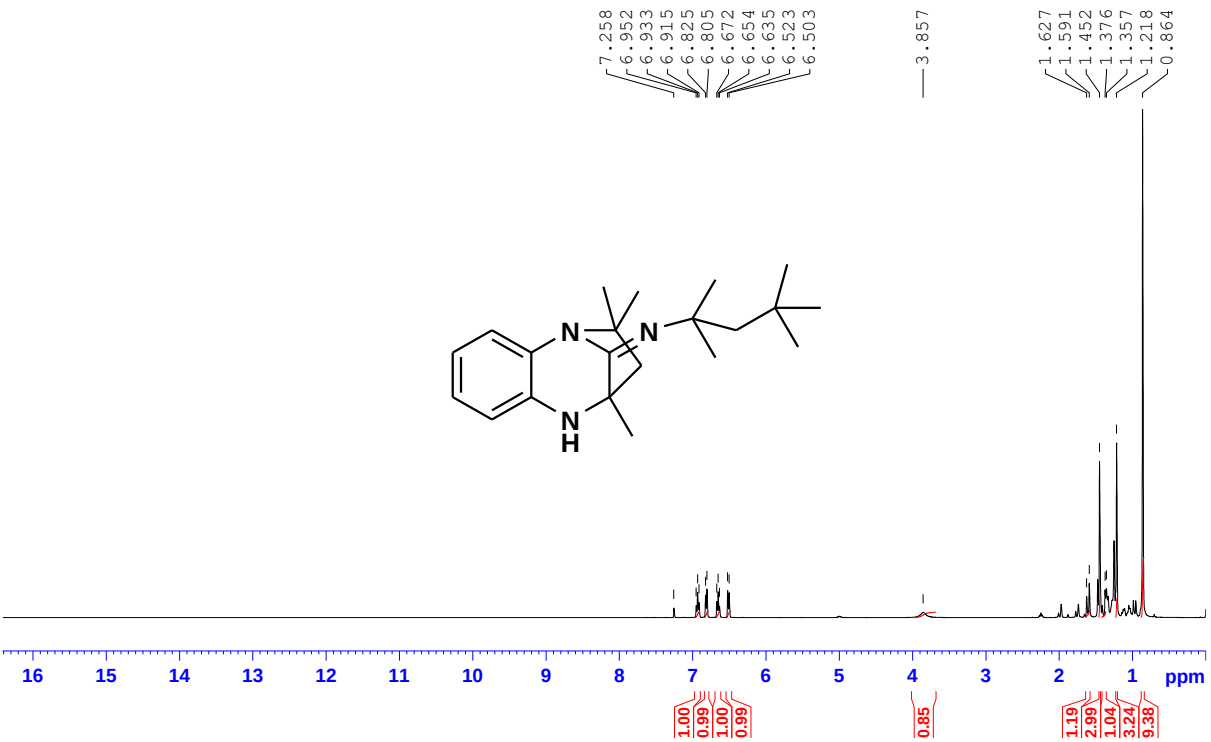
**6.14b**


167.75
158.80
140.38
134.45
123.68
123.37
121.92
114.75
77.48
77.16
76.84
56.24
52.32
51.83
50.63
31.90
31.81
29.17
26.22
or
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

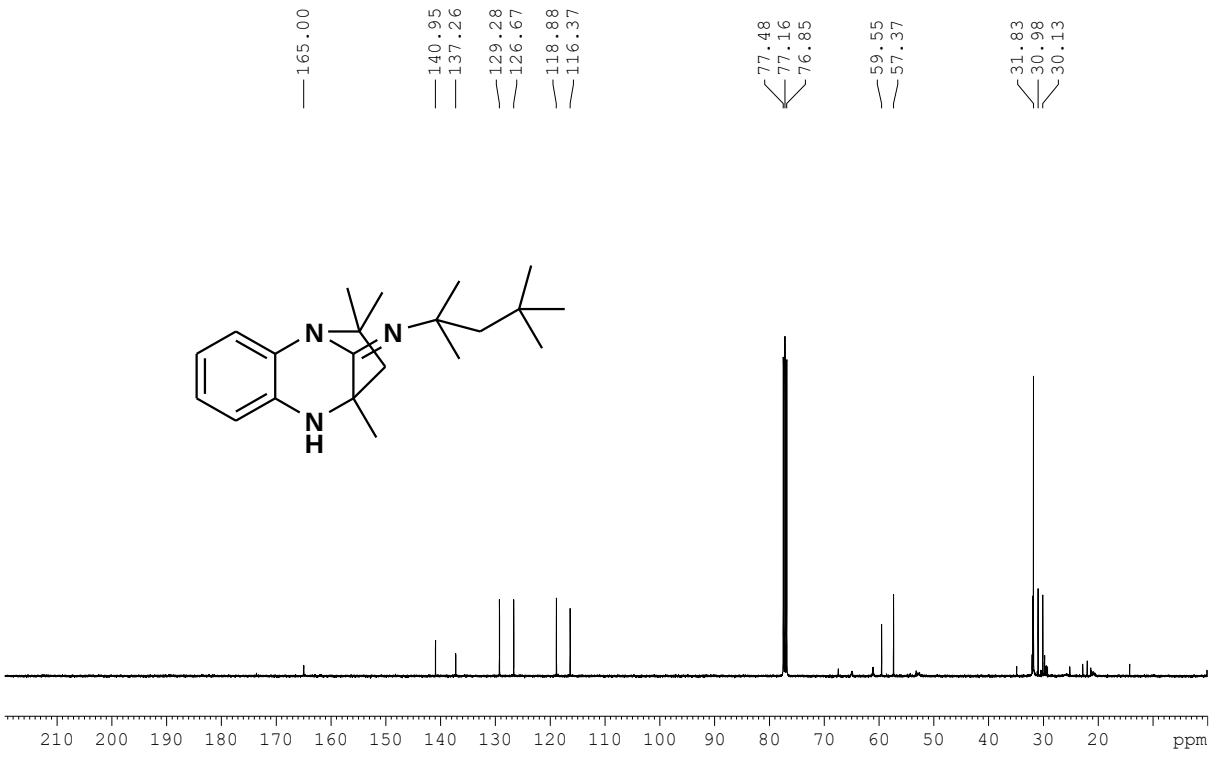



*Diazepine -based* ***acetone-d$_6$ (6.8)***

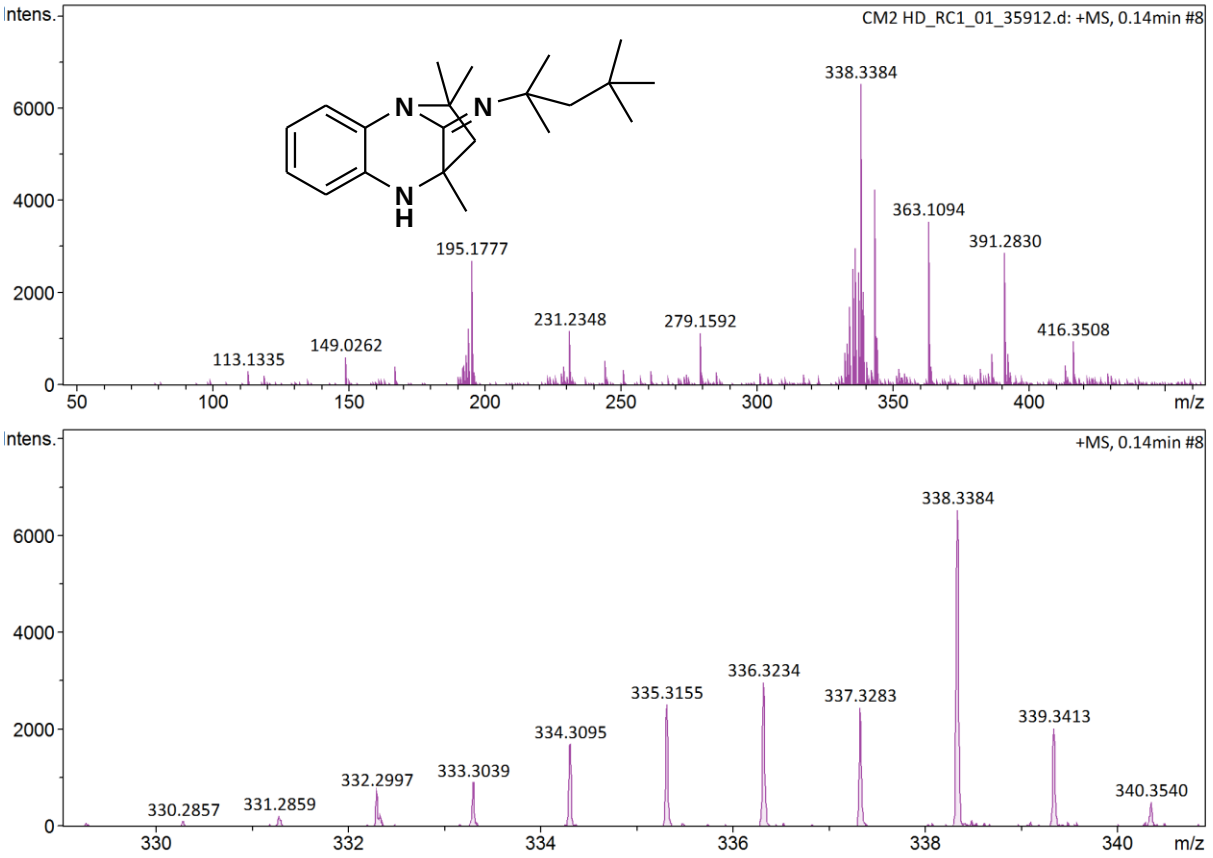
**6.8i**



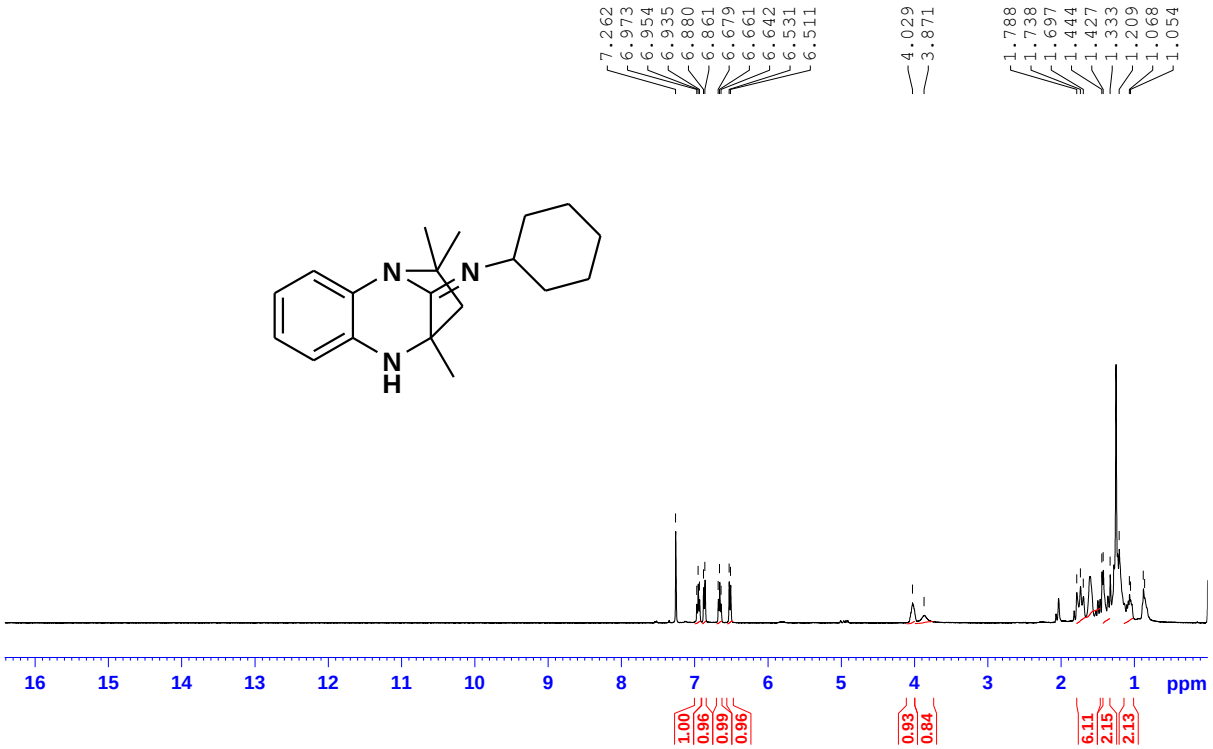

**6.8i**


165.00
140.95
137.26
129.28
126.67
118.88
116.37
77.48
77.16
76.85
59.55
57.37
31.83
30.98
30.13
N
N
N
H
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

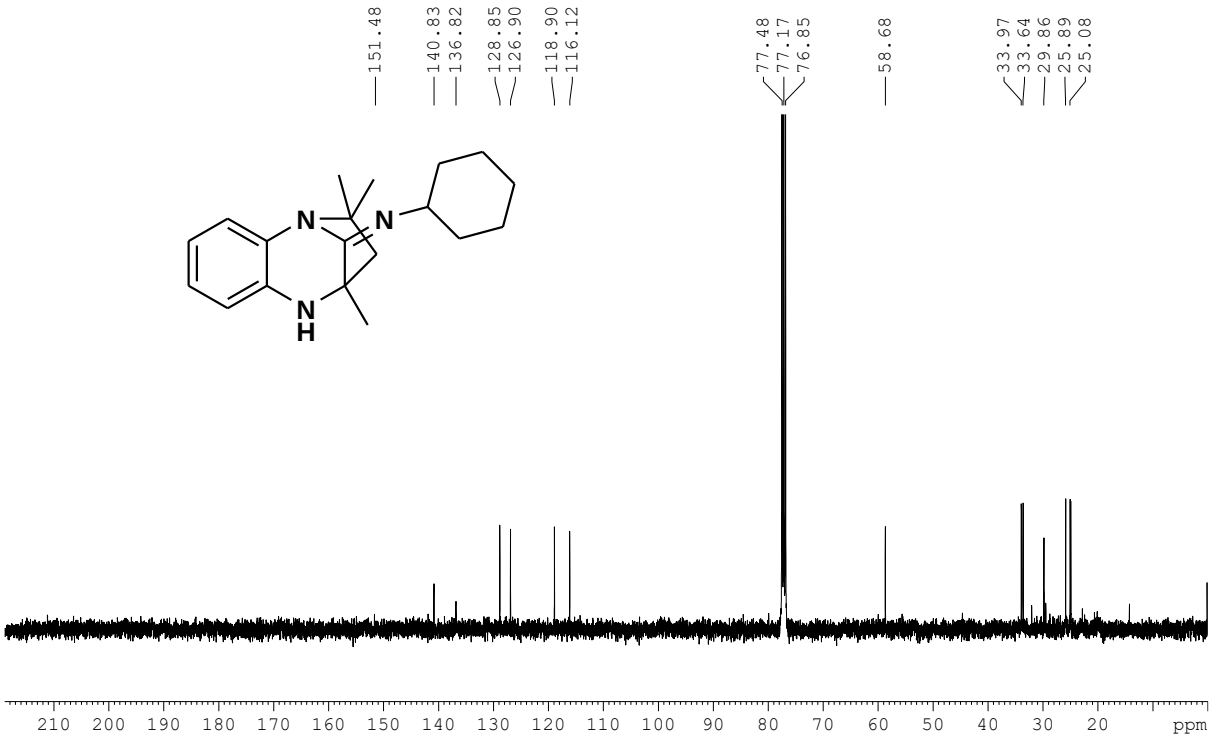
**6.8i**


Intens.
CM2 HD_RC1_01_35912.d: +MS, 0.14min #8
6000
4000
2000
0
338.3384
363.1094
391.2830
195.1777
231.2348
279.1592
416.3508
149.0262
113.1335
50 100 150 200 250 300 350 400 m/z
Intens.
+MS, 0.14min #8
6000
4000
2000
0
338.3384
336.3234
335.3155
337.3283
339.3413
334.3095
333.3039
332.2997
330.2857
331.2859
340.3540
330 332 334 336 338 340 m/z

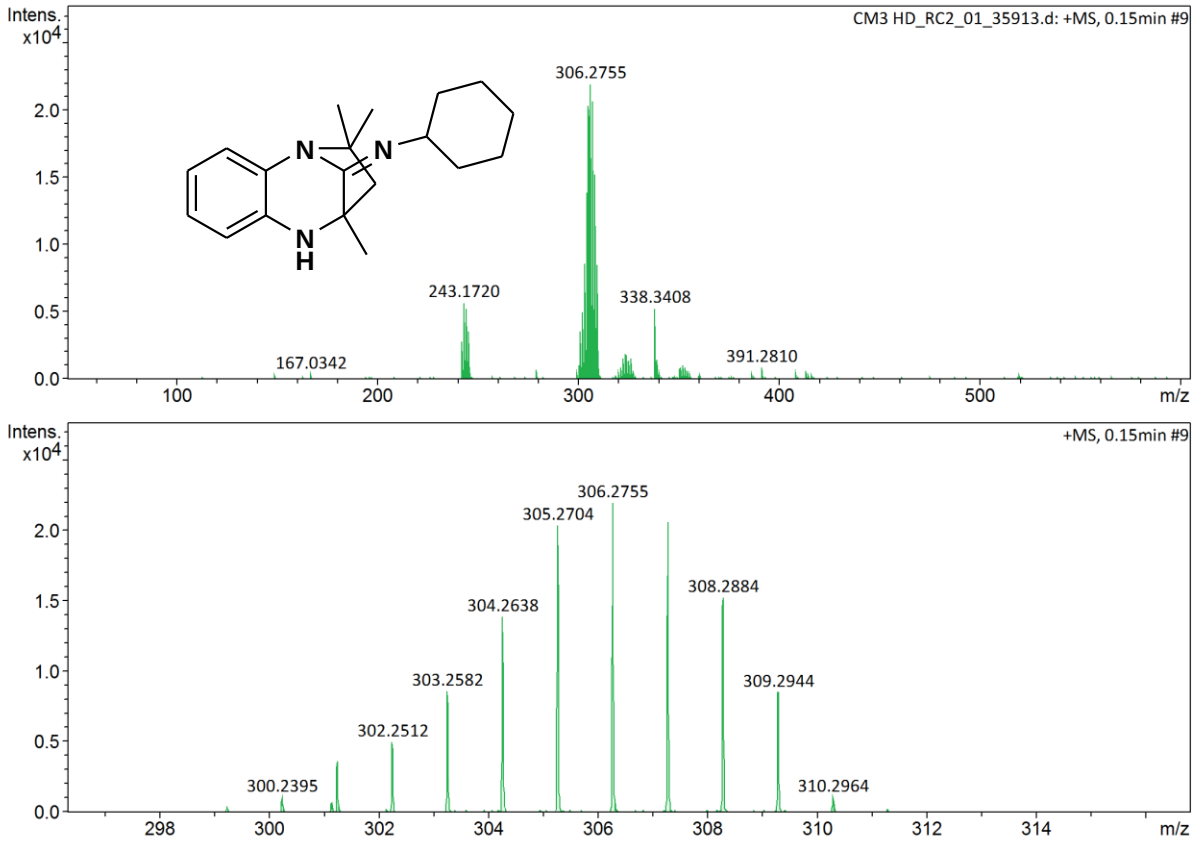
**6.8j**


7.262
6.973
6.954
6.935
6.880
6.861
6.679
6.661
6.642
6.531
6.511
4.029
3.871
1.788
1.738
1.697
1.444
1.427
1.333
1.209
1.068
1.054
N
N
N
H
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
1.00 0.96 0.99 0.96
0.93 0.84
6.11 2.15 2.13

**6.8j**

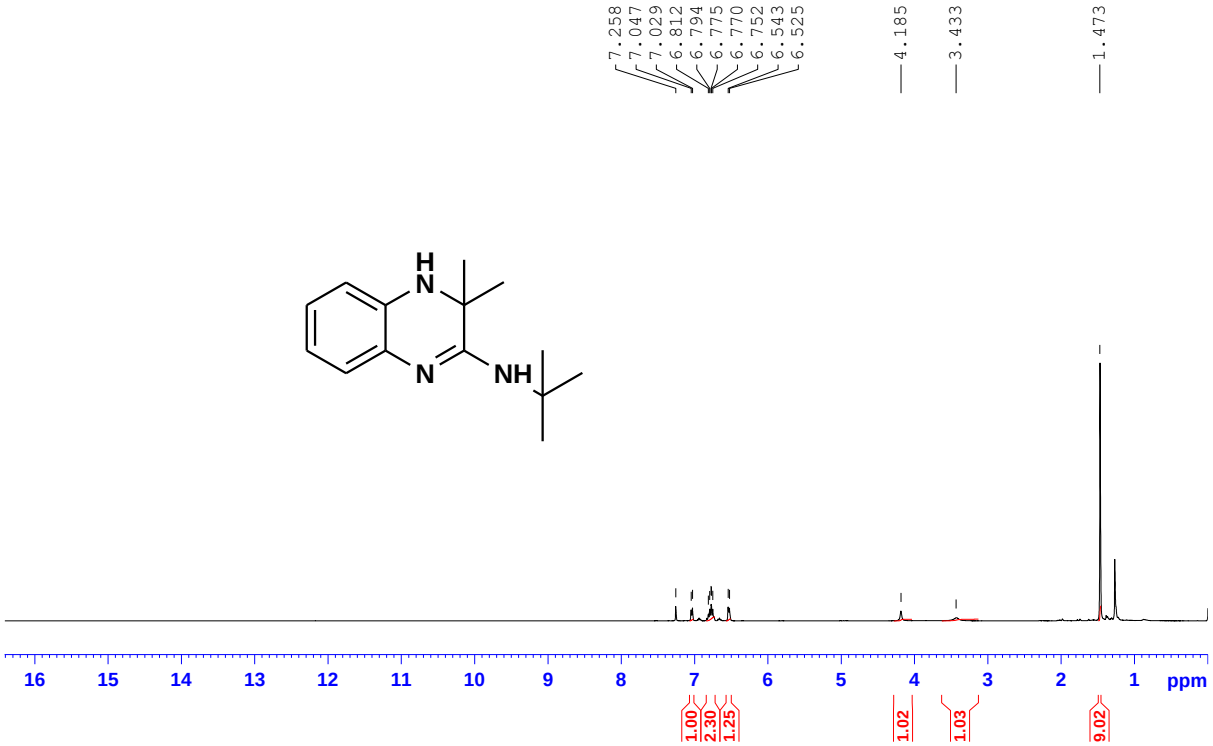

151.48
140.83
136.82
128.85
126.90
118.90
116.12
77.48
77.17
76.85
58.68
33.97
33.64
29.86
25.89
25.08
N
N
N
H
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

**6.8j**

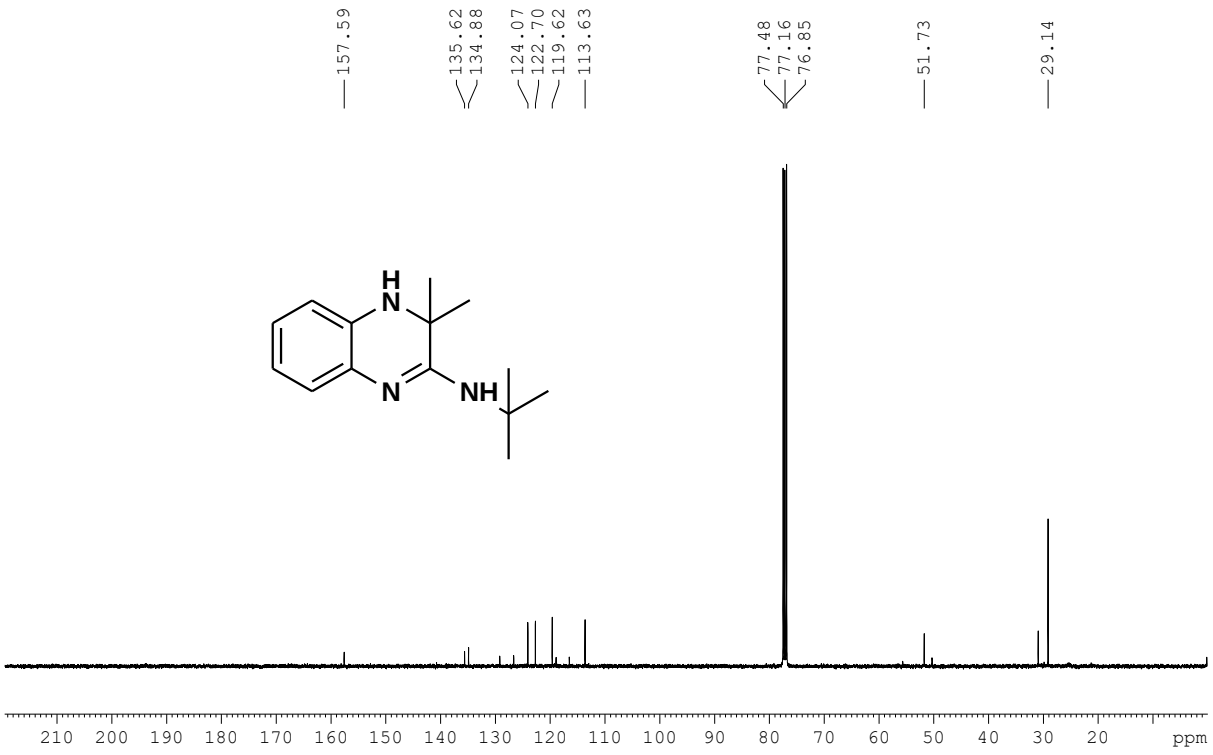



*Quinoxaline-based (**6.14**) **acetone-$d_6$** page*

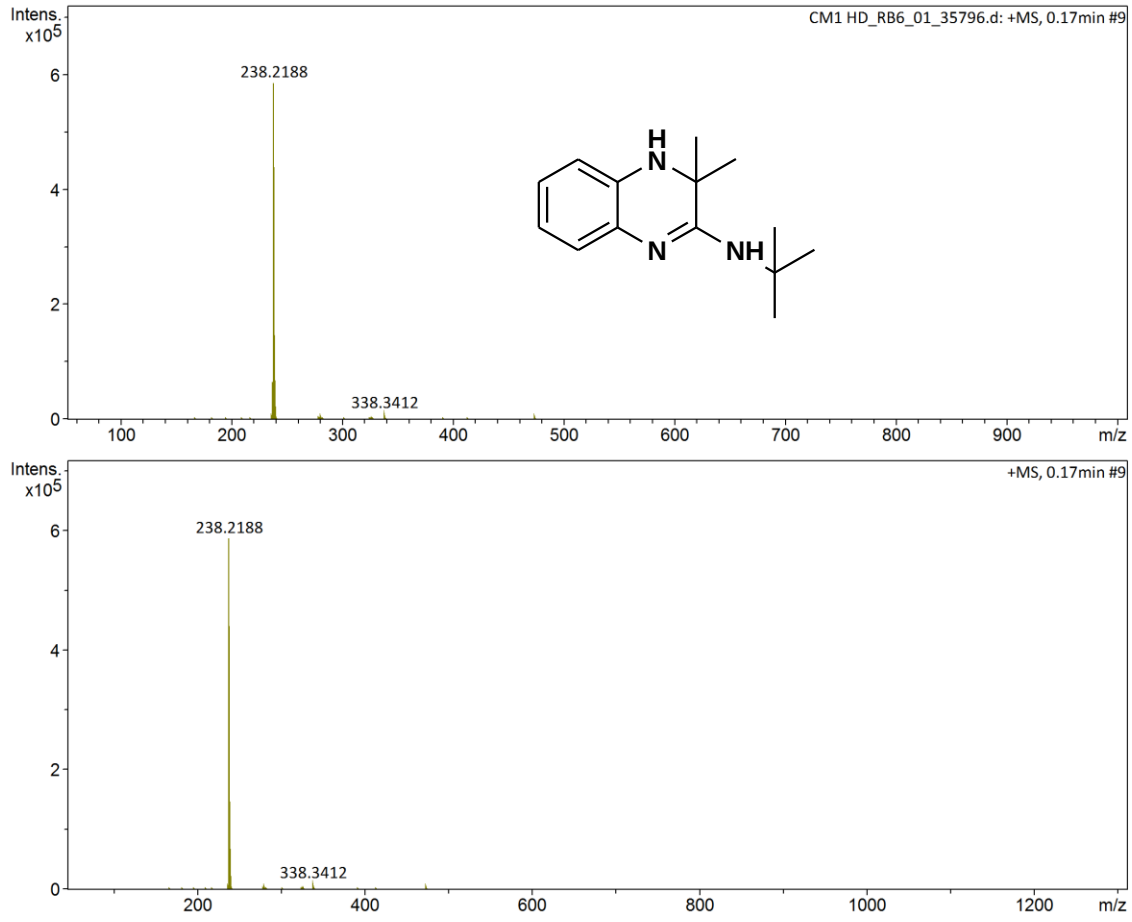
**6.14h**



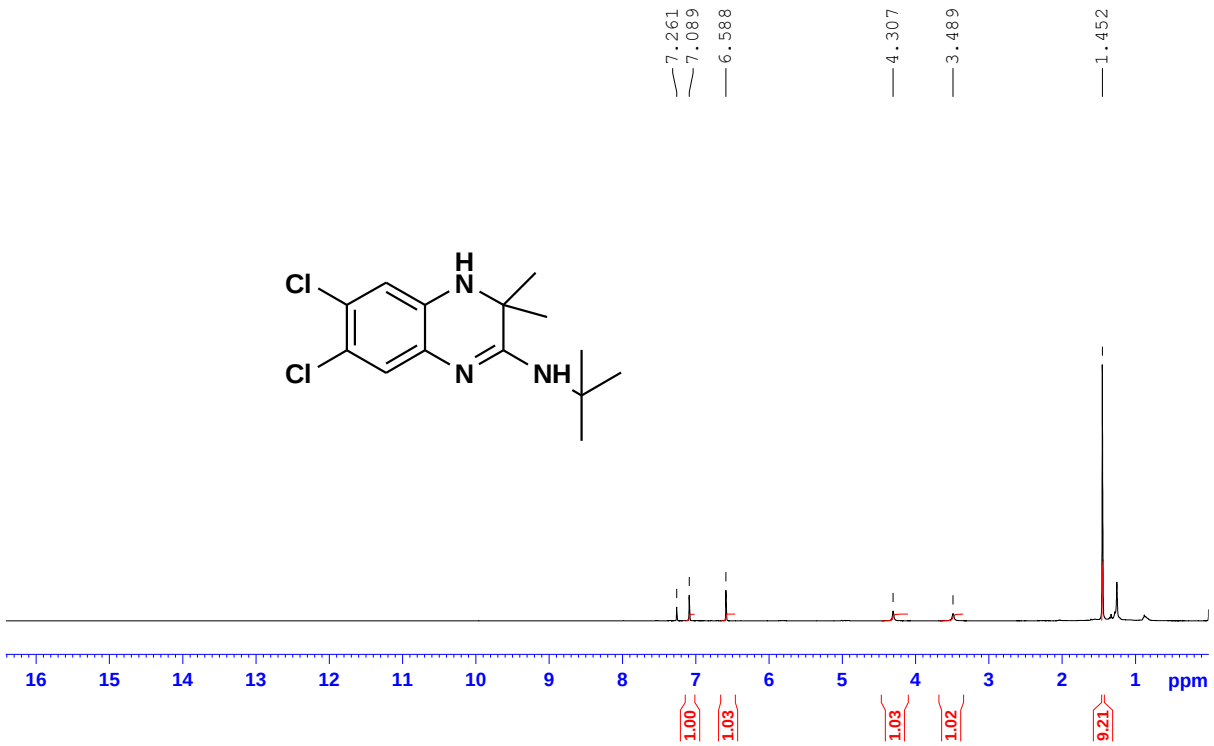

**6.14h**


157.59
135.62
134.88
124.07
122.70
119.62
113.63
77.48
77.16
76.85
51.73
29.14
H
N
N
NH
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

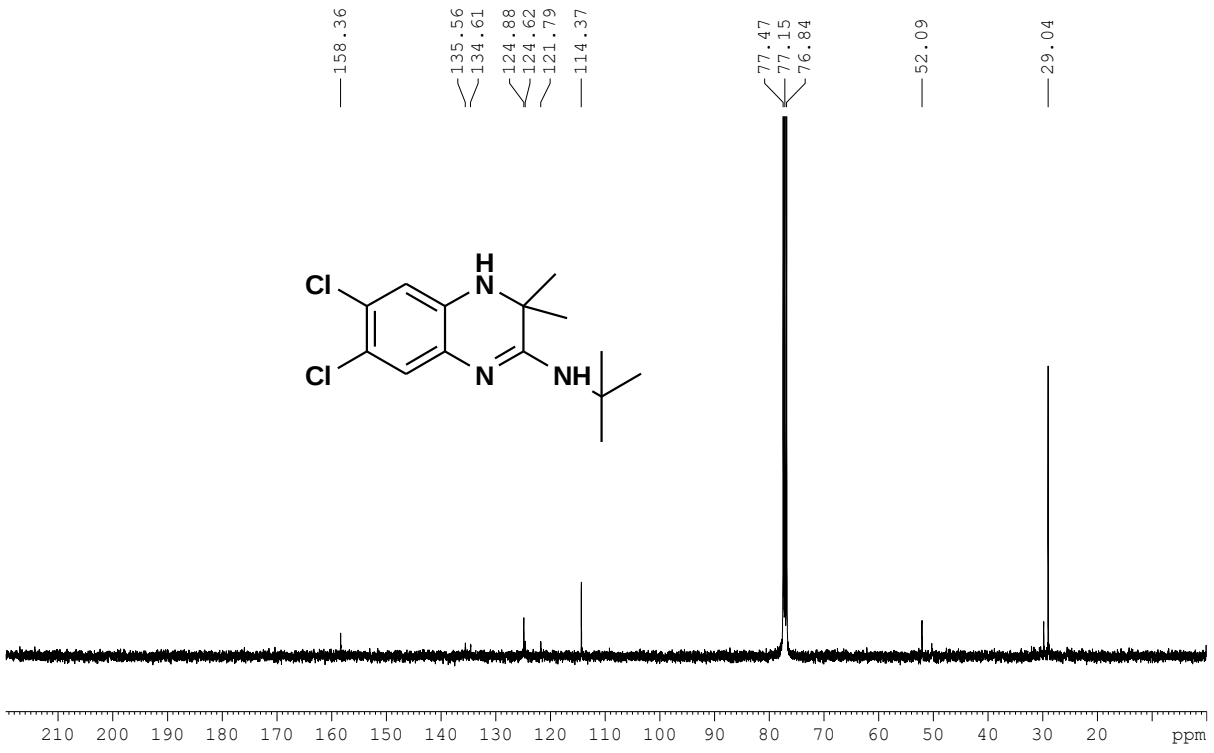
**6.14h**


Intens.
x10^5
CM1 HD_RB6_01_35796.d: +MS, 0.17min #9
238.2188
338.3412
H
N
N
NH
100 200 300 400 500 600 700 800 900 m/z
Intens.
x10^5
+MS, 0.17min #9
238.2188
338.3412
200 400 600 800 1000 1200 m/z

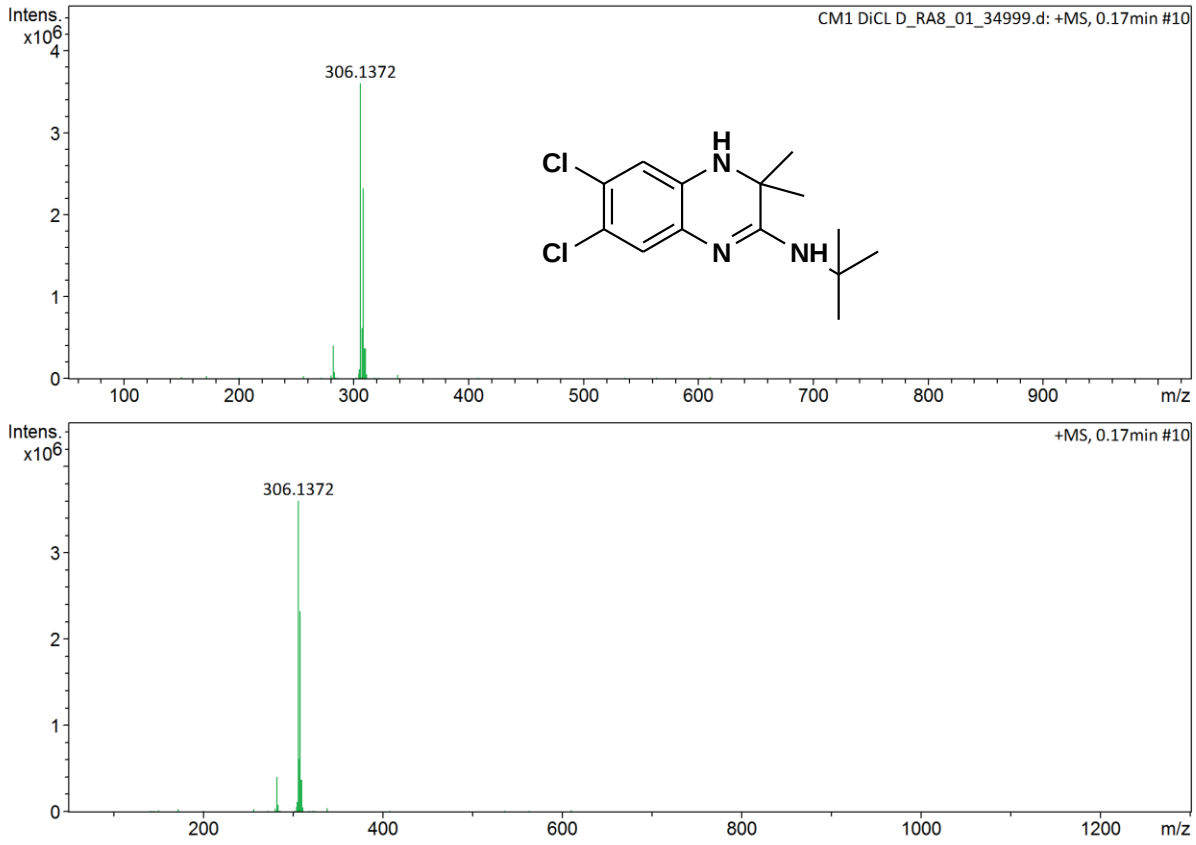
**6.14i**


7.261
7.089
6.588
4.307
3.489
1.452
Cl
Cl
H
N
N
NH
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
1.03
1.03
1.02
9.21

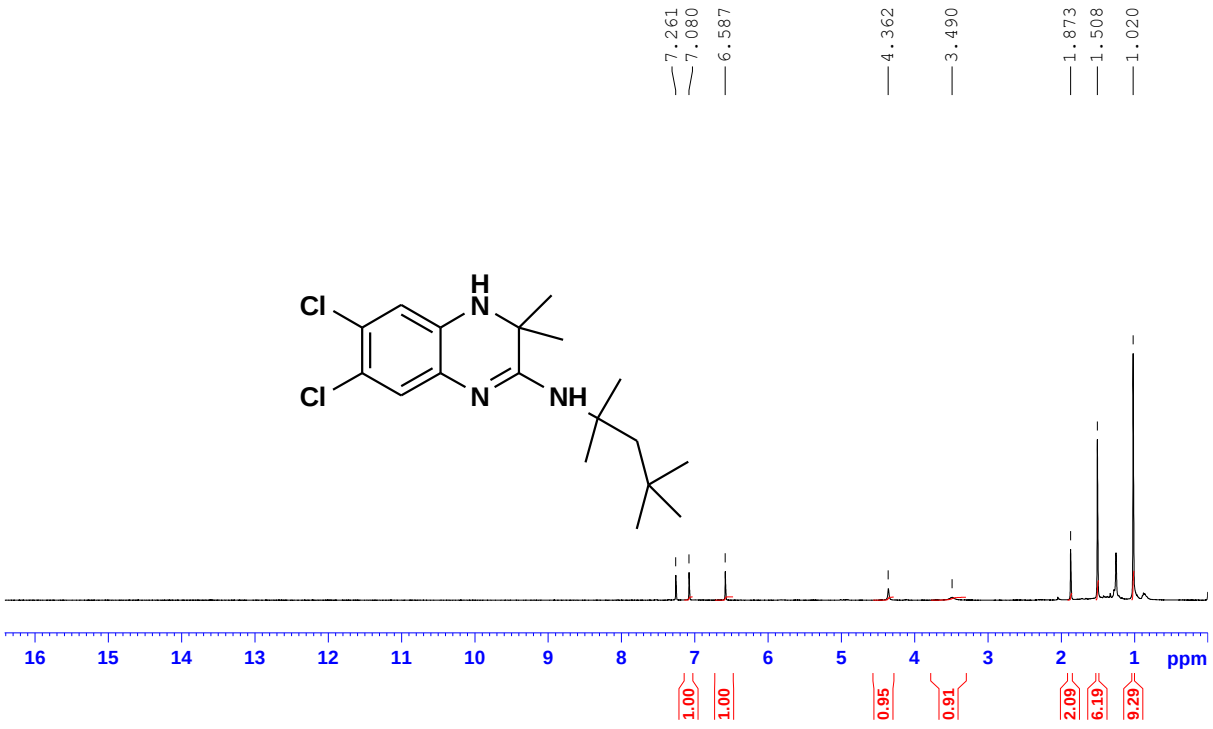
**6.14i**


158.36
135.56
134.61
124.88
124.62
121.79
114.37
77.47
77.15
76.84
52.09
29.04
Cl
Cl
H
N
N
NH
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

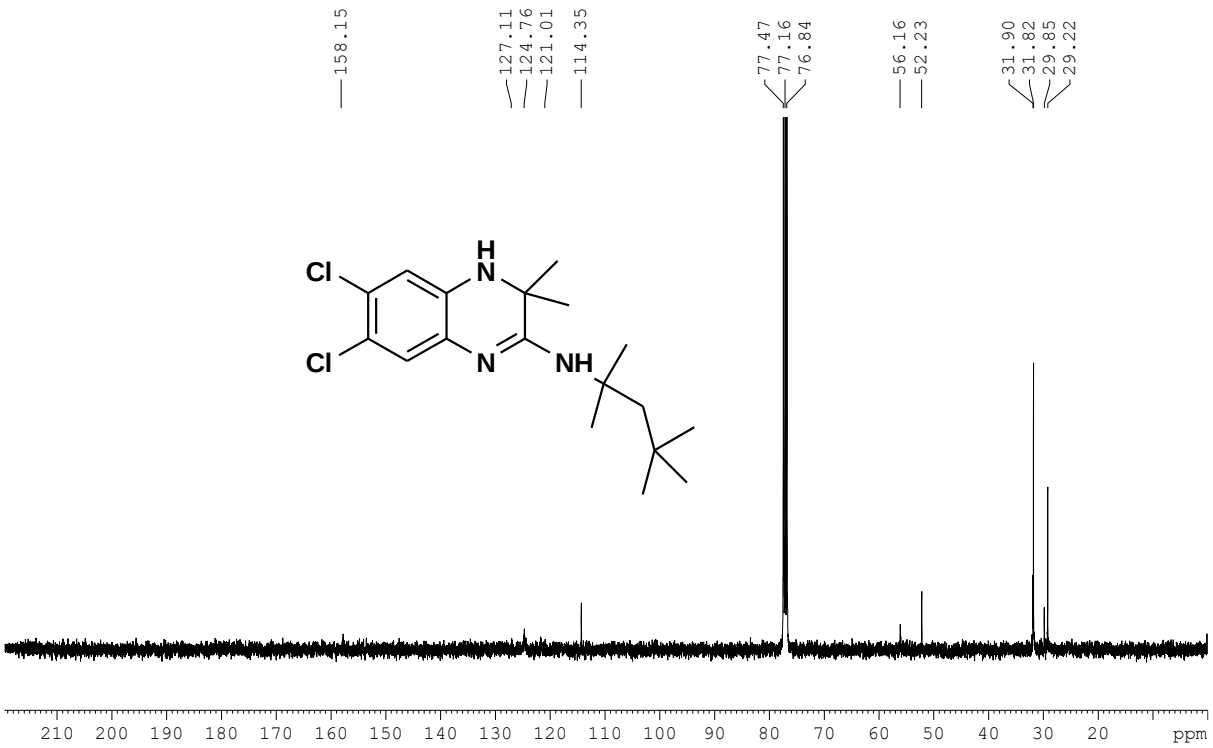
**6.14i**


CM1 DiCL D_RA8_01_34999.d: +MS, 0.17min #10
Intens.
x10^6
306.1372
Cl
Cl
H
N
N
NH
m/z
+MS, 0.17min #10
306.1372

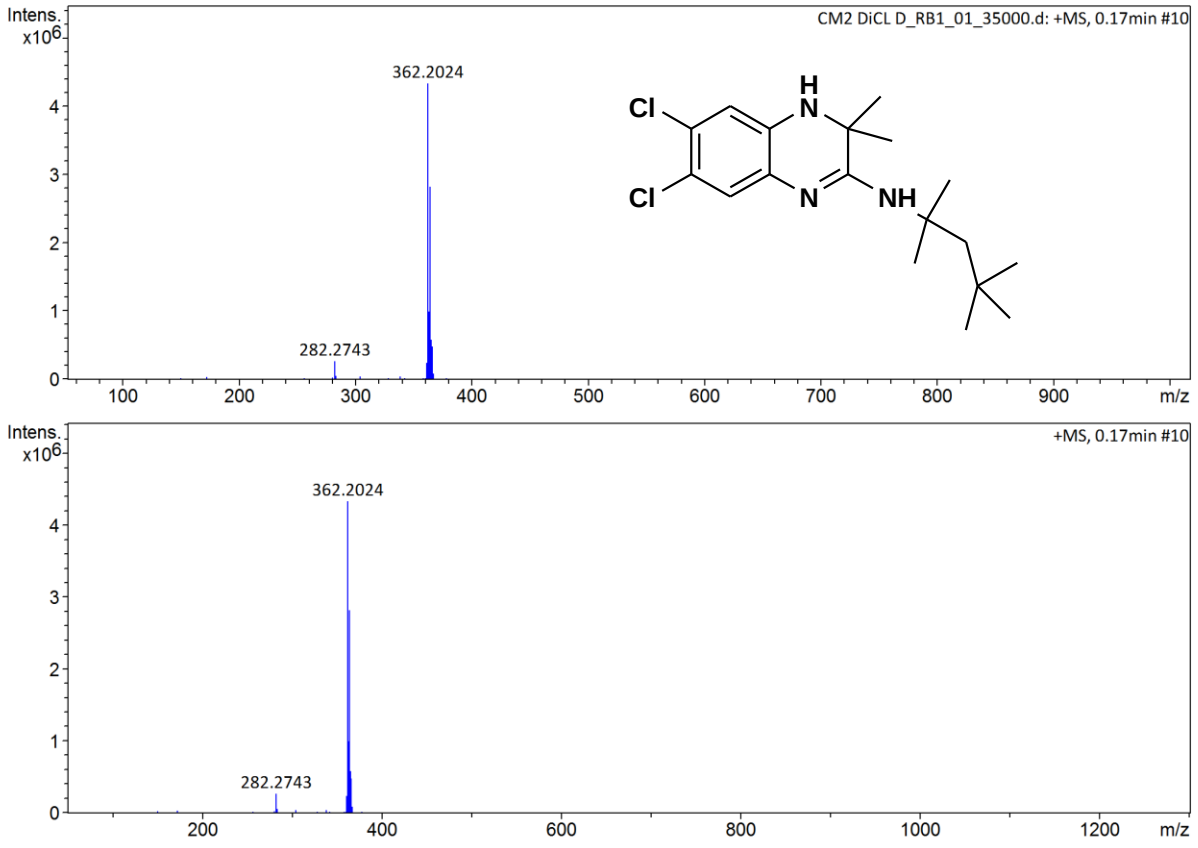
**6.14j**


7.261
7.080
6.587
4.362
3.490
1.873
1.508
1.020
Cl
Cl
H
N
N
NH
ppm
1.00
1.00
0.95
0.91
2.09
6.19
9.29

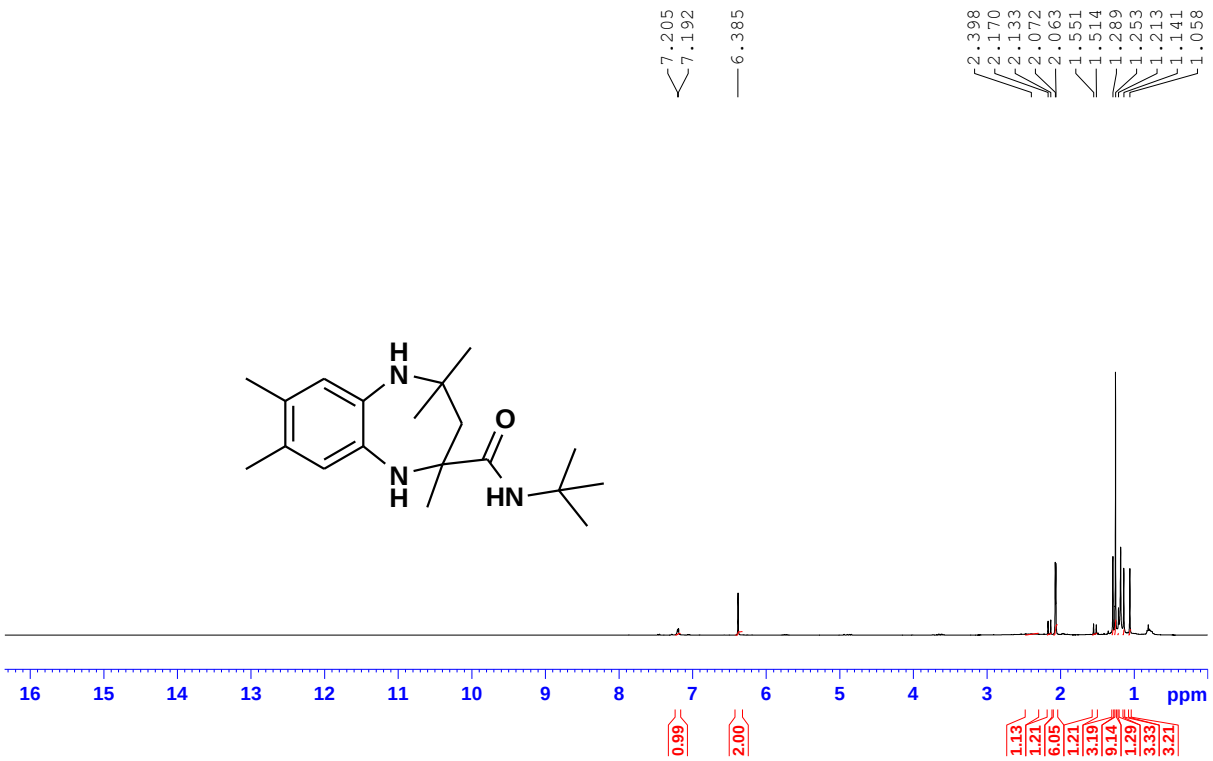
**6.14j**


158.15
127.11
124.76
121.01
114.35
77.47
77.16
76.84
56.16
52.23
31.90
31.82
29.85
29.22
Cl
Cl
H
N
N
NH
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

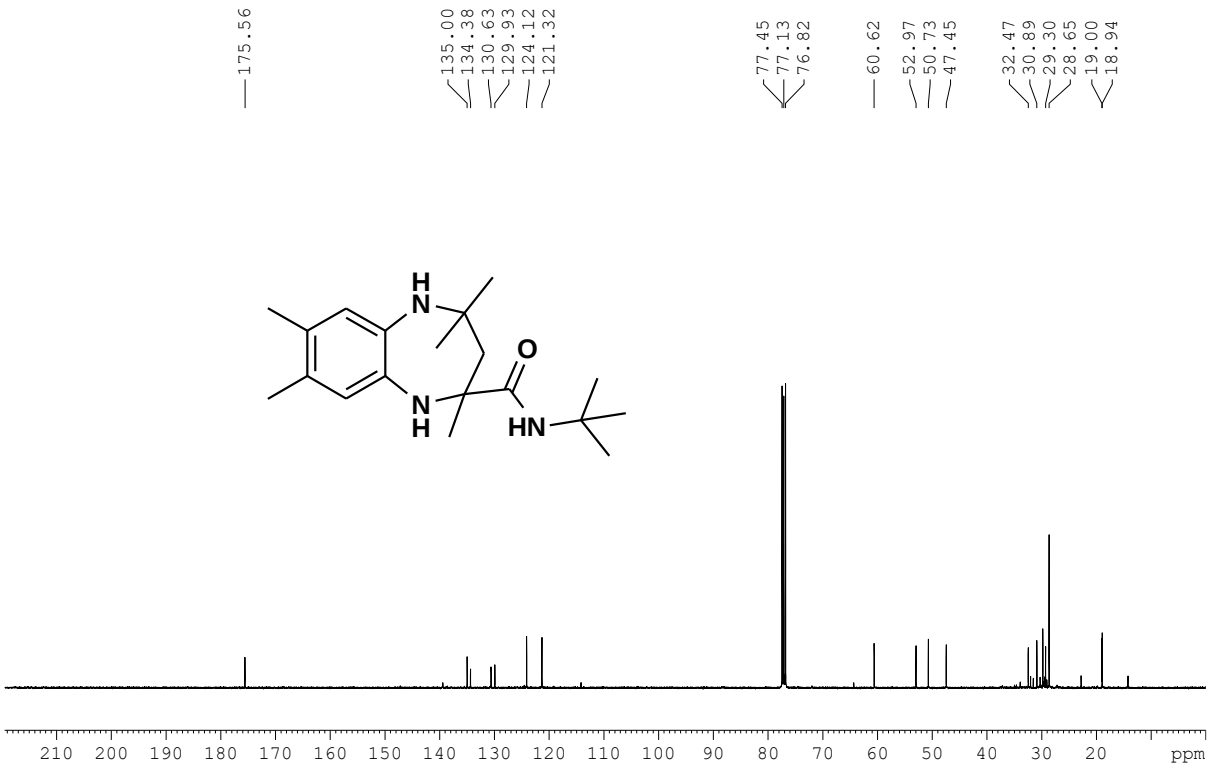
**6.14j**


Intens.
x10^6
CM2 DiCL D_RB1_01_35000.d: +MS, 0.17min #10
362.2024
282.2743
Cl
Cl
H
N
N
NH
100 200 300 400 500 600 700 800 900 m/z
Intens.
x10^6
+MS, 0.17min #10
362.2024
282.2743
200 400 600 800 1000 1200 m/z

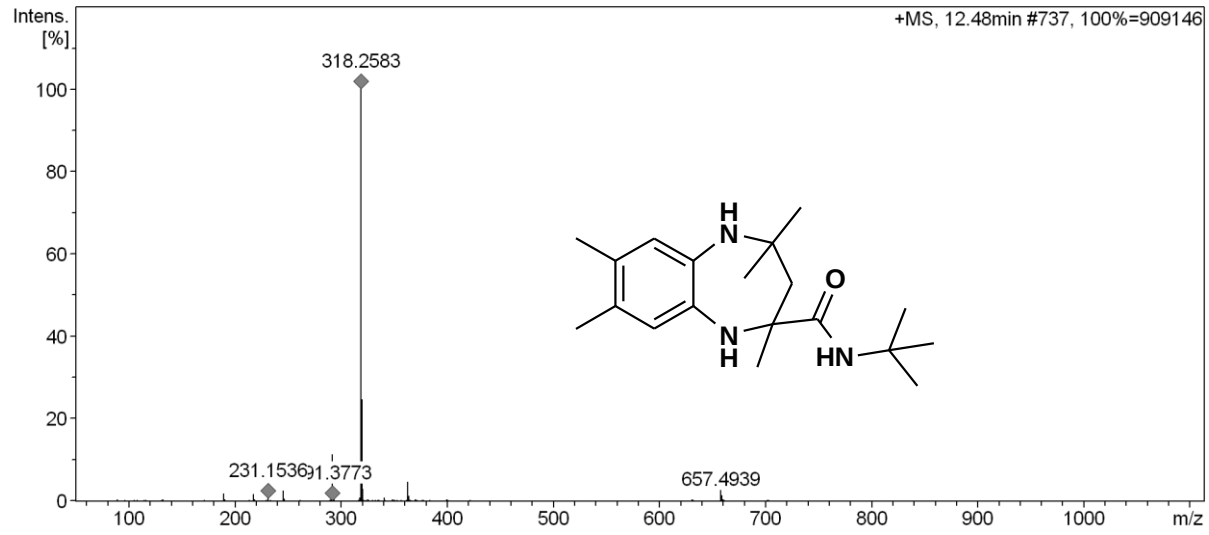
**6.13a**


7.205
7.192
6.385
2.398
2.170
2.133
2.072
2.063
1.551
1.514
1.289
1.253
1.213
1.141
1.058
H
N
N
H
O
HN
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.99
2.00
1.13
1.21
6.05
1.21
3.19
9.14
1.29
3.33
3.21

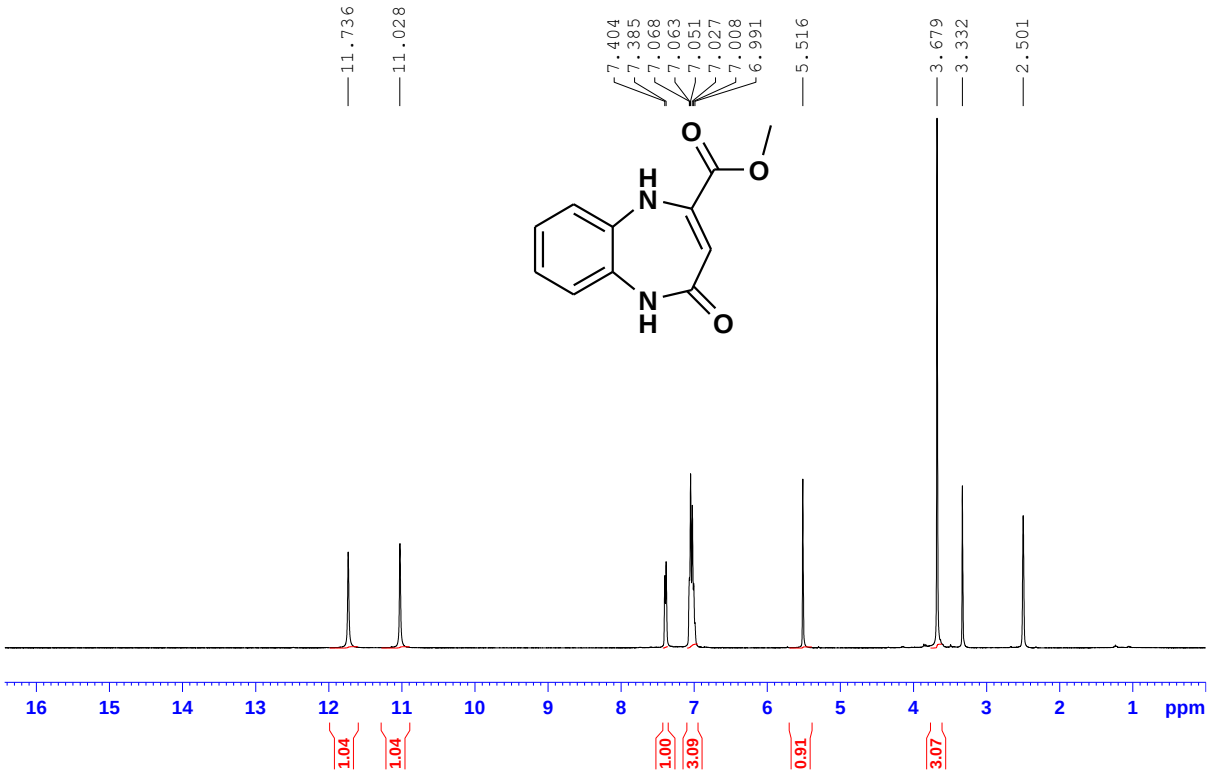
**6.13a**


175.56
135.00
134.38
130.63
129.93
124.12
121.32
77.45
77.13
76.82
60.62
52.97
50.73
47.45
32.47
30.89
29.30
28.65
19.00
18.94
H
N
O
N
H
HN
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

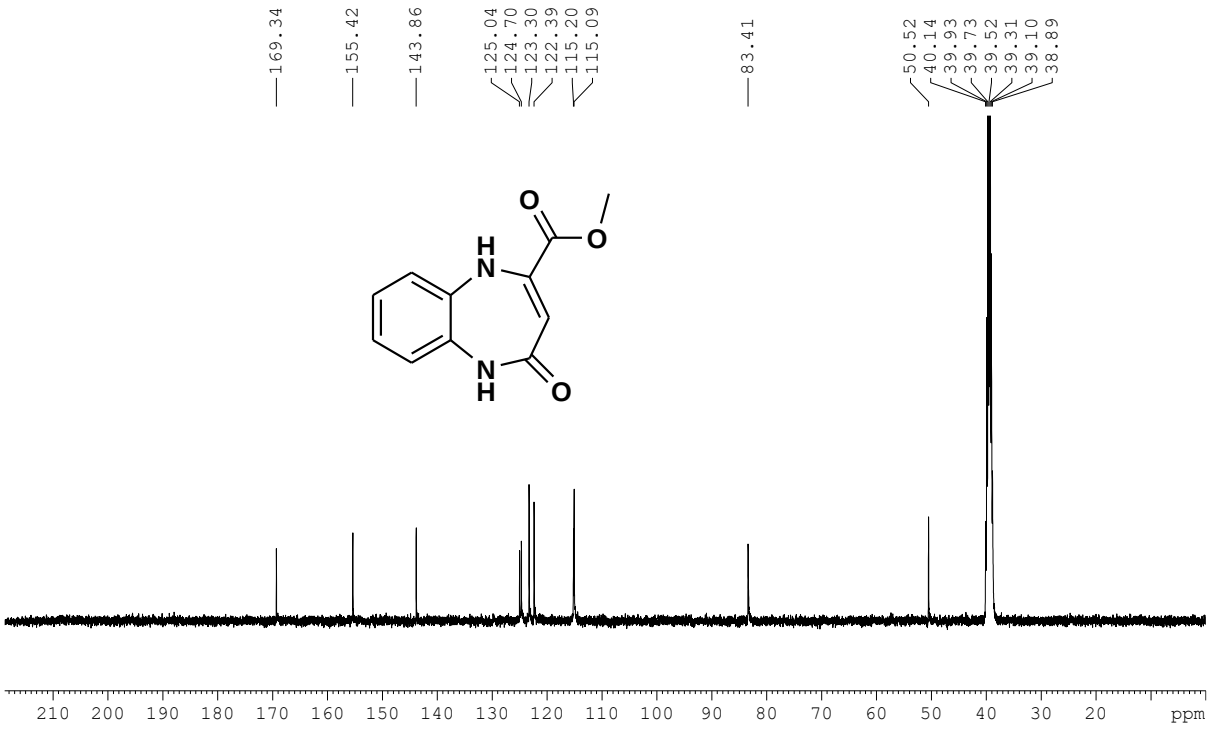
**6.13a**


Intens.
[%]
+MS, 12.48min #737, 100%=909146
318.2583
231.1536
321.3773
657.4939
H
N
O
N
H
HN
100
80
60
40
20
0
100 200 300 400 500 600 700 800 900 1000 m/z

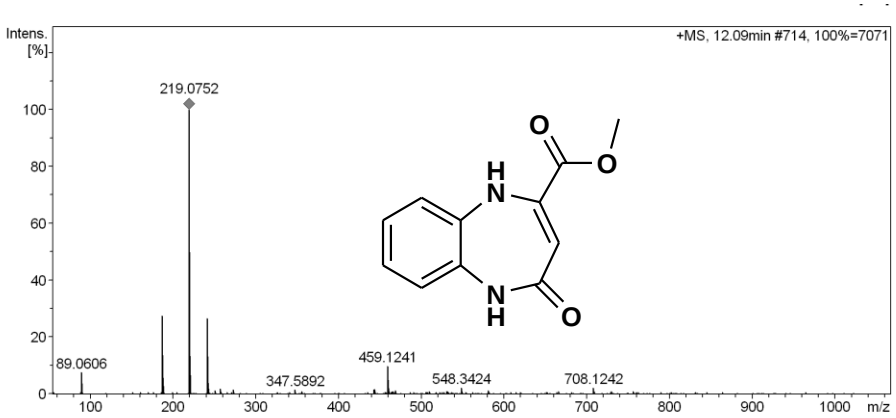
**6.16a**

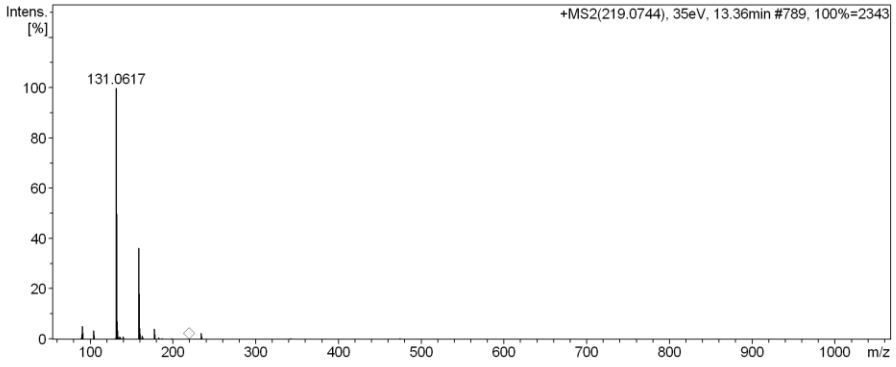

11.736
11.028
7.404
7.385
7.068
7.063
7.051
7.027
7.008
6.991
5.516
3.679
3.332
2.501
O
O
H
N
N
H
O
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
1.04
1.04
1.00
3.09
0.91
3.07

**6.16a**

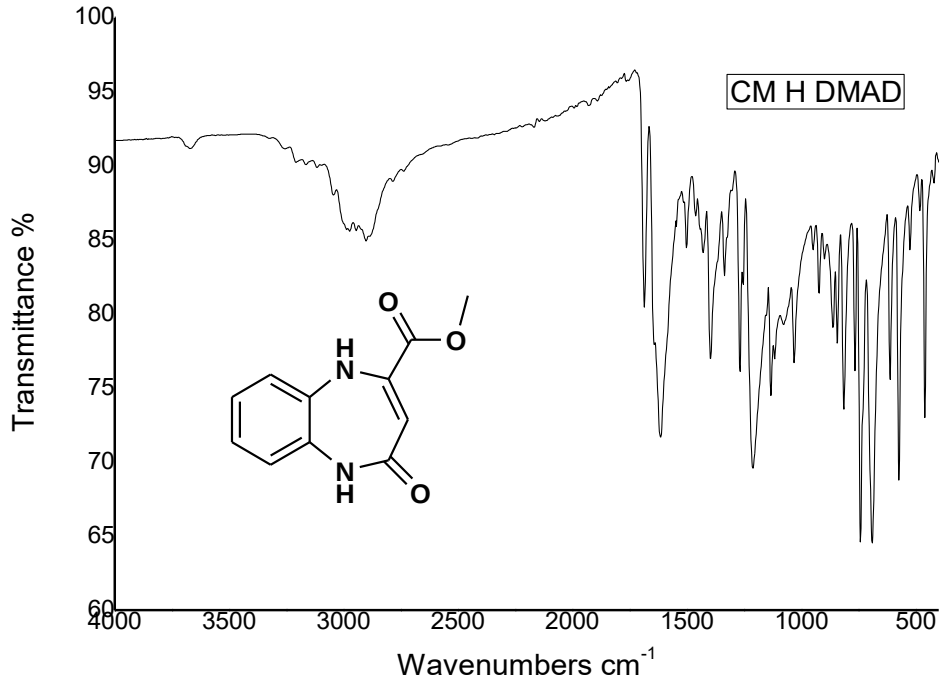

169.34
155.42
143.86
125.04
124.70
123.30
122.39
115.20
115.09
83.41
50.52
40.14
39.93
39.73
39.52
39.31
39.10
38.89
O
O
H
N
N
H
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

**6.16a**

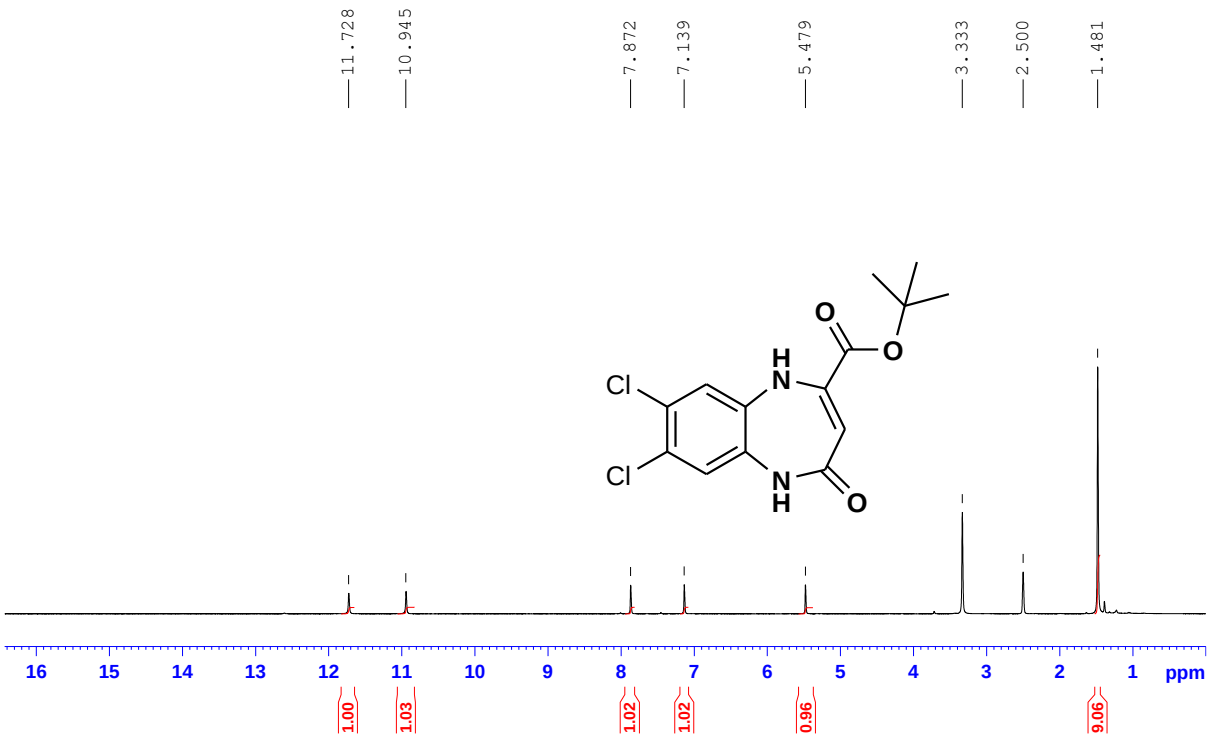

Intens.
[%]
+MS, 12.09min #714, 100%=7071
219.0752
89.0606
347.5892
459.1241
548.3424
708.1242
m/z


Intens.
[%]
+MS2(219.0744), 35eV, 13.36min #789, 100%=2343
131.0617
m/z

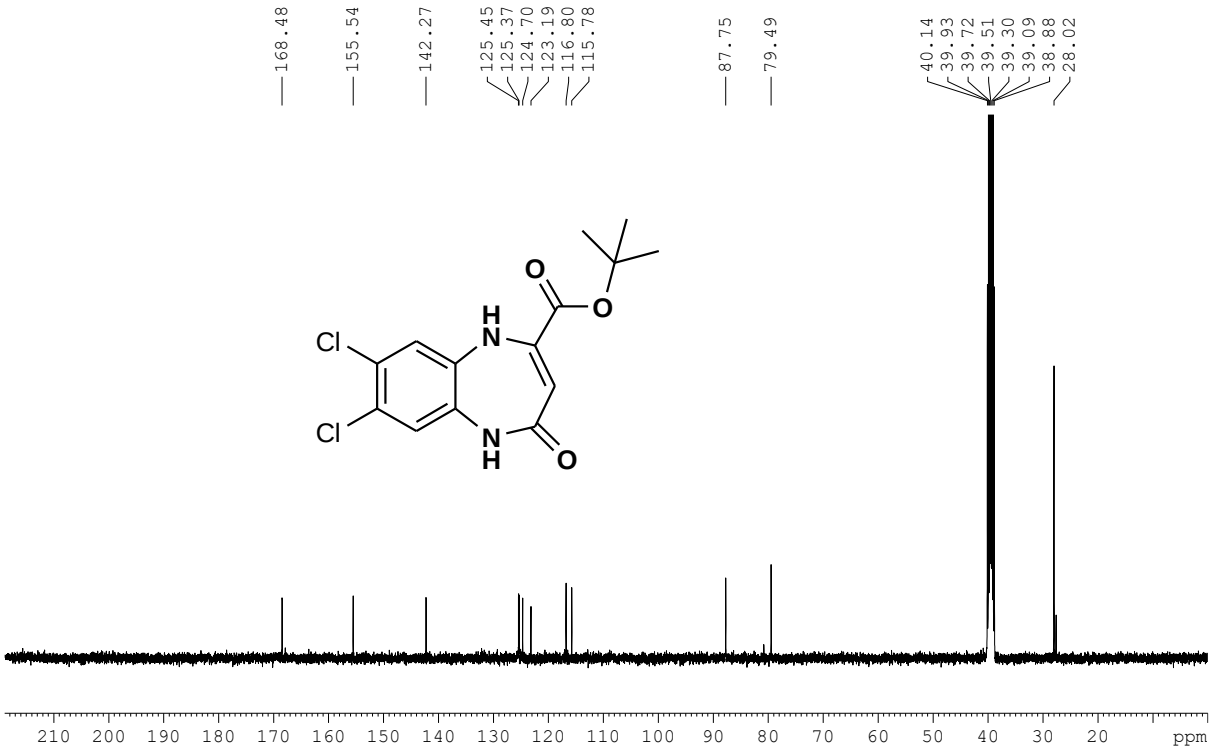
**6.16a**


CM H DMAD
Transmittance %
Wavenumbers cm-1

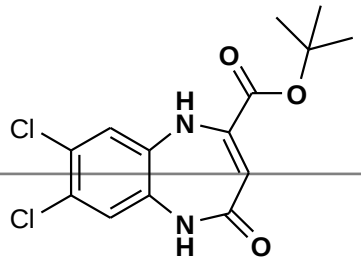
**6.16i**

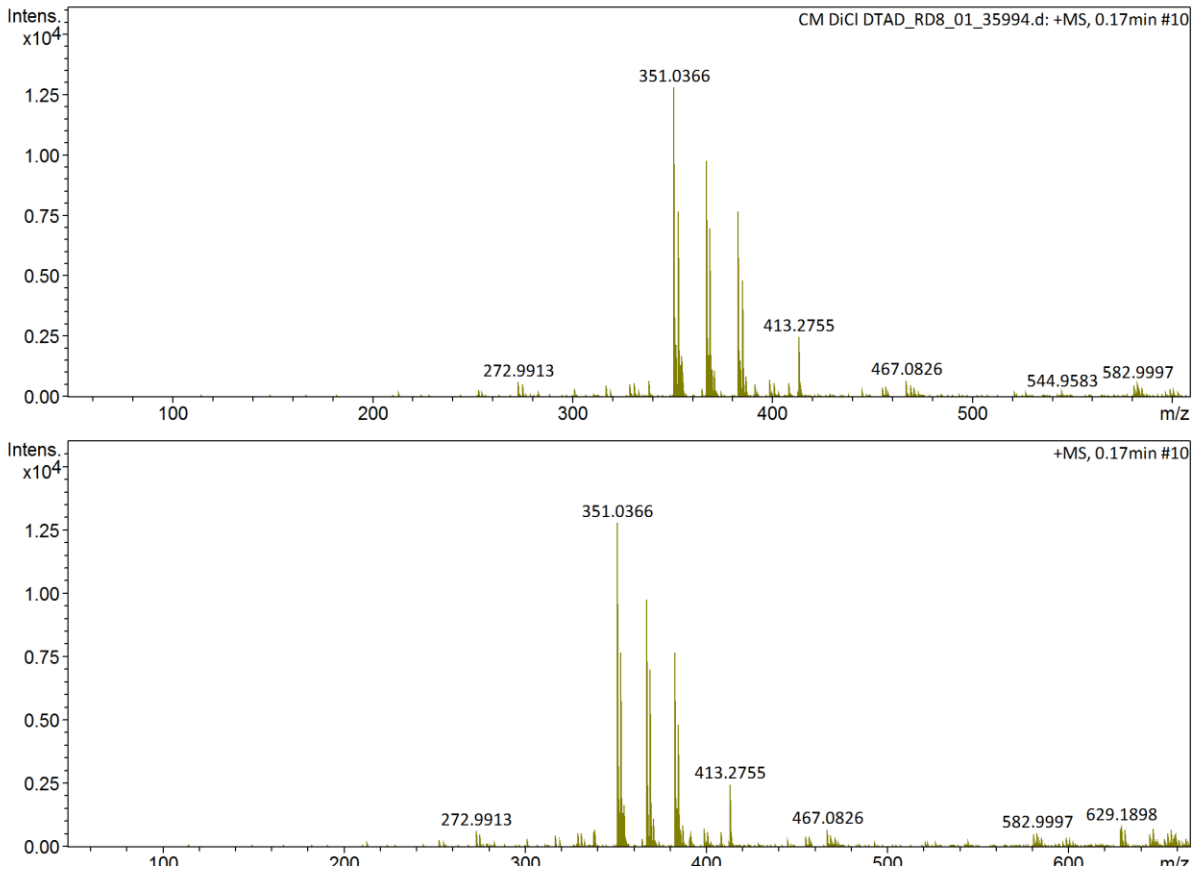

11.728
10.945
7.872
7.139
5.479
3.333
2.500
1.481
Cl
Cl
H
N
N
H
O
O
O
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
1.00
1.03
1.02
1.02
0.96
9.06

**6.16i**


168.48
155.54
142.27
125.45
125.37
124.70
123.19
116.80
115.78
87.75
79.49
40.14
39.93
39.72
39.51
39.30
39.09
38.88
28.02
Cl
Cl
H
N
N
H
O
O
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

**6.16i**


Cl
Cl
H
N
N
H
O
O
O


Intens.
x10^4
CM DiCl DTAD_RD8_01_35994.d: +MS, 0.17min #10
351.0366
413.2755
272.9913
467.0826
544.9583
582.9997
m/z
Intens.
x10^4
+MS, 0.17min #10
351.0366
413.2755
272.9913
467.0826
582.9997
629.1898
m/z