APPLICATION OF BIOCATALYSTS IN THE SYNTHESIS OF ANTI-TUBERCULOSIS COMPOUNDS AND ANALOGUES.

A thesis submitted to the faculty of science, University of the Witwatersrand, in fulfilment of the requirements for the degree of Doctor of Philosophy

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JUNE 6, 2022

560296

DECLARATION

I declare that this thesis is my own, unaided work. It is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

Signature

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Professor Dean Brady.

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Abstract

The current advancements in genomic engineering and DNA technology have expanded the range and applications of enzymes in synthetic organic chemistry. Biocatalytic transformations continue to replace traditional chemical methods at an exponential rate. With the world now faced with various sustainability and climate change challenges, synthetic chemists need to rethink how we might prepare important target molecules in the future.

In this thesis, we present an overview of the design, investigation, and application of enzyme biocatalysts towards the synthesis of important active pharmaceutical ingredients, placing a particular interest in **anti-tuberculosis drugs**. The target molecules in this work include **benzimidazole** and **benzothiazole** fused to **triazole** rings (part one) and **quinazolinone** derivatives (part two). The enzymes of focus in this study include commercially available biocatalysts—Laccases (EC1.10.3.2), Nitrile hydratases (EC 4.2.1.84), and Lipases (E.C. 3.1.1.3).

The synthesis of hydroxy 2-aryl-*1H*-benzimidazoles and 2-arylbenzothiazoles from the cyclisation and oxidation reaction of arylamines and hydroxy benzaldehydes was investigated using laccase as an oxidant. Subsequently, the obtained hydroxy heterocycles were treated with potassium carbonate and propargyl bromide in acetone to form 2-aryl-*1H*-benzimidazole and 2-arylbenzothiazole ether alkynes in decent yields.

A novel approach for synthesising triazole rings through the laccase-catalysed 1,3-dipolar cycloaddition reaction between alkynes and azide derivatives was outlined. By treating the obtained 2-aryl-*1H*-benzimidazole and 2-arylbenzothiazole alkynes with phenyl-azides in the presence of a catalytic amount of laccase (*Trametes versicolor*), target benzothiazole and benzimidazole fused 1,2,3-triazole derivatives were achieved. Our method effectively catalysed the condensation reaction for a broad range of substrates, yielding both the 1,4-Disubstituted-*1H*-1,2,3-triazoles and the 1,5-disubstituted-*1H*-1,2,3-triazoles in adequate to moderate yields.

For the first time, we present a novel multistep biocatalytic process for the synthesis of novel quinazolinone derivatives. We effectively catalysed the hydrolysis of nitriles to amides using whole cells containing nitrile hydratase. This is then followed by a condensation reaction between the obtained amides with aldehyde esters using microwave irradiation and a novel laccase/DMSO oxidation to obtain quinazolinone esters in excellent yields. Subsequently, we hydrolysed the quinazolinone esters to acids using immobilised lipase (CAL B). Finally, using HBTU as a coupling reagent, we effectively catalysed the direct amidation reaction of the quinazolinone acid to obtain the target quinazolinone amide derivatives in excellent yields.

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List of Abbreviations

2-MeTHF	2-Methyltetrahydrofuran
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
AIDS	Acquired immunodeficiency syndrome
APCs	antigen-presenting cells
ART	antiretroviral therapy
CALB	Lipase B from Candida antarctica
COSY	Heteronuclear single quantum coherence
DABCO	1,4-diazabicyclo[2.2.2]octane
dd	Doublet of doublets
ddd	Doublet of doublet of doublets
DDQ	2,3-Dicloro-5,6-dicyano-1,4-benzoquinone
DMF	N,N-dimethylformamide
DMSO	Dimethyl Sulfoxide
ee	Enantiomeric excess
EMY	effective mass yield
EPA	Environmental Protection Agency
EPR	Electron Paramagnetic Resonance
ET	electron transfer
EtOAc	Ethyl acetate
FDA	Food and Drug Administration
FMO	frontier molecular orbital
HAA	hydroxyanthanilic acid
HAT	hydrogen-atom transfer
HAT	Human African Trypanosomiasis
HBT	N-hydroxy benzotriazole
HBTU	*N,N,N′,N′*-Tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate, *O*-(Benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate

HCMV	human cytomegalovirus
HCV	Hepatitis C virus
HDAC6	Histone deacetylase 6
HIV	Human immunodeficiency virus
HMBC	Heteronulcear multiple bond correlation
HOMO	Highest occupied molecular orbital
HPI	N-Hydroxyphthalimide
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear multiple quantum coherence
Hz	Hertz
IR	Infra-red
KDa	Kilo Dalton
LMS	laccase-mediator systems
LUMO	unoccupied molecular orbital
MCOs	multicopper oxidases
MD	molecular dynamics
MHz	Megahertz
MI	mass intensity
MTB	Mycobacterium tuberculosis
mV	millivolts
MW	Microwave
NMR	Nuclear magnetic resonance
PAHs	polycyclic aromatic hydrocarbons
PI4KIIIα	type III phosphatidylinositol 4-kinase alpha
RTIL	room temperature ionic liquids
SHE	Standard Hydrogen Electrode
TB	Tuberculosis
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
UV	Ultraviolet
VA	violuric acid
WHO	World Health Organization

Table of Contents

DECLARATION i

Abstract ii

acknowledgement iv

List of Abbreviations v

Chapter One 1

INTRODUCTION AND BACKGROUND 1

BIOCATALYSIS IN SYNTHETIC ORGANIC CHEMISTRY 2

- **SUSTAINABLE DEVELOPMENT** 2
- **GREEN CHEMISTRY** 2
- **BIOTECHNOLOGY** 5
- **BIOCATALYSTS IN ORGANIC SYNTHESIS** 7

LACCASE ENZYMES 10

- **INTRODUCTION** 10
- **OCCURRENCE IN NATURE** 10
- **FUNGAL LACCASES** 11
- **BACTERIAL LACCASES** 12
- **PLANTS AND INSECT LACCASES** 13
- **MOLECULAR STRUCTURE OF LACCASES** 14
- **MODE OF ACTION OF LACCASES** 16
- **LACCASE MEDIATOR SYSTEMS (LMS)** 16
- **LACCASES IN THE SYNTHESIS OF HETEROCYCLIC COMPOUNDS** 19
  - DIMERISATION AND TRIMERISATION (C—C BOND FORMATION) 19
  - NUCLEAR AMINATION 22
  - THIO-BOND FORMATION (C–S BOND FORMATION) 23

NITRILE HYDRATASE ENZYMES 25

- **INTRODUCTION** 25
- **STRUCTURAL PROPERTIES OF NHases** 26

- **CATALYTIC MECHANISMS OF NHases** 26
- **NHases IN ORGANIC SYNTHESIS** 28

LIPASE ENZYMES 29

- **INTRODUCTION** 29
- **NATURAL SOURCES OF LIPASES** 30
- **FUNGAL LIPASES** 30
- **BACTERIAL LIPASES** 31
- **LIPASE STRUCTURES** 31
- **APPLICATION OF LIPASES IN ORGANIC SYNTHESIS** 33
  - ENANTIO-SELECTIVE HYDROLYSIS 33
  - TRANS-ESTERIFICATION/AMINATIONS 34

TUBERCULOSIS (TB) 36

- **INTRODUCTION** 36
- **LIFE CYCLE OF TB** 36
- **DRUG-RESISTANT TB** 38

AIMS AND OBJECTIVES 40

CHAPTER TWO 42

SYNTHESIS OF PROPARGYL BENZIMIDAZOLE AND BENZOTHIAZOLE DERIVATIVES 42

BACKGROUND 43

- **BENZIMIDAZOLES** 43
- **BENZOTHIAZOLES** 45

RESULTS AND DISCUSSION 48

- **SYNTHESIS OF HYDROXY 2-ARYLBENZOTHIAZOLES** 48
- **SYNTHESIS OF PROPARGYL 2-ARYLBENZOTHIAZOLES** 51
- **SYNTHESIS OF HYDROXY 2-ARYL-*1H*-Benzimidazoles** 52
- **SYNTHESIS OF PROPARGYL 2-ARYL-*1H*-BENZIMIDAZOLES** 54

CONCLUSION 56

CHAPTER THREE 57

A BIOCATALYTIC SYNTHESIS OF NOVEL 2-ARYL-*1H*-BENZIMIDAZOLES AND 2-ARYLBENZOTHIAZOLES FUZED TRIAZOLES 57
BACKGROUND 58
- **TRIAZOLES** 58

RESULTS AND DISCUSSION 61
- **SYNTHESIS OF PHENYL AZIDES** 62
- **SYNTHESIS OF TRIAZOLES** 62
  - **ENZYME ACTIVITY STUDY** 63
  - **SOLVENT SCREENING** 64
  - **ENZYME SCREENING** 65
  - **SUBSTRATE SCREENING** 66
  - **DISTINGUISHING BETWEEN TRIAZOLE 20e AND 21e** 70
  - **NOVEL TRIAZOLE FUZED DERIVATIVES** 72

CONCLUSION 75
CHAPTER FOUR 76
A BIOCATALYTIC SYNTHESIS OF NOVEL QUINAZOLINONES 76
BACKGROUND 77
- **QUINAZOLINES** 77

RESULTS AND DISCUSSION 81
- **SYNTHESIS OF 2-AMINOBENZAMIDES** 82
- **SYNTHESIS OF QUINAZOLIN-4(*1H*)-ONE DERIVATIVES** 83
- **OXIDATION OF 2-PHENYLQUINAZOLIN-4(1*H*)-ONE** 85
- **SYNTHESIS OF 2,3-DIHYDROQUINAZOLIN-4(3*H*)-ONES** 86
- **SOLVENT SCREENING FOR THE DIRECT AMIDATION OF ESTERS (29d-f)** 89
- **SOLVENT SCREENING FOR THE HYDROLYSIS OF ESTER 29** 91
- **LIPASE SCREENING** 93

CONCLUSION 99
CHAPTER FIVE 100
CONCLUSION AND FUTURE WORK 100
CHAPTER SIX 102
EXPERIMENTAL SECTION 102
GENERAL 102

- **THE FOLLOWING METHODS WERE USED FOR THE PREPARATION OF 2-ARYLBENZOTHIAZOLES DERIVATIVES 4a–f** 103
  - METHOD A 103
  - METHOD B 103
  - METHOD C 103
    - Synthesis of 3-(5-chlorobenzo[*d*]thiazol-2-yl)phenol (4b) 104
    - Synthesis of 3-(5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)phenol (4c) 105
    - Synthesis of 2-(benzo[*d*]thiazol-2-yl)-4-bromophenol (4d) 105
    - Synthesis of 2-(5-chlorobenzo[*d*]thiazol-2-yl)-4-methoxyphenol (3e) 106
    - Synthesis of 4-(5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)phenol (3f) 106
- **PREPARATION OF PROPARGYL BENZOTHIAZOLE DERIVATIVES 6a–f** 107
    - Synthesis of 5-chloro-2-(3-(prop-2-yn-1-yloxy)phenyl)benzo[*d*]thiazole (6b) 107
    - Synthesis of 2-(3-(prop-2-yn-1-yloxy)phenyl)-5(trifluoromethyl) benzo[*d*] thiazole (6c) 108
    - 2-(4-(prop-2-yn-1-yloxy)phenyl)-5-(trifluoromethyl)benzo[*d*]thiazole (6f) 108
- **THE FOLLOWING METHODS WERE USED FOR THE PREPARATION OF 2-ARYL-1*H*-BENZIMIDAZOLES DERIVATIVES 5/8** 109
  - Method D. 109
  - Method E. 109
    - Synthesis of 3-(1-(3-hydroxybenzyl)-1*H*-benzo[*d*]imidazol-2-yl)phenol (8a) 109
    - Synthesis of 3-(6-Chloro-1*H*-benzo[*d*]imidazol-2-yl)phenol (5h) 110
- **PREPARATION OF PROPARGYL BENZIMIDAZOLE DERIVATATIVES 7/9** 111
    - Synthesis of 2-(3-(prop-2-yn-1-yloxy)phenyl)-1H-benzo[*d*]imidazole (7a) 111
    - Synthesis of 2-(4-(prop-2-yn-1-yloxy)phenyl)-1*H*-benzo[*d*]imidazole (7c) 111
- **PREPARATION OF PHENYL AZIDE 15a-f** 112
- **THE FOLLOWING METHODS WERE USED FOR THE PREPARATION OF 1,2,3-Triazoles Derivatives (20/21a-h)** 113
  - Method F: 113
  - Method G. 113
  - Method H. 113
    - Synthesis of 4-(Bromomethyl)-1-(4-nitrophenyl)-1*H*-1,2,3-triazole (20a) 114
    - Synthesis of 5-bromo-1-(4-nitrophenyl)-1H-1,2,3-triazole (21a) 114
    - Synthesis of 4-butyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazole (20b) 115
    - Synthesis of 4-(hex-5-yn-1-yl)-1-(4-nitrophenyl)-1*H*-1,2,3-triazole (20c) 115
    - Synthesis of 4-(3-Methoxyphenyl)-1-(4-nitrophenyl)-1*H*-1,2,3-triazole (20e) 116

- Synthesis of 5-(3-methoxyphenyl)-1-(4-nitrophenyl)-1H-1,2,3-triazole (21e)..... 116
- Synthesis of 4-(3-Fluorophenyl)-1-(4-nitrophenyl)-1*H*-1,2,3-triazole (20f) 117
- Synthesis of 5-(3-methoxyphenyl)-1-(4-nitrophenyl)-1H-1,2,3-triazole (21f) 118
- Synthesis of 3-((1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy) benzaldehyde (20g) 118
- Synthesis of 3-((1-(4-nitrophenyl)-1H-1,2,3-triazol-5-yl)methoxy) benzaldehyde (21g) 119
- Synthesis of 3-((1-(3,4-Dimethylphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy) benzaldehyde (20h)..... 120

PREPARATION OF NOVEL BENZIMIDAZOLE AND BENZOTHIAZOLE FUSED TRIAZOLE DERIVATIVES (23/24) 120

- Synthesis of 5-Chloro-2-(3-((1-(4-isopropylphenyl)-1*H*-1,2,3-triazol-4-yl) methoxy)phenyl) benzo[*d*]thiazole (23b) 120
- Synthesis of 5-chloro-2-(3-((1-(4-isopropylphenyl)-1*H*-1,2,3-triazol-5-yl)methoxy)phenyl) benzo[*d*]thiazole (24b) 121
- Synthesis of 2-(3-((1-Phenyl-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)benzo[*d*] thiazole (23c) 122
- Synthesis of 2-(3-((1-(2-bromo-4-chlorophenyl)-1*H*-1,2,3-triazol-5-yl)methoxy)phenyl)-5-(trifluoromethyl)benzo[*d*]thiazole (24c)..... 123
- Synthesis of 2-(3-((3-(4-Chlorophenyl)-3*H*-pyrazol-5-yl)methoxy)phenyl)-1*H*-benzo[*d*]imidazole (23f)..... 123
- Synthesis of 5-Chloro-2-(3-((1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[*d*]imidazole (23g)..... 124
- Synthesis of 5-Chloro-2-(3-((1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[*d*]imidazole (24g)..... 125
- Synthesis of 5- Bromo-2-(3-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[*d*]imidazole (23h) 125
- Synthesis of 5-Bromo-2-(3-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[*d*]imidazole (23J) 126
- Synthesis of 2-(4-((1-Phenyl-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-*1H*-indole (23k) 127
- Synthesis of 1-(4-((1-Phenyl-1*H*-1,2,3-triazol-4-yl)methoxy)benzyl)-2-(4-((1-phenyl-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-1*H*-benzo[*d*]imidazole (23l)..... 128
- Synthesis of 2-(3-((1-(4-Nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-(trifluoromethyl) benzo[*d*]thiazole (23n)..... 129
- Synthesis of 2-(3-((1-(4-nitrophenyl)-1H-1,2,3-triazol-5-yl)methoxy)phenyl)-5-(trifluoromethyl)benzo[d]thiazole (24n)..... 130
- Synthesis of 2-(3-((1-phenyl-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-(trifluoromethyl) benzo[*d*]thiazole (23o)..... 130
- Synthesis of 2-(3-((1-(2-Bromo-4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-1*H*-benzo[*d*]imidazole (23r)..... 131

- Synthesis of 2-(4-((1-(2,4-Dimethylphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-1*H*-benzo[*d*]imidazole (23s) 132
- Synthesis of 1-(4-((1-(2,4-Dimethylphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzyl)-2-(4-((1-(2,4-dimethylphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-1*H*-benzo[*d*] imidazole (23t) 133

GENERAL PROCEDURE FOR THE PREPARATION OF 2-AMINOBENZALDEHYDE DERIVES 26a-b 134

- 2-aminobenzamide (26a) 134
- 2-amino-5-nitrobenzamide (26b) 134

GENERAL PROCEDURE FOR THE SYNTHESIS OF ETHER BENZALDEHYDES 27(d-f) 135

- Ethyl 4-(4-formylphenoxy)butanoate (27d) 135
- Ethyl 4-(3-formylphenoxy)butanoate (27e) 136
- Ethyl 4-(4-formyl-2-methoxyphenoxy)butanoate (26f) 136

GENERAL PROCEDURE FOR THE PREPARATION OF 2-SUBSTITUTED QUINAZOLINONE DERIVATIVES 29 137

- 2-phenylquinazolin-4(3*H*)-one (29a) 137
- 2-(3,4-dimethoxyphenyl)quinazolin-4(3*H*)-one (29b) 138
- 2-(3,4,5-trimethoxyphenyl)quinazolin-4(*3H*)-one (29c) 138
- Ethyl 4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanoate (29d) 139
- Ethyl 4-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanoate (29e) 139
- Ethyl 4-(2-methoxy-4-(6-nitro-4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy) butanoate (29f) 140

GENERAL PROCEDURE FOR THE PREPARATION OF 2-SUBSTITUTED QUINAZOLINONE ACIDS 30 141

- Method I. 141
- Method J. 141
- Method K 141
  - 4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanoic acid (30d) 141
  - 4-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanoic acid (30e) 142
  - 4-(2-methoxy-4-(6-nitro-4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy) butanoic acid (30f) 143

GENERAL PROCEDURE FOR THE PREPARATION OF 2-SUBSTITUTED QUINAZOLINONE AMIDES 32 144

- *N*-benzyl-4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanamide (32a) 144
- *N*-(4-methoxybenzyl)-4-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy) butanamide (32b) 145
- 4-(2-methoxy-4-(6-nitro-4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)-*N*-(4-methoxybenzyl)butanamide (32c) 145

- *N*-benzyl-4-(2-methoxy-4-(6-nitro-4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy) butanamide (32d) 146
- *N*-isopropyl-4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanamide (32f) 146
- *N*-benzyl-4-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanamide (32g) 147
- *N*-isobutyl-4-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanamide (32h) 147
- *N*-*i*sobutyl-4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanamide (32j)..... 148
- *N*-(4-methoxyphenethyl)-4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy) butanamide (32l)..... 148
- *N*-isopropyl-4-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanamide (32n) 149
- *N*-(4-methoxybenzyl)-4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy) butanamide (32o)..... 149
- *N*-(2-ethylhexyl)-4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy) butanamide (32p) 150
- *N*-(3,4-dimethoxyphenethyl)-4-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy) butanamide (32q) 150
- *N*-(3,4-dimethoxyphenethyl)-4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy) butanamide (32r) 151
- *N*-(4-chlorophenethyl)-4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy) butanamide (32s) 151
- *N*-(4-chlorophenethyl)-4-(2-methoxy-4-(6-nitro-4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanamide (32t) 152

REFERENCES 153

APPENDIX: NMR SPECTRA 1

Chapter One

Introduction and Background

SUSTAINABLE DEVELOPMENT

As a result of ever-expanding human endeavours, the world is now faced with unanticipated negative environmental and health impacts. This comes because of human-induced carbon emissions, extensive manufacturing, use, and release of chemicals in excessive amounts. As a result, global environmental and health concerns were raised and ultimately brought about the definition of sustainable development in the 1980s.[1]

The idea of sustainability remains one of the key issues to address. Although it is a widely used phrase; sustainable development has many different meanings and as a consequence, it arouses a lot of various responses.[2] However, the generally used meaning defines sustainability as: "Humanity has the ability to make development sustainable—to ensure that it meets the needs of the present without compromising the ability of future generations to meet their own needs." When applied to the field of chemical production and synthesis, sustainability has brought forth the concept of Green Chemistry.[2,3]

GREEN CHEMISTRY

The term 'Green Chemistry' was first introduced by Anastas of the US Environmental Protection Agency (EPA) to address environmental issues associated with the production and characteristics of chemical products.[4,5] A brief definition of green chemistry is "The design of chemical products and processes that are more environmentally benign and reduce negative impacts to human health and the environment".[6] The leading goal of green chemistry is to attain sustainable industrial and societal development through the design of environmentally friendly products and processes.[7] Anastas and Warner (1998) proposed the *12 Principles of Green Chemistry* as guidelines on how chemical processes ought to be implemented[8]:

THE 12 PRINCIPLES OF GREEN CHEMISTRY[8]

1. **Prevention**. Waste prevention is always better than cleaning up waste after it is produced.
2. **Atom Economy**. Utilize systems that maximise the incorporation of all reactants in the final product.
3. **The Lesser amount of hazardous/toxic chemicals**. Avoid or eliminate the use of harmful substances or toxic waste generation by using synthetic methodologies that pose little or no toxicity to human health and the environment.
4. **Safer chemical products by design**. Develop products that can accomplish their intended purpose while remaining benign and harmless to the environment.
5. **Safer solvents and auxiliary substances**. Where probable, substitute harmful non-renewable solvents with eco-friendly and bio-renewable ones.
6. **Design for energy efficiency**. Design processes that aim to minimize the environmental and economic impacts.
7. **Use of Renewable Feedstocks**. Employ renewable raw materials or feedstocks instead of materials that cannot be replenished.
8. **Reduce Derivatives.** Avoid processes that make use of derivatization (use of blocking or protecting/deprotecting groups).
9. **Catalysis.** Selective catalytic reagents ought to be utilized instead of stoichiometric reagents.
10. **Design for Degradation.** Chemical products should be designed to undergo biodegradation at the end of their function and not persist in the environment.
11. **Real-time analysis for Pollution Prevention.** Analytical methodologies need to be developed to monitor and control chemical processes to prevent pollution.
12. **Inherently safer processes**. Substances and the form of substances used in chemical processes should be carefully chosen to minimize the potential for chemical accidents, environmental contamination because of spillage, or explosions and fires.

Green chemistry eliminates waste at the source, it primarily focuses on pollution prevention strategies rather than waste remediation (first of the twelve principles).[4] In the past decade, there has been a great shift towards replacing non-renewable fossil feedstocks – crude oil, coal, and natural gas – with renewable biobased sustainable raw materials for the manufacture of both chemical and energy products (7th principle of green chemistry).[4,5] The switch to renewable biobased resources will afford a favourable decrease in carbon emissions and environmental pollution levels.[5]

New strategies are often being sought out to address the underlying issues associated with waste generation and the use of hazardous reagents and solvents.[5] Atom economy (mol wt of product/sum of mol wts of starting materials) and the E factor (kgs waste/kg product) are two underlying concepts of green chemistry that are used to measure the overall environmental suitability of a chemical process.[9] Other proposed green chemistry metrics for verifying the efficiency or environmental performance of chemical processes include the effective mass yield (EMY). Which is defined as the percentage of the mass of the desired product relative to the mass of materials used during the preparation process.[10] A fourth metric is mass intensity (MI), introduced by Constable *et al.* (2002), defined as the total mass of materials used to produce a product divided by the mass of the product.[11]

The E factor is granted the upper hand when compared to the alternative green chemistry metrics.[4] It directly identifies the amount of waste produced taking into account the product yield.[4,5] The ideal E factor is zero, meaning when describing how wasteful a process is, the ideal goal is to achieve zero amounts of waste.[4] It also reveals the relative quantity of waste that is produced by various parts of a process including reagents, solvents losses, all process aids, and fuels.[5,11] This metric could be used by various industry segments including petrochemicals, fine chemicals, and pharmaceuticals.[11] **Table 1**, published in 1992, summarizes a range of E factors for the various segments of the chemical industry.

Table 1: Factors in the Chemical Industry.

INDUSTRY SECTOR	TONNAGE	E FACTOR (KG WASTE/KG PRODUCT)
Oil refining	10^6–10^8	<0.1
Bulk chemicals	10^4–10^6	<1–5
Fine chemicals	10^2–10^4	5 to >50
Pharmaceuticals	10–10^3	25 to >100

The publication of **Table 1** has presented an incentive to the chemical industry, specifically the fine chemical and pharmaceutical sectors, to reduce the amount of waste they produce during production.[9] Despite that, it may be challenging for the Pharmaceutical sector to consistently adhere to this metric in its operations. Particularly due to lack of clarity surrounding how 'total waste' is ultimately classified and accounted for.[11]

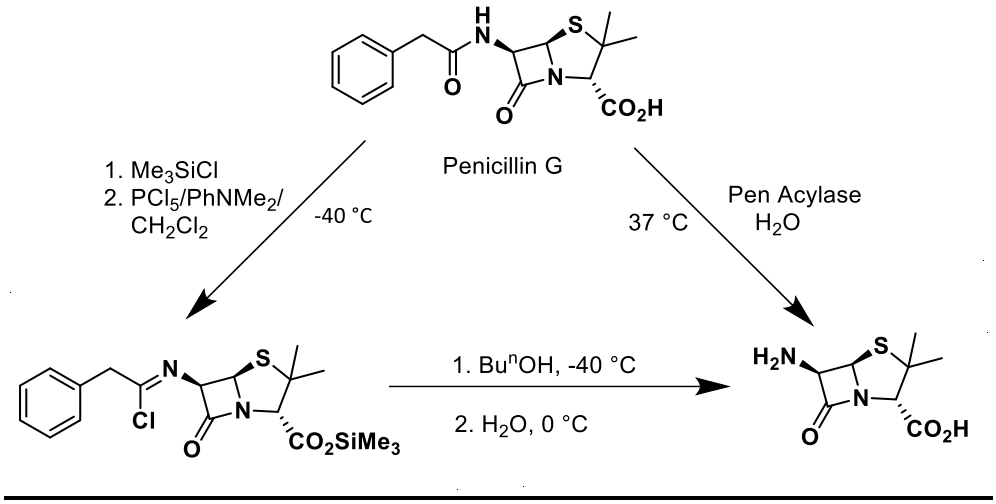
One of the important principles of green chemistry is the concept of using renewable feedstocks and catalysts in chemical production. Therefore, the need to direct chemical synthesis towards renewables such as biocatalysts to mediate synthetically challenging transformations with excellent levels of chemo-, regio- and stereo-selectivity is very important.[2,3]

BIOTECHNOLOGY

Biotechnology has progressed considerably in recent years and this is mainly due to new advancements in genomic engineering practices.[12] It is defined as the application of biotechnology-based tools such as micro-organisms and enzymes to manufacture bio-based and sustainable products such as fuel, chemicals, and plastics from renewable resources.[13] Biocatalytic processes can be conducted in organic solvents, aqueous solutions, or in biphasic-systems. This enables enzymes and catalytically active cells to selectively and efficiently catalyse reactions for both polar and non-polar organic compounds.[14]

The use of industrial biotechnology in synthetic chemistry is on the edge of substantial progress.[14] There were only a few applications of biocatalysis in various industrial

applications such as pharmaceuticals, fragrances and flavours, and other fine chemicals up until the mid-1980s. However, during the late 1980s, biocatalysis in fine chemicals was greatly influenced by two developments of societal and industrial importance. First, to serve for the development of catalysts systems that evaded the use of toxic and hazardous reagents and solvents.[15] A leading example of this is the industrial synthesis of the key intermediate in penicillins; 6-aminopenicillanic acid (6-APA) (**Scheme 1**).[15,16]



Scheme 1: Enzymatic versus chemical synthesis of 6-APA.[15]

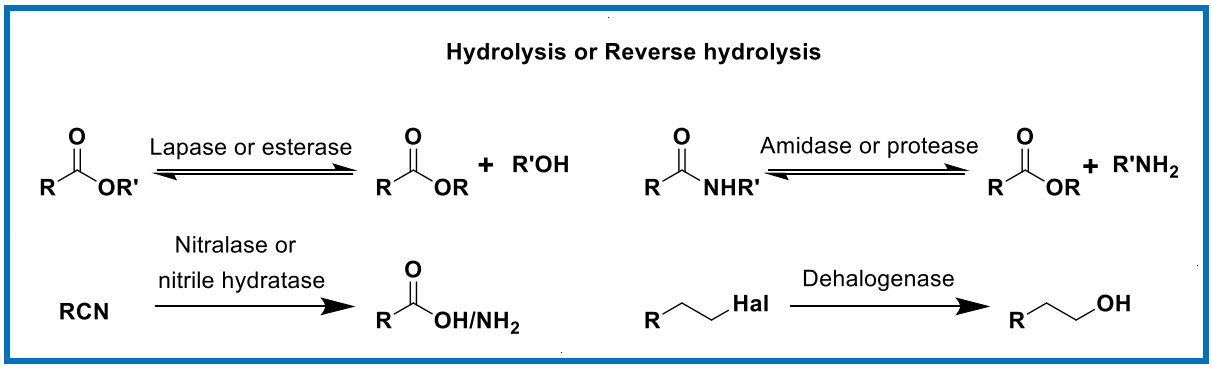
The second development was the realization of the importance of stereoisomers in a biological system. For instance, health and human services agencies like the FDA introduced legislation that binds pharmaceutical companies to market chiral drugs as the single, active enantiomer. This generated a pressing need for the development of green and economical methods for the synthesis of pure enantiomers. This gap may perhaps be occupied by the use of inorganic complexes (utilizing chiral ligands) or by biocatalytic methods as enzymes are typically known to be very enantioselective.[15]

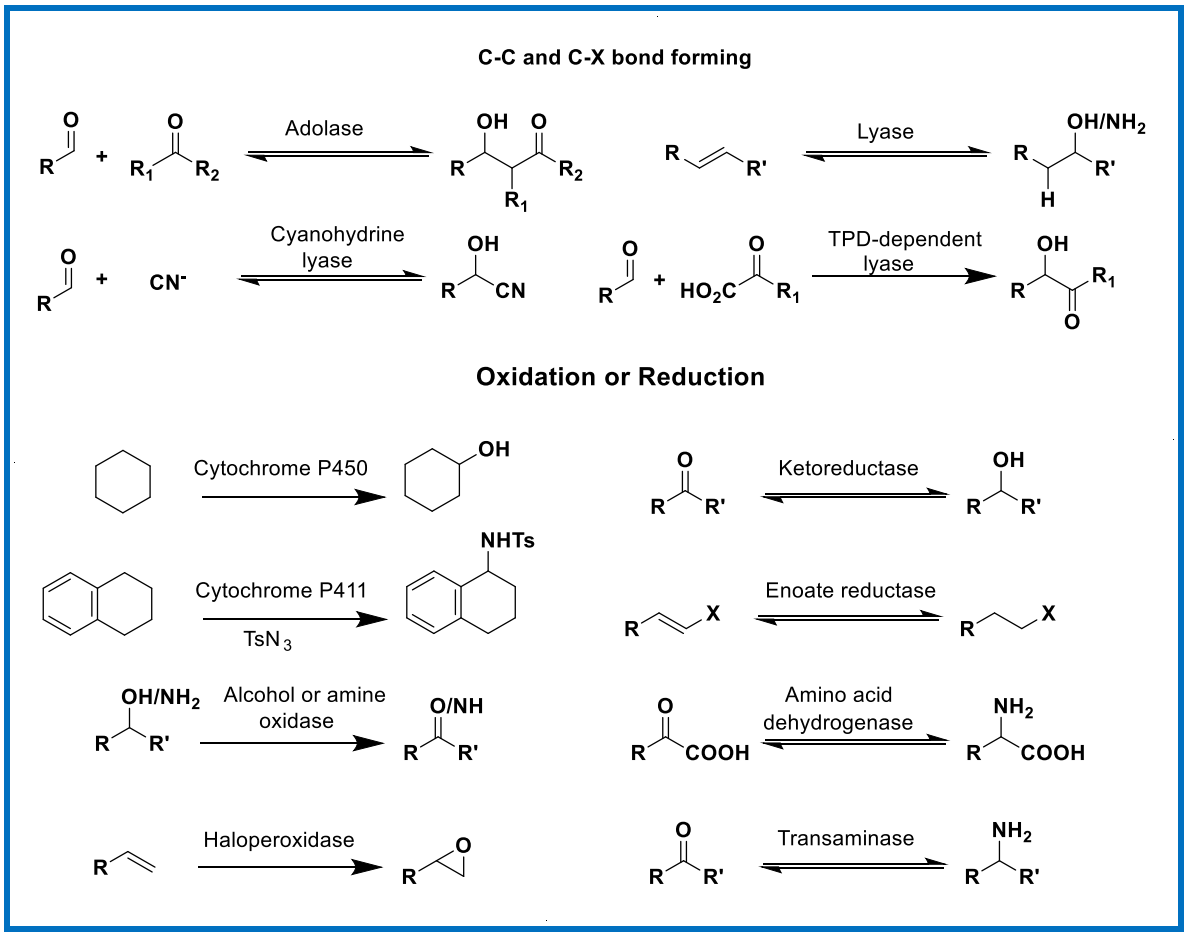
The application of biocatalysis in industrial synthesis was revolutionized when Zaks and Klibanov demonstrated the lipase catalysed transesterification reaction between tributyrin and various alcohols in an organic medium at elevated temperatures.[15,17] The discovery of functional enzymes in organic solvents opened a broader scope in organic synthesis than formerly accredited that enzymes work well only in aqueous media. With these new advancements in mutagenesis and directed evolution techniques, researchers can produce enzymes with improved activity, substrate specificity, chemo-, regio- and enantio- selectivity.[18]

BIOCATALYSTS IN ORGANIC SYNTHESIS

Biocatalysis is increasingly becoming the method of choice for the synthesis of novel small molecules, complex natural products, and also key intermediates for the pharmaceutical and chemical industry.[19,20] The use of biocatalysts in organic synthesis, whether it is isolated enzymes or whole cells offers clear economic and environmental benefits.[21] When compared to classical conversional methods, biocatalysis can present several significant advantages.[19,21] Biocatalysis obeys ten of the twelve principles of green chemistry; atom economy, improved environmental impact, cost-effective, enzymes are basically non-hazardous, renewable, and biodegradable, they reduced solvent usage and operate at near-ambient reaction temperatures.[21]

Up to now, the use of biocatalysts in synthetic organic chemistry has been in moderation, predominantly in the enantio-selective synthesis of chiral molecules.[19,21] However, the emergence of the field of protein engineering and directed evolution; allowed for the development of new improved biocatalysts with tailor-designed properties and capable of performing multistep synthetic processes.[20,22] **Scheme 2** demonstrates examples of a diverse range of commercially available enzymes being utilized as efficient alternatives towards obtaining important target structures. Over the years, the synthesis of valuable organic compounds has taken a considerable step in the forward direction. This is achieved through important functional group interconversions such as hydrolysis, oxidation-reduction, C—C, and C—X bond formation while using enzymes and microorganisms as catalysts in the synthetic approaches.[19,20]





Scheme 2: Commercially available enzymes as alternative catalysts.

These biocatalysts are remarkably efficient at catalysing important transformations in organic synthesis and in that way, they present an attractive substitute to their chemical counterparts.[19] It is important to develop synthetic routes in which different biocatalysts can be combined to create complex organic molecules. In this thesis, we focus on the development of effective and versatile biocatalytic methodologies for the synthesis of important organic heterocycles. We highlight our advances in performing hydration of nitriles with nitrile hydratases, performing effective oxidative ring closing reactions of five and six-membered aromatic rings using green oxidants such as laccase, and also conducting lipase catalysed hydrolysis or (direct) amide bond formation reactions.

This work is aimed at reducing the environmental impact of the synthesis of pharmaceuticals, which is per mass the most polluting type of synthesis. This method development may lead South Africa towards a Global Bio-economy Strategy. As successful green methods are typically more cost-effective (by reducing waste) this

would enhance the production opportunities in South Africa and help aid in addressing substantial socio-economic imbalances that still plague the country. This work is aligned with the Department of Science and Innovation (DST) Bioeconomy strategy; the Department of Trade and Industry (dti) and DST play a coordination role and specializes in the development of Green and Sustainable Chemistry.

The University of the Witwatersrand (Wits) has a long history of research and progress in the area of biocatalysis (both enzymes and microbes). The Wits Molecular Sciences Institute (MSI) houses the DS/NRF Biocatalysis Initiative, facilitating the integration of biocatalysis into synthetic organic chemistry. The research spans over a broad range of interdisciplinary research in organic synthesis utilizing biocatalysts such as nitrile hydratase, nitrilase, lipases, esterases and proteases, transaminase, laccases, aldolase, ketoreductase, and lipoxygenase. for chemo-, regio-, and stereoselective bond-forming and -breaking reactions.

LACCASE ENZYMES

INTRODUCTION

Laccases (benzenediol: oxygen oxidoreductases, EC1.10.3.2) are multi-copper polyphenol oxidases that are also referred to as blue copper oxidases or large blue copper proteins.[23,24] Laccases are categorised in the family multicopper oxidases (MCOs), along with ascorbate oxidase, bilirubin oxidase, peroxidases, and ceruloplasmin.[25,26] MCOs catalyse oxidation reactions of several substituted organic molecules (particularly phenolic compounds) with the concurrent four-electron reduction of O_2 into water.[24,25] The substrates catalysed by laccases consist of a selection of organic and inorganic compounds such as phenols, anilines, polyamines, polyphenols, lignin derivatives, and many others; and this occurs via a one-electron oxidation process.[24,27]

Over the past few decades, there have been considerable advancements in the purification and characterization of laccases. Several new laccases have been evolved or are known from genome sequencing. This thesis aims at assessing potential applications, substrate specificity, advantages, limitations, and future of laccases. Particular attention is devoted to evaluating their role and efficiency in organic synthesis.

OCCURRENCE IN NATURE

Laccases, like other oxidoreductase enzymes, are abundant and postulated to play various natural functions.[23,28] They carry out a range of cellular or microbial processes such as biosynthesis of the cell wall in plants, pigment production of bacterial or fungal spore, and degradation of complex natural polymers such as lignin.[24,28,29] Because of their ability to both polymerize and depolymerize natural occurring polymeric substances, laccases are involved in both lignification and delignification processes.[28,30]

In 1883 Yoshida first discovered laccases in latex produced from the Japanese lacquer tree (*Rhus vernicifera*).[31,32] Fungal laccases are commonly described in the literature, with over 100 laccases having been purified and characterized from fungal cultures of

basidiomycetes and ascomycetes and the amount is still rising.[28] Laccases are ubiquitous, they are found in all domains of life. They have been detected amongst various phylogenetic classes, such as plants, insects and bacteria.[24] There is also evidence for their existence in lichens[33]; oysters[34] and sponges[35].

FUNGAL LACCASES

It has been reported that laccases primarily originate from fungi.[23,36] The presence of laccases has been demonstrated in more than 60 fungal strains, belonging to several classes such as *Deuteromycetes, Ascomycetes* and *Basidiomycetes*.[37,38] Among fungal species, ascomycetes, deuteromycetes and basidiomycetes fungi produce various laccase variants, where the white-rot fungi *Trametes Versicolor*, *T. hirsute* (*C. hirsutus*), *T. ochracea*, *T. villosa*, *T. gallica*, *Cerrena maxima*, *Coriolopsis polyzona*, *Lentinus tigrinus*, and *Pleurotus eryngii* are the commonly demonstrated fungal laccases in literature.[24,27,32,38]

The great abundance of white-rot basidiomycete fungi and their unique oxidative potency makes them attractive for various biotechnological applications.[28,38] Most purified laccases occur as extracellular glycoproteins, however, some strains of white-rot fungi have been demonstrated to contain intracellular laccases.[39] Generally, fungal laccases exist as monomeric proteins, however, others also exhibit homodimeric, heterodimeric, and multimeric structural forms. Depending on both environmental conditions and enzyme origin, fungal laccases are produced in multiple isoforms.[28]

Fungal laccases present a wide range of substrate specificity and carry out a variety of natural processes such as sporulation, melanin synthesis, fruiting body formation, stress defence, mutagenic processes, and degrading lignin molecules.[23,38,39] Fungal laccases have received significant attention in biotechnology because of their high redox potential—they are capable of oxidizing substrates with an oxidative potential above 400 mV.[39] Fungal laccases have been applied in bioremediation processes to degrade polycyclic aromatic hydrocarbons (PAHs)[40], decolourisation of dyes[41], detoxification of phenolic compounds[42], biodegradation of plastics[43] and pharmaceutical substances.[44]

BACTERIAL LACCASES

Laccase activity is not commonly observed in bacteria, only limited bacterial laccases have been reported in the literature.[45] However, there is developing evidence in genome analysis suggestive of a widespread existence of bacterial laccases in soil and the rhizosphere of a range of grasses and cereals.[38,45] Laccase activity in bacteria was first discovered in *Azospirillum lipoferum*, from a rice rhizosphere extraction in 1993.[39] Several laccases were then gradually detected in bacteria from various species, such as *Bacillus subtilis*, *Klebsiella*, *Marinomonas mediterranea*, *Proteobacterium*, *Streptomyces griseus*, and *Escherichia coli*.[24,27] Furthermore, bacterial laccases have also been identified in microorganisms of the Archaea domain like *Haloferax volcanii*.[39,46]

The most well-known of the bacterial laccases is the endospore coat protein CotA from *Bacillus subtilis*.[39,47] The spore coat protein cotA was the first prokaryotic laccase for which the three-dimensional structure was elucidated, with an overall fold consisting of three cupredoxin domains (**Figure 1**).[28,39,47] Tertiary structures of a limited number of other bacterial laccases with two-domain structures have been found in groups known as small laccases i.e. *Streptomyces, Amycolatopsis,* and *Nitrosomonas* (**Figure 1**).[39] Their role in bacteria includes processes such as copper homeostasis in *Escherichia coli*[48], cell pigmentation[49], and protection against hydrogen peroxide and UV light.[50]

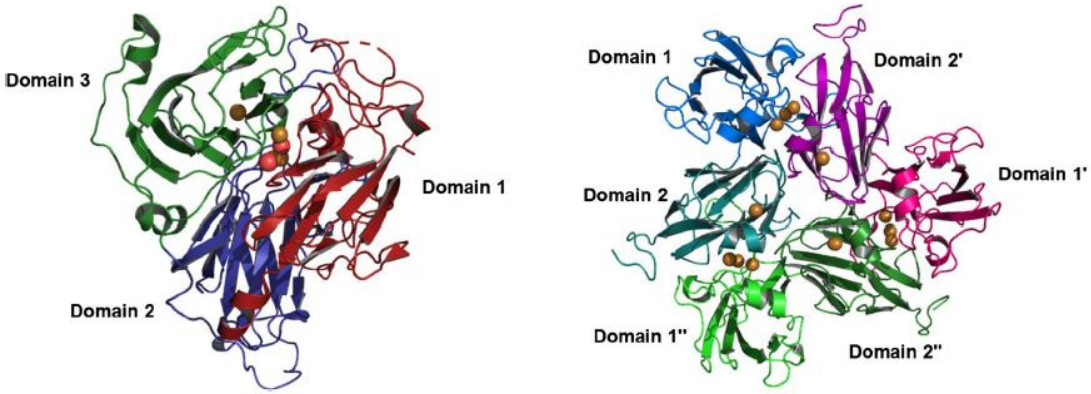


Figure 1: Three-domain laccase from Bacillus subtilis (PDB 1GSK) and the two-domain laccase from Streptomyces coelicolor (PDB 3CG8).[39]

Bacterial laccases have been found to exhibit unique biochemical properties; they are relatively stable under different conditions such as high temperatures, pH, organic solvents, and metal ion conditions.[39,51] Compared with fungal laccases, bacterial laccases

are generally more vigorous and stable enzymes. However, their application has been limited by their low redox potential (<400 mV).[39] Even so, bacterial laccases are still considered as promising biological tools for many industrial applications.[51] Applications of bacterial laccases include degradation of synthetic dyes[52], degradation of pharmaceutical pollutants[53,54], bio-sensors[55], and in the pulp & paper industry.[56]

PLANTS AND INSECT LACCASES

Since their discovery from the Japanese lacquer tree (*Rhus vernicifera*), there have been a limited number of studies on plant laccases.[39] The typical laccases found in plants have been identified in apples, potatoes, trees, cabbages, mango, lacquer, prune, sycamore, pine, peach, beets, turnips and many other plant species.[27] Plant laccase families are even greater in number than fungal laccase families—about 22 laccase genes have been identified in rice (*Oryza sativa*)[53,57], 17 in the *Arabidopsis* laccase family.[58] Additionally, 84, 44, and 46 laccase gene family were identified in cotton from cultivated *allotetraploid* genes; *Gossypium hirsutum* and its two predecessors *diploids G. arboreum* and *G. raimondii*.[53,59]

Similar to bacterial laccases, plant laccases possess a low redox potential (0.41 V for *R. vernicifera*), however, their molecular design and reaction mechanisms are similar to those of fungal laccases.[39] Plant laccases have been defined and associated with various processes such as participating in lignin synthesis, pigmentation and also pigment breakdown, responses to external stresses and root elongation.[24,39,53]

There are only a few reports of the use of plant laccases in bioremediation processes. Wang *et al*. (2004) described a phytoremediation process using secretory laccase (LAC1), expressed in *Gossypium arboretum* roots to catalyse the oxidation of toxic phenolic compounds.[39,60] Laccases from certain plants have been successfully confirmed to exhibit activity on the degradation of dye substances using suspension cells and purified laccases.[39]

The least purified and characterized of all identified laccases are the insect laccases.[53] Recently proteins with characteristic laccase features and properties have been categorized in insects of the classes that include *Calipora*, *Lucilia*, *Manduca*, *Papilio*, *Schistocerca*, *Bombyx* and *Rhodnius*.[27,31] insect laccases have been identified to play a

significant role in the insect's physiology such as cuticle sclerotization and melanization, wound healing and the development of the immune system.[39,53]

MOLECULAR STRUCTURE OF LACCASES

Laccases are extra- or intracellular glycosylated proteins that are often found as isozymes that oligomerize to form multimeric (monomeric, dimeric or tetrameric) protein complexes.[23,24,32] The average molecular weight of laccases varies with different sources—in general, the average molecular weight observed for glycosylated protein monomer ranges from 50-130 KDa.[23,24] The covalently linked carbohydrate moiety in laccases usually comprises of *N*-acetylglucosamine, mannose, and galactose and can account for 10–45% of the protein molecule by weight.[32,36,61] It is believed the carbohydrate moiety serves an important role in the molecular structure of laccases as it provides the conformational stability of the enzyme.[32] In addition, it helps prevent enzyme inactivation by free radicals and protects it from proteolysis.[27,32]

As MCOs, the active site of laccase enzymes usually consists of four copper atoms in specific oxidation states.[39] The copper atoms are classified into three redox sites (T1, T2 & T3): one copper of the first type (T1), one copper of the second type (T2) and two coppers of the third type (T3). The T2 and T3 coppers together make up what is known as the tri-nuclear cluster (**Figure 2**).[32,39]

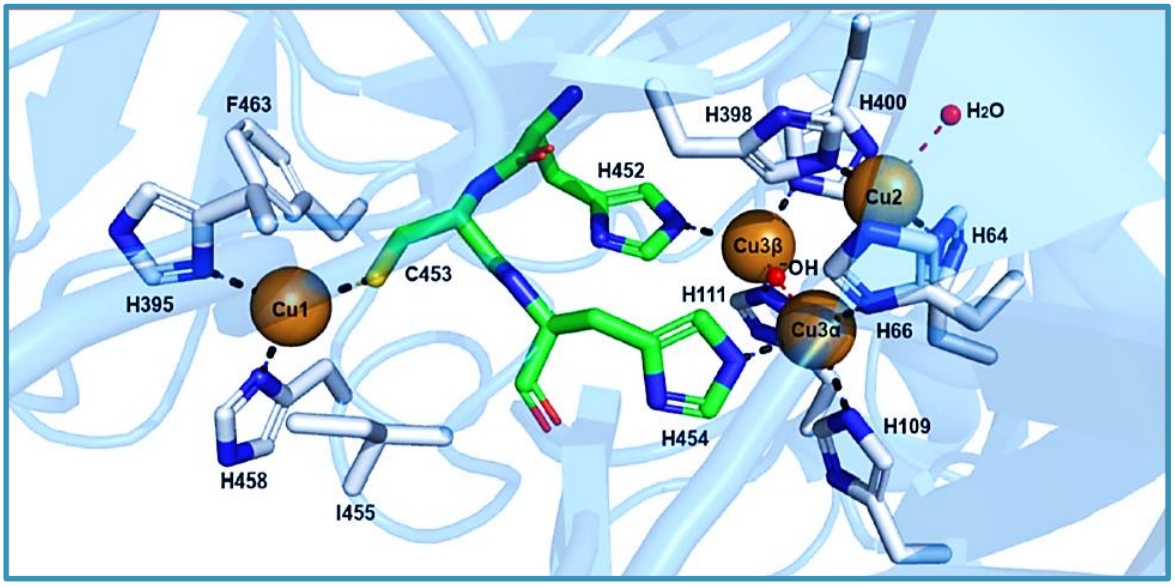


Figure 2: Laccase active site from *Trametes versicolor* (PDB1KYA). The amino acids of the histidine-cysteine pathway are in green.[39]

The copper atoms are classified based on the metal ion's surroundings and the characteristic electronic absorption properties.[39] The type 1 copper atom (T1) (paramagnetic) provides the enzyme with its characteristic blue colour; exhibits a charge-transfer band at 600 nm.[27,62] As shown in **Fig. 2** above, the T1 copper forms a trigonal coordination with a single cysteine and two histidine equatorial ligands.[24,39] The axial ligand of T1 varies with laccase origin; methionine is commonly found in bacteria, while leucine or phenylalanine occurs in fungal laccases.[39] There are claims that the oxidation potential of laccases is influenced by the ligand at the axial position, perhaps even regulating enzyme activity. Kumar *et al*. (2003), demonstrated how amino acid residues mutation from phenylalanine to methionine extensively lowered the reduction potential of a fungal laccase from *Trametes villosa*.[26,63]

The type 1 copper site has been identified to be the site with a high redox potential of about +740 mV, therefore it is the site where substrate oxidation takes place.[32,63] Copper of the second type (T2) (paramagnetic), does not display any blue colour; shows weak absorption in the visible region and paramagnetic properties in EPR studies.[24,32,63] Copper of the third type (T3) is EPR undetectable; diamagnetic spin-coupled copper-copper pair (absorbs at 330 nm).[24,63] The binuclear copper pair exhibits anti-ferromagnetism as a result of the bridging hydroxyl ligand coupling them, therefore making them diamagnetic and EPR undetectable.[24]

Together the type two (T2) and type three (T3) copper atoms form a tri-nuclear cluster, the site where molecular oxygen binds and a four-electron reduction takes place, releasing water.[27,39] The cluster is located approximately 12 Å away from the T1 site, and the T2 and T3 copper atoms are in close proximity, positioned approximately 4 Å apart.[24,64] The T2 copper atom is coordinated by histidine side chains and each of the T3 copper atoms is coordinated to three histidine ligands (a total of 6 histidines at the T3 site).[39]

Some variants of laccases do not possess the T1 copper (no "blue" maximum (600 nm) is seen in the absorption spectrum) and are commonly referred to as the yellow laccases.[28,65,66] Several laccases contain a range of metals other than copper; laccase from *Phellinus ribis* lacks the T1 copper atom and contains one manganese atom. Whereas laccase from *Polyporus ostreatus* consists of only one copper, one iron, and two zinc atoms.[28]

MODE OF ACTION OF LACCASES

The mechanism of action of laccases takes place through a one-electron oxidation of a substrate (phenolic compounds), coupled with a four-electron reduction of molecular oxygen to water.[28,32] As a result of this oxidation an oxygen-centered free radical is generated, which is typically unstable and susceptible to a second enzymatic catalysis reaction into a quinone molecule.[29,32] The catalytic cycle of laccase has been identified to follow three main steps:[24,37]

a) Copper of first type (T1) is reduced by accepting electrons from the substrate,
b) Electron transfer from T1 copper site to the tri-nuclear site (T2&T3), and
c) Reduction of molecular oxygen to water by the tri-nuclear centre (T2&T3).

The T1 copper coordinates with substrates through the imidazole ring from one of its Histidine ligands and this is where outer-sphere electron transfer (ET) from the substrate to the tri-nuclear cluster occurs through a non-covalent binding process.[24,67] When the fully reduced laccase (T2&T3 site) interacts with molecular oxygen two intermediates of the enzyme are produced; the two well-known forms are recognized as the peroxide intermediate and native intermediate.[68] The peroxide intermediate is formed through a 2e- process from the interaction of molecular oxygen with the reduced tri-nuclear cluster. A native intermediate is formed when the peroxide intermediate undergoes a second 2e- process and decomposes into the native intermediate. The latter is reported to carry out an important function in the catalytic cycle of the enzyme by acting as oxygen radical during the reaction with molecular oxygen.[24,68]

LACCASE MEDIATOR SYSTEMS (LMS)

Laccases possess an extensive scope of substrate specificity, and their relatively high redox potentials (500 to 800 mV vs. Standard Hydrogen Electrode (SHE)) makes them effective and versatile enzymes.[67,69] However, when the substrate possesses a greater redox potential compared to the enzyme or it is too large to fit into the active site of the enzyme (due to steric hindrance), the oxidation capabilities of the laccase are restricted.[67] Nonetheless, when the enzyme is applied in the presence of small redox

mediator molecules in what is called laccase-mediator systems (LMS), one can overcome this problem and boost the oxidation capacity of the enzyme.[69] The thought behind using mediator systems was to utilise low molecular weight compounds (**Figure 3**); that upon reaction become reactive oxidized intermediates, which then diffuse out of the enzymatic pocket allowing the enzyme to oxidize substrates that are bulky and have high redox potentials.[27]

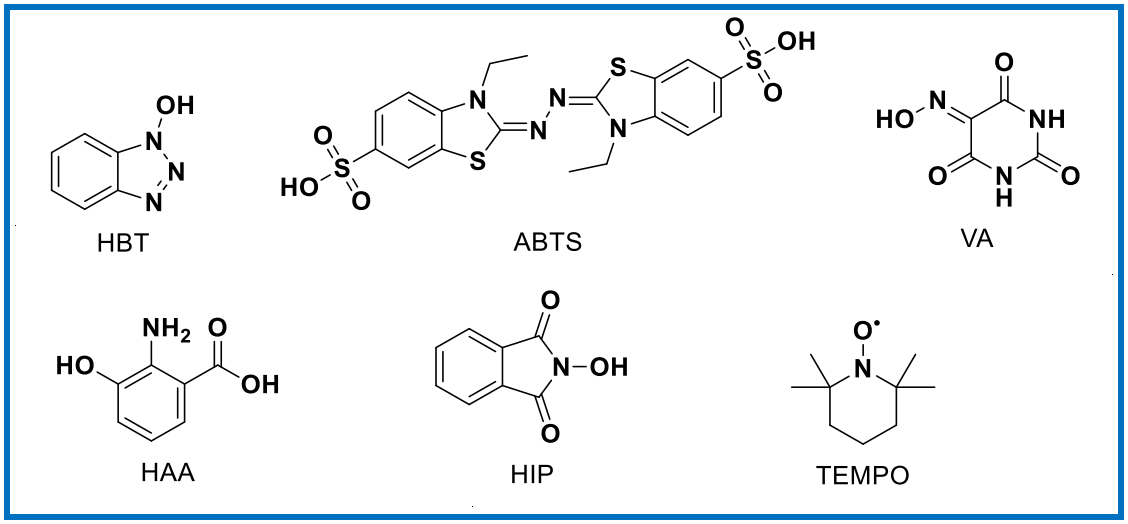
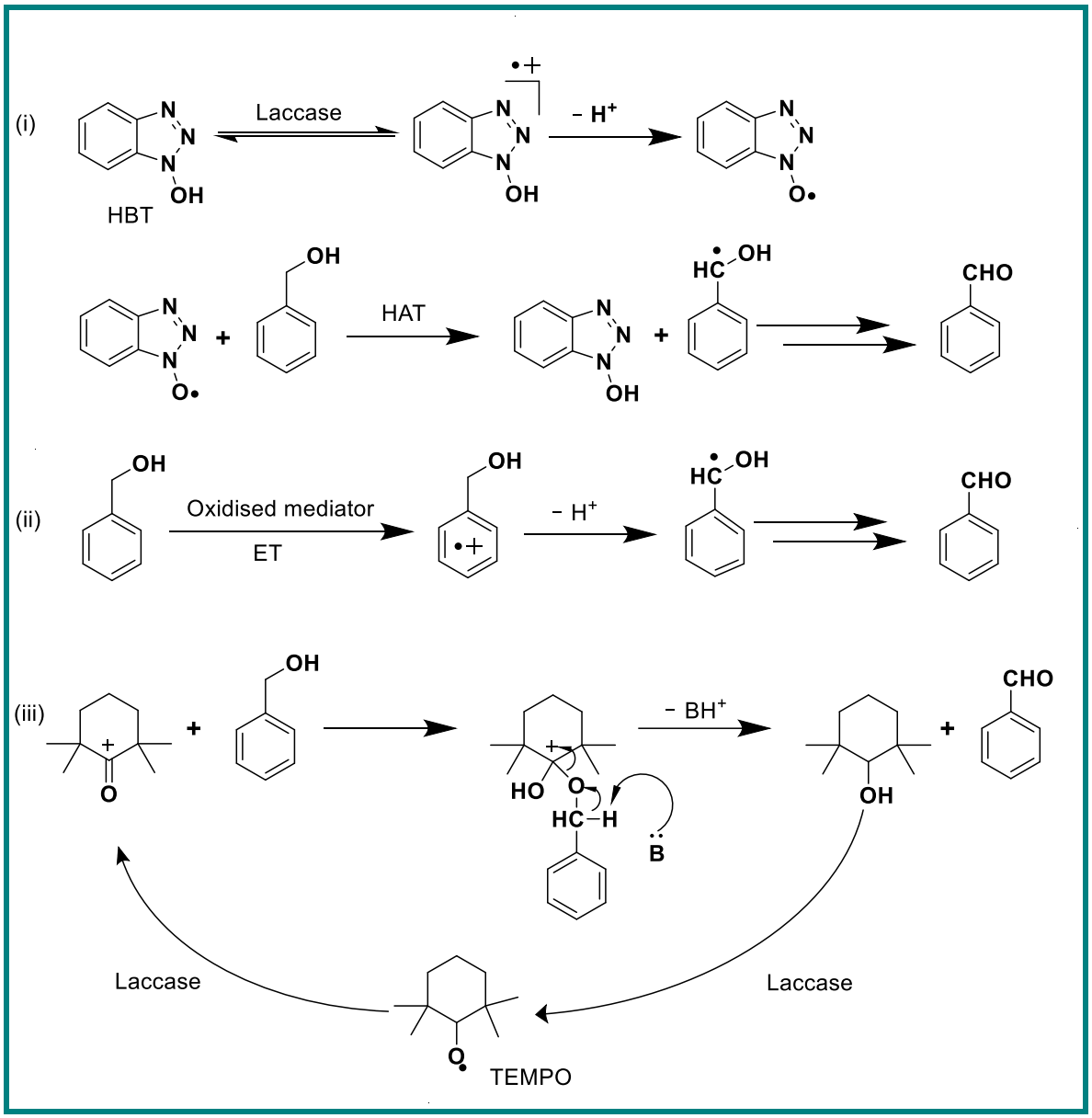


Figure 3: Examples of laccase mediator molecule.

Ideally, when oxidized, a redox mediator should generate stable radical species that do not deactivate the enzyme and oxidize the substrate through mechanisms that are different from that of the enzyme.[67,69] The mechanism of the mediator–substrate oxidation is dependent on the type of mediator molecule and is reported to occur through either electron transfer (ET), radical hydrogen-atom transfer (HAT), or ionic oxidation routes.[32,67] Commonly used mediators include *N*-hydroxy benzotriazole (HBT), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), violuric acid (VA), 3-hydroxyanthanilic acid (HAA), *N*-Hydroxyphthalimide (HPI), and (2,2,6,6-Tetramethylpiperidin-1-yl)oxyl (TEMPO).[32] Mediators that have the N–OH structural features, such as HBT, VA, HAA, and HPI, favour the HAT pathway, while ABTS reacts via an ET mechanism. And other mediators like TEMPO and its analogues follow an ionic oxidation pathway (**Scheme 3**).[32,67]



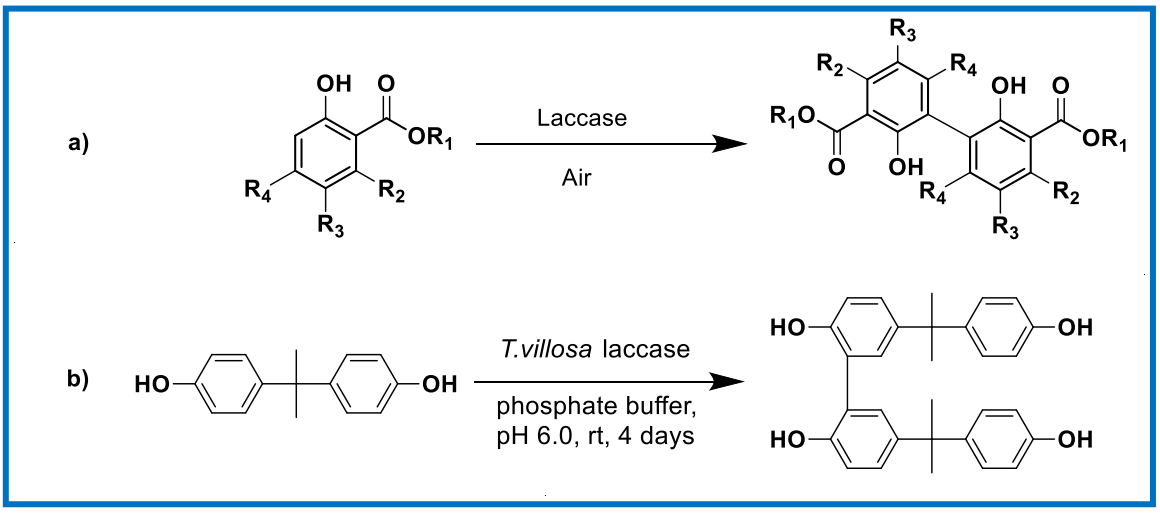
Scheme 3: mechanism of the mediator–substrate oxidation via the i) HAT pathway, (ii) the electron transfer (ET) mechanism and (iii) the oxidation pathway.[67]

Laccase mediator systems must be economically and environmentally friendly. However, many of the synthetic mediators are found to be costly, toxic, and can inhibit enzyme activity when used at higher concentrations.[28] There are several natural occurring laccase-mediators found in the environment, and these are phenolic compounds such as phenol red, catechol, hydroquinone, vanillin, acetovanillone, syringaldehyde, 2,4,6-trimethylphenol, *p*-coumaric acid.[28,70] Redox- mediators are widely used in environmental remediation processes and industrial applications for the oxidation of aromatic methyl groups, bleaching of textile dyes, oxidation of benzyl alcohols and polycyclic aromatic compounds.[27,69]

LACCASES IN THE SYNTHESIS OF HETEROCYCLIC COMPOUNDS

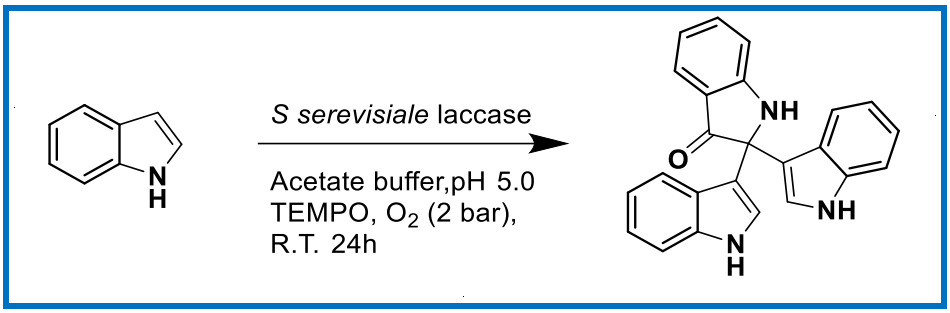
▪ DIMERISATION AND TRIMERISATION (C—C BOND FORMATION)

A few biocatalysis routes have been reported in the literature for self-coupling reactions (C–C BOND); for the synthesis of various dimers and trimers.[71] A laccase-catalysed oxidative dimerization of salicylic esters under aerobic conditions was achieved at room temperature, with the highest yield (isolated) of the corresponding biaryls of 85% **(**Scheme 4a).[72] Another laccase-catalysed dimerization reaction was reported on a self-coupling reaction of bisphenol, oxidized by purified laccase from *Trametes villosa* in a phosphate buffer (pH 6) at room temperature (Scheme 4b).



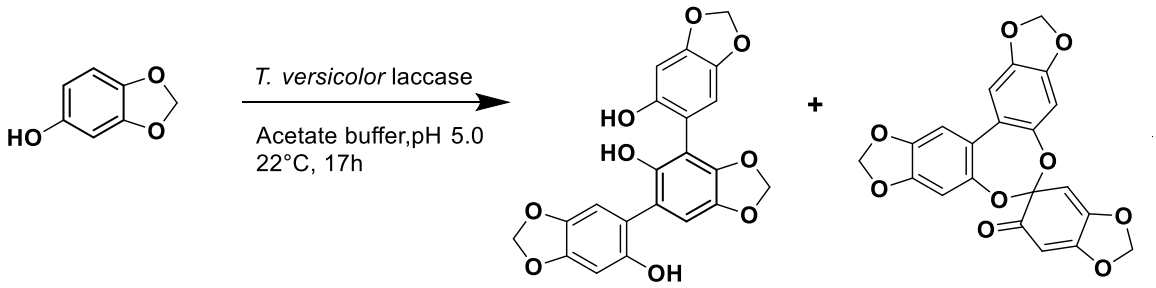
Scheme 4: Laccase-catalysed dimerization of salicylates.

The formation of the relative ratio of dimeric and/or trimeric products can be significantly influenced by the conditions of the reaction. This includes type of substrates, the use of buffer and/co-solvent, redox-mediators and enzyme origin.[73] Ganachaud *et al*. (2008) reported the trimerization of indole (**Scheme 5**) through laccase catalysis in the presence of various mediators such as ABTS, HBT, and TEMPO.[74] The products obtained from using TEMPO as a mediator improved the yield of the isolated product 2,2-Bis(30-indolyl)indoxyl from 23% to 51%.[73,74]



Scheme 5: Trimerization of indole in the presence of Laccase.

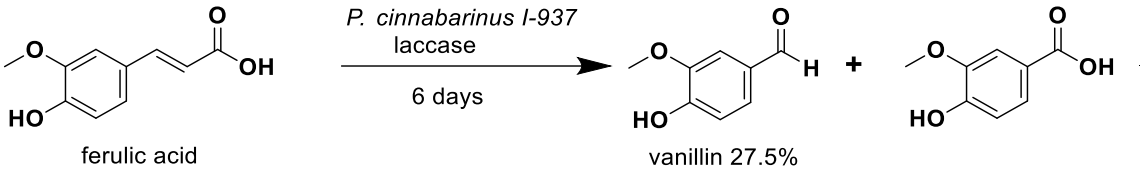
Another study demonstrated the influence of laccase source, mediator, and solvent system on the formation and ratio of the trimerization reaction of sesamol (a naturally occurring phenolic antioxidant; **Scheme 6**).[75] The reactions were conducted using *T. versicolor* and *A. bisporus* laccases and it was determined that the outcome of the sesamol oxidation is greatly influenced by the catalyst and the oxidant used.[73,75]



Scheme 6: Oxidative trimerization of sesamol in the presence of Laccase.

OXIDATIVE DECOMPOSITION AND CYCLISATION

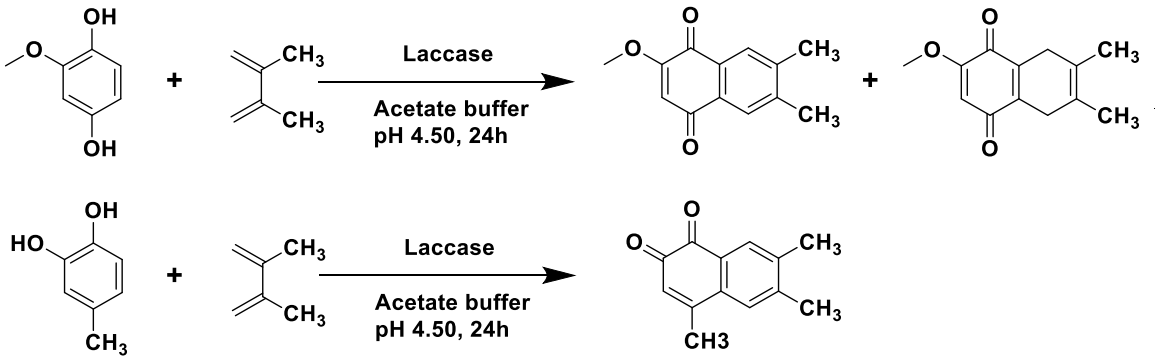
The white-rot fungus has been shown to degrade acids to alcohols and aldehydes under aerobic conditions.[76,77] The oxidative decomposition of ferulic acid in the culture of the fungus, *Trametes sp*. yielded coniferyl alcohol along with other products such as dihydroconiferyl alcohol, coniferylaldehyde, vanillyl alcohol, vanillic acid, 2-methoxyhydroquinone and 2- methoxy quinones.[24,77] Falconnier *et al.* (1994) reported another oxidative metabolism of feluric acid, where the white-rot fungus laccase *Pycnoporus cinnabarinus* I-937 was used to metabolise ferulic acid into vanillin (**Scheme 7**).[32,78]



Scheme 7: A biotransformation of ferulic acid by laccase.

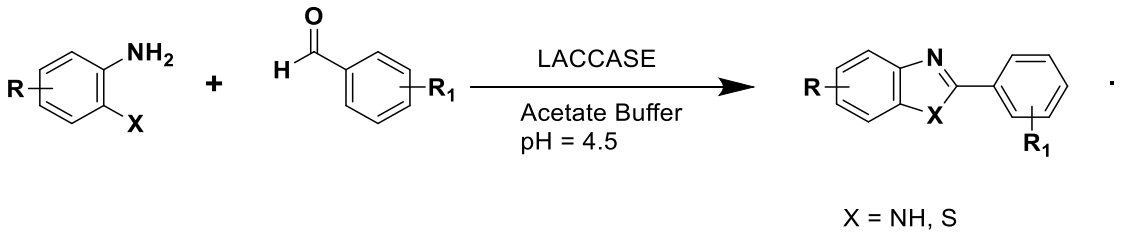
The propenoic side chain of ferulic acid was oxidatively cleaved to give the corresponding vanillic acid in molar yield of 27.5%.[78]

Witayakran *et al.* (2007) reported the one-pot intermolecular Diels-alder reactions between dienes and quinones to generate *o*- and *p*-naphthaquinones *in situ* with laccase in aqueous medium.[79,80] The reactions were carried out variously at 70°C, 3°C and room temperature in an open vessel containing an acetate buffer (pH 4.5) (**Scheme 8**).[32]



Scheme 8: Laccase catalysed synthesis of naphthaquinones through Diel-Alder reactions.[32]

We previously reported the use of commercial laccases Novoprime and Suberase® at room temperature for the synthesis of 2-aryl-1*H*-benzimidazoles and 2-arylbenzothiazoles in good to excellent yields (**Scheme 9**).[81]



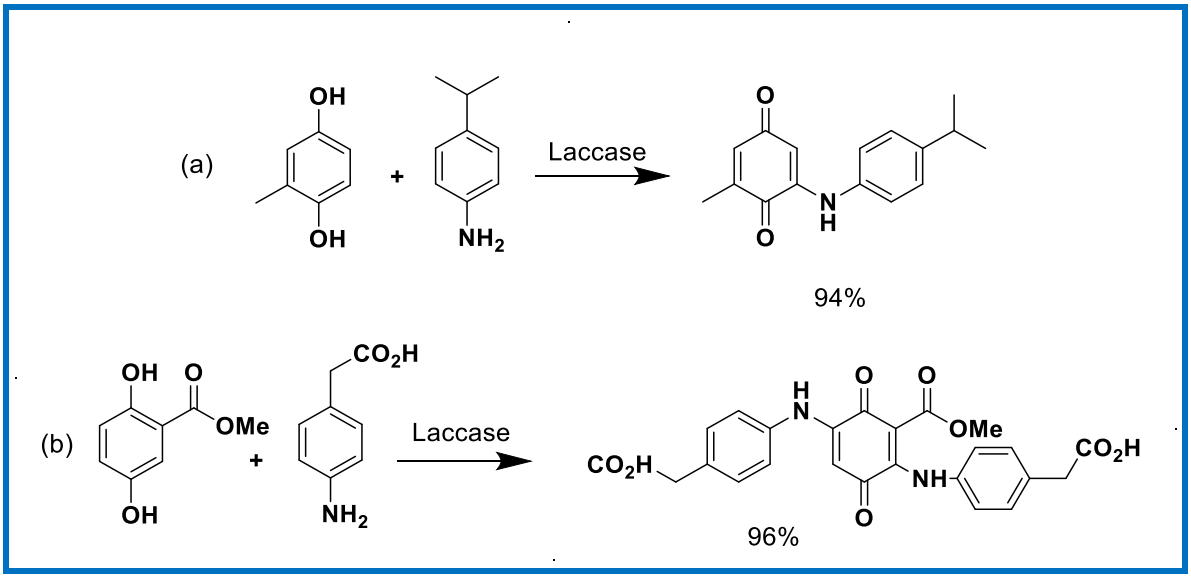
Scheme 9: Synthesis of 2-aryl-1H-benzimidazole and 2-aryl-benzothiazole derivatives

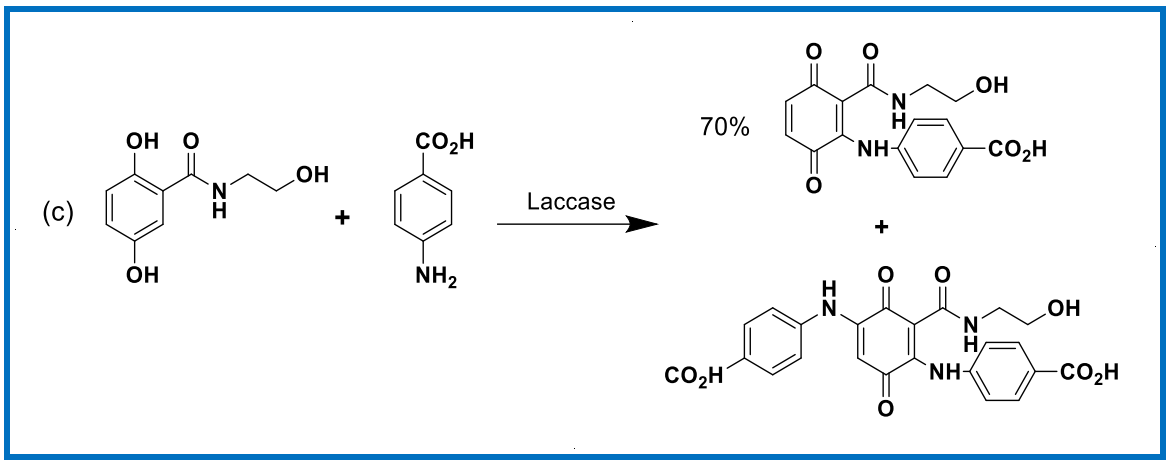
The oxidative cyclisation reactions were conducted in the presence of atmospheric oxygen in an acetate buffer (0.1 M, pH 4.5) using acetonitrile as a co-solvent.[81]

▪ NUCLEAR AMINATION

Laccase-catalysed aminations represent one of the most widely studied enzyme reactions and is a powerful tool for the construction of biologically important C–N bond molecules.[82] There have been several studies on laccase-catalysed amination reactions in the literature and amongst the reactions investigated are novel antibiotics.[82,83] The major drawback associated with the laccase-catalysed aminations, particularly, the amination of hydroquinones, is regioselectivity. It has been reported that the reaction tends to lead to the formation of mono- or di-aminobenzoquinones products (usually a combination) depending on reaction conditions such as the molar ratio of substrates, buffers/co-solvents, laccase concentrations, reaction time, temperature, and pH of solution .[24,84]

Niedermeyer *et al.* (2005), investigated the nuclear amination of *p*-hydroquinones with primary aromatic amines catalysed by fungal laccases (EC 1.10.3.2) from *Trametes spec.* and *Myceliophthora thermophila*.[84] And it was discovered that one of the laccases favoured the formation of mono- over di-aminated products where else the other formed more di-aminated over mono-aminated products in various reaction conditions (**Scheme 10**).[24,84]

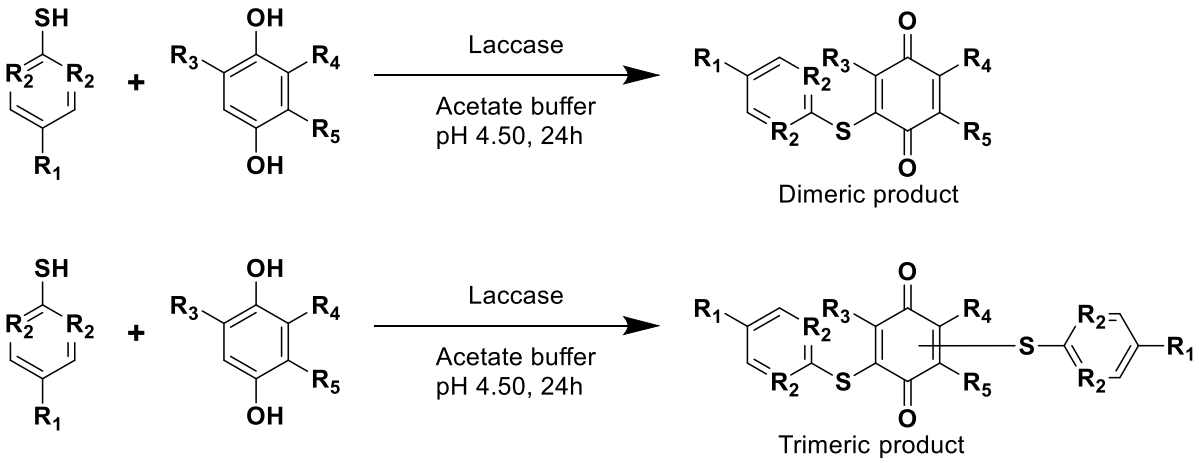


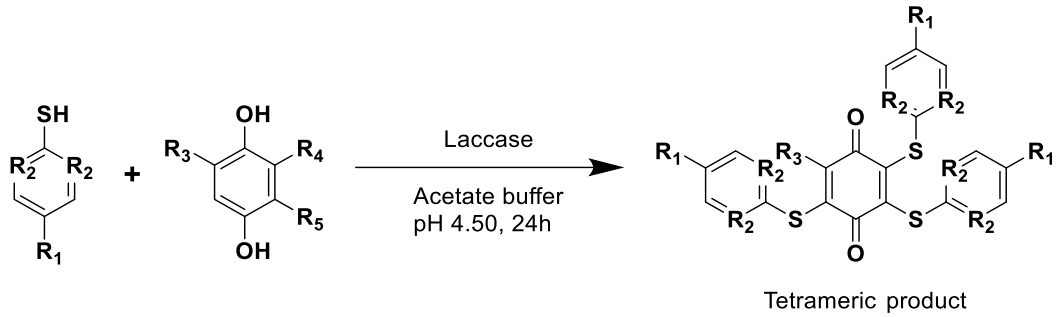


Scheme 10: (a) monoamination (b) diamination (c) mono- and di-amination of *p*-hydroquinone catalysed by laccase.[24]

▪ THIO-BOND FORMATION (C–S BOND FORMATION)

Little is known in the literature about the laccase catalysed C–S bond formation reactions.[24] Therefore, it is of significant value for both green chemistry and biotechnology to extend the scope of application of laccases for reactions involving C–S bond, as these compounds possess several important biochemical and pharmaceutical benefits.[24,85] Schlippert *et al*. (2016) described laccase-catalysed reactions involving C–S bond, using laccases from *Pycnoporus cinnabarinus* and *Myceliophthora thermophila* to generate a library of thiolated products, ranging from dimers to tetramers (**Scheme 11**).[85]





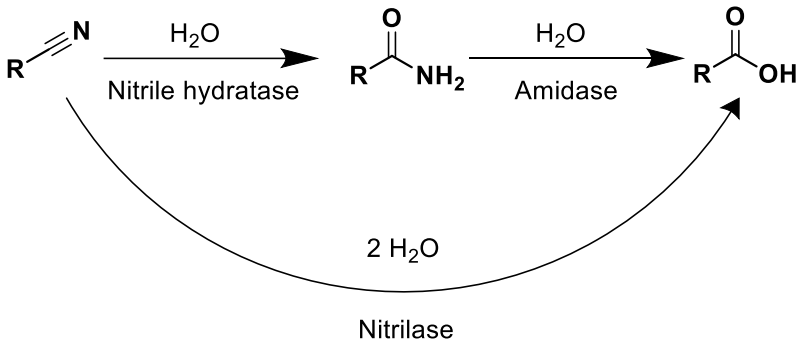
Scheme 11: Laccase catalysed C-S bond formation reaction.

A series of *p*-hydroquinones and aromatic thiols were reacted via Michael addition reaction in the presence of a laccase and it was discovered that laccases are suitable biocatalysts for the synthesis of heteromolecular thiolated products under eco- and environmentally friendly conditions.[85]

NITRILE HYDRATASE ENZYMES

INTRODUCTION

Nitriles are abundant cyano group (R—CN) containing compounds found in the natural environment.[86] They are synthesised by many plants and are used as starting materials or intermediates in biological systems and also in synthetic organic chemistry.[86,87] Because of the cyano (CN) moiety, nitriles are generally associated with toxic inorganic cyanides.[88] Nonetheless, the general occurrence of nitriles in nature resulted in the prevalence of bacteria and fungi able to degrade nitriles to their corresponding carboxylic acids and amides.[89–91] The natural metabolism of nitriles follows two pathways to produce organic acids utilizing nitrile—hydrolysing enzymes.[87,92] In the first pathway, nitriles undergo direct hydrolysis to carboxylic acid by the action of nitrilase (EC 3.5.5.1). In the second pathway, nitriles are first converted into amides by nitrile hydratases (EC 4.2.1.84) and successively hydrolysed to carboxylic acids and ammonia by amidase (EC 3.5.1.4) (**Scheme 12**).[87,93]



Scheme 12: Enzymatic conversion of nitriles to carboxylic acids.[93]

Nitrile hydratases (NHases; EC 4.2.1.84) are metalloenzymes involved in the catalysis of the biological hydrolysis of nitriles to their corresponding amides.[94,95] There are two classes of NHase enzymes—based on the type of metal ion incorporated in the active site of the enzyme. There are those with a non-haem iron (III) centre (Fe—Nhase) and those with a non-corrin cobalt(III) centre (Co-Nhase) at their active site.[96,97] The Co—Nhase have been found to be more vigorous and possess a broader substrate specificity. In recent years, NHases from various organisms (and several mutants) have been efficiently used for the industrial production of chemicals and pharmaceuticals and also in the environmental elimination of nitriles from wastewater.[96]

STRUCTURAL PROPERTIES OF NHases

NHases are generally made of two types of subunits (α and β subunits), each with a distinct amino acid sequence. They typically exist as a stable αβ (46 kDa in size) heterodimer or $(\alpha\beta)_2$ (92 kDa in size) heterotetramer and bind one metal atom per αβ unit.[96] The metal ion at the catalytic site of NHase is deeply submerged at the interface of the α and β subunits and for catalysis to occur, the substrate must reach the interior of NHase.[87] The Crystal structure for the Co^{+3}—NHase displays how the transportation of substrate and product to and from the active-site happens and this process occurs through a tunnel located at the interface of α- and β- subunits of *P. thermophila* JCM3095 (**Figure 4**).[87,98]

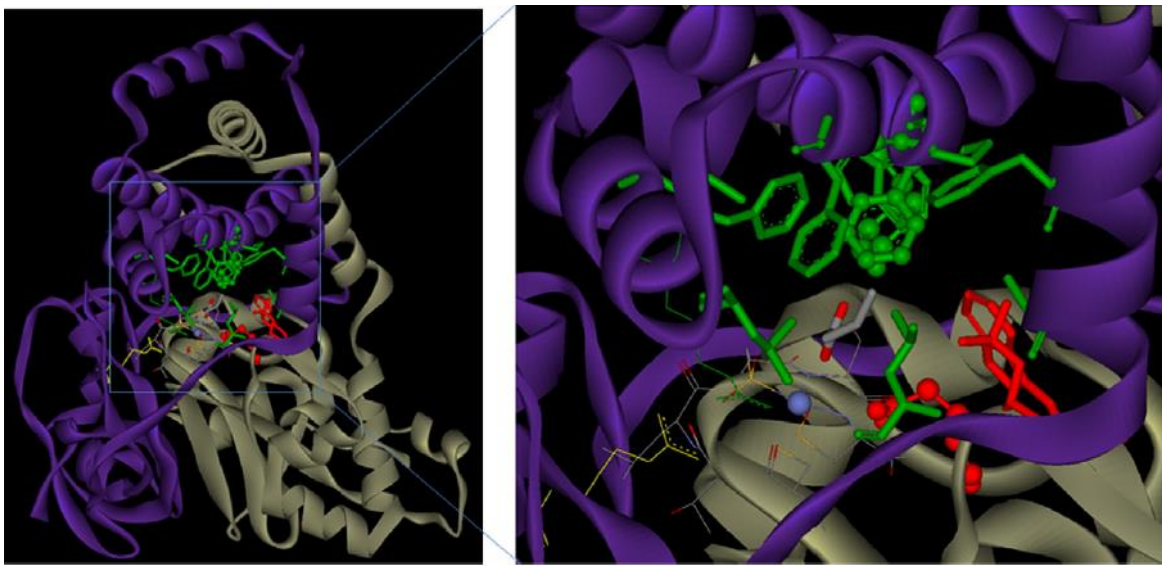


Figure 4: Crystal structure of NHase of P. thermophila JCM 3095 (PDB 1ugp).[87]

Data from molecular dynamics (MD) simulations of the related Fe-centred NHase also demonstrated how this tunnel plays a key role in the stereospecificity and substrate affinity profile of NHase.[87,99]

CATALYTIC MECHANISMS OF NHases

Even though there are extensive theoretical and experimental investigations, the exact catalytic mechanism of NHase has not yet been fully explained.[100] However, by performing quantum-mechanical studies of the active site of NHase protein structures

along with experimental investigations, several plausible mechanisms have been reported in recent years.[87,101] Using metals such as Fe, Co, Pt, Rh, Ru, and Ni researchers were able to simulate or mimic the active site of NHase to investigate the full reaction pathway of the enzyme. Huang *et al.* (1997) proposed three conventional mechanisms followed by the metal ion in NHase: (i) Inner sphere mechanism, (ii) Outer-sphere mechanism and (iii) Second-outer sphere mechanism (**Figure 5**).[87,102] In all three mechanisms, the metal ion is proposed to be acting as a Lewis-acid when activating the nitrile for hydration.[102]

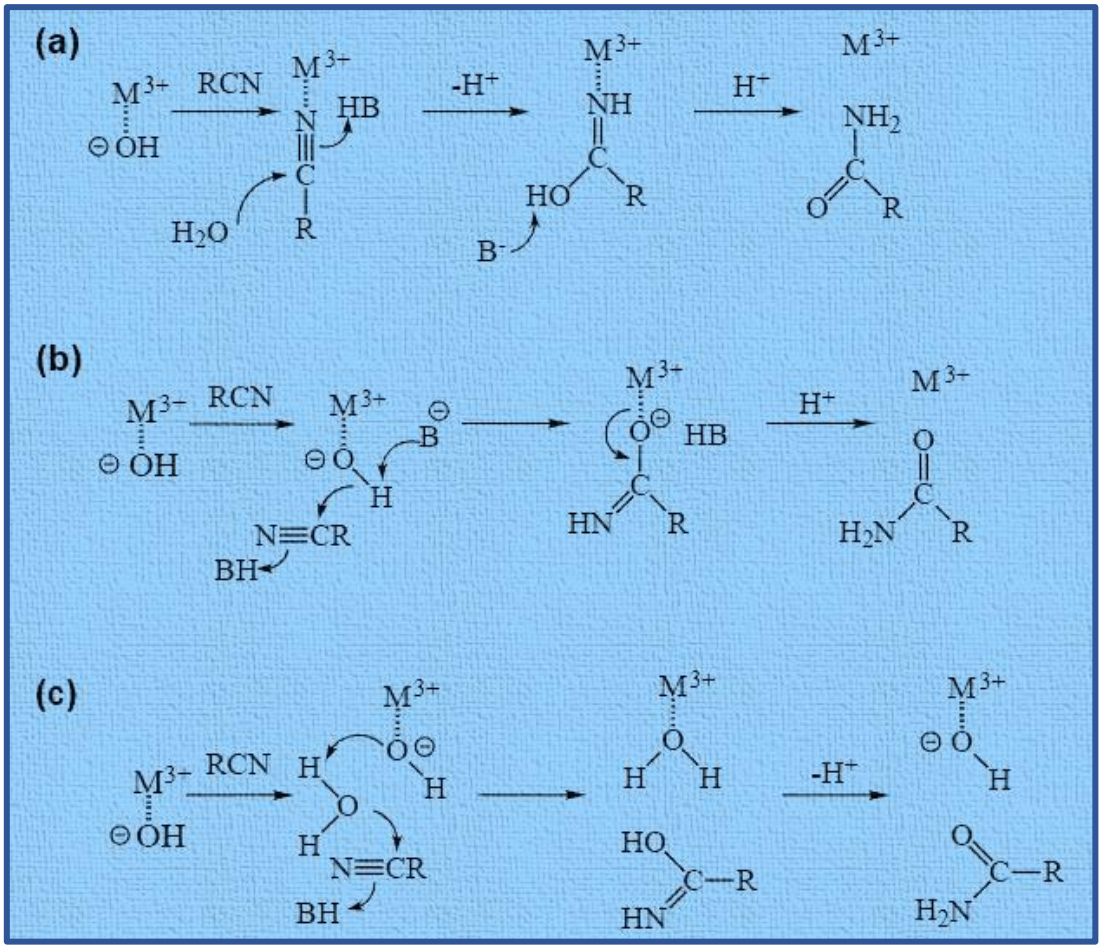


Figure 5: Possible catalytic mechanisms of NHases, (a) Inner-sphere mechanism, (b) Outer-sphere mechanism and (c) Second-outer sphere mechanism.[87,102]

The inner-sphere mechanism involves ligand exchange, where the nitrile group from the substrate substitute a hydroxide ligand on the metal-centre and the subsequent metal-bound nitrile undergoes hydrolysis by a base-activated water molecule. The resultant metal-bound intermediate then rearranges to release the amide product (**Figure 5 A**).[87,103]

The outer-sphere mechanism also includes ligand exchange; it is proposed that the nitrile carbon from the substrate undergoes a nucleophilic attack in the active site pocket of the enzyme.[87,103] It is reported that the metal-bound substrate co-ordinates to the metal via oxygen, which then rearranges to the amide product (**Figure 5 B**).[87] In the second outer-sphere mechanism, the nitrile undergoes hydrolysis by a metal-bound hydroxide-activation, where the metal-hydroxide causes deprotonation of a free water molecule. The hydrolysed intermediate generated eventually rearranges to form the amide product (**Figure 5 C**).[87,102]

NHases IN ORGANIC SYNTHESIS

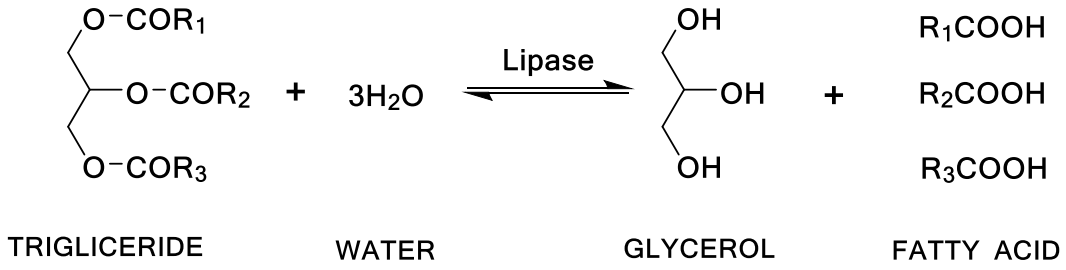
Unlike conventional synthesis, NHase-catalysed hydration of organic nitrile to their subsequent amides is an appealing and feasible alternative.[87] Within many NHase expressing strains, nitrile hydratases from *Rhodococcus rhodochrous, Pseudomonas chlororaphis, Nocardia sp*. are amongst the commonly studied in the literature and are extensively utilized for the synthesis of many amide products.[87,104] They have been applied in the production of multiple organic amides such as acrylamide[105], benzamide, 2,6-difluorobenzamide, thiophenecarboxamide, furanecarboxamide indoleacetamide[106], nicotinamide[107], butyramide[108] and 5-cyanovaleramide[109] from various nitriles.[87,105]

High yields and substrate-specificity make NHase desirable for industrial applications. The production of acrylamide was the first successful NHase-catalysed industrial biotransformation process.[87,104] NHase mediated conversion of acrylamide from acrylonitrile has been employed by Mitsubishi Rayon Corporation, where close to 25% of the global total production of acrylamide is produced (95,000 tonnes per annum).[104] It was reported that NHase also has applications in the detoxification and environmental bioremediation of nitrile polluted wastewater (Acrylonitrile Waste Effluents).[110] NHase enzymes can be used to gain access to complex organic amide and carboxyl compounds found in both fine chemicals and pharmaceuticals.[104] And extensive research is being sorted out for the discovery of nitrile biocatalysts that are more efficient, stable, have a broad range of substrates and are thermally robust.[104,111]

LIPASE ENZYMES

INTRODUCTION

Lipases (triacylglycerol acylhydrolases—E.C. 3.1.1.3) are lipolytic enzymes that function to catalyse the hydrolysis of long-chain triglycerides to glycerol, and free fatty acids.[112,113] Under aqueous conditions, lipases cleave off the carboxyl ester bonds found in triacylglycerols and thereby releasing glycerol and fatty acids (**Scheme 13**).[114] In nature, they are responsible for the breakdown of lipids or the transfer of lipids from one organism to the next.[112] In addition, lipases have a widespread of functions, this includes interesterification, esterification, alcoholysis, acidolysis and aminolysis.[113,115,116] These reactions typically occur with exceptional regio-, chemo- and enantio- selectivity, making lipases one of the leading biocatalysts in synthetic organic chemistry.[117]



Scheme 13: Lipase-catalysed hydrolysis of triglyceride.

Lipases are not limited by reaction media—their notable ability to perform in both aqueous and non-aqueous environments makes them important biocatalysts for biotechnological applications.[115] The natural substrates of lipases are lipids that generally lack affinity for water, however, the reaction is carried out at the lipid–water interface (in a bi-phasic system).[114] Their ability to catalyse a wide range of chemical transformations made them popular in food manufacturing, detergents, leather, textiles, paper production, cosmetics, fine chemicals, biodiesel production, medical diagnostic tools and pharmaceutical industries.[113,115] The growing importance of lipases is reflected by the number of publications reporting various biotechnological versatility aspects of the enzyme—with over a 1000 publications each year.[114,118]

NATURAL SOURCES OF LIPASES

Lipase was first discovered by J. Eberle in a pancreas in 1834[119] and in 1848 C.I. Bernard also observed lipase activity while using pancreatic secretions to emulsify and saponify neutral fats.[120] Lipases are found everywhere, spread-out the Earth's flora and fauna[118]**:** they can be found in mammals [121], in higher plants[122], fungi[123], yeast[124] and bacteria.[125] Microbial lipases are the most common (mostly bacteria and fungi), whereas plant and mammalian lipases are reported to be very expensive to produce and purify.[115] Microbial lipases have emerged as one of the leading lipases in modern biotechnology, as they possess a wide range of activities, including substrate specificity, high yield production, are easy to genetically modify, and are stable in various pH, temperature and solvents.[124,126,127]

FUNGAL LIPASES

Lipase-producing fungi are found in several natural and unnatural habitats, including oil-contaminated soils, coal tips, industrial wastes and food industry wastes (dairy, vegetable oils, seeds and spoiled food).[115,126] Some of the commonly described lipase-producing fungi include *Aspergillus*, *Candida, Ashbya, Acremonium, Mucor, Rhizopus, Penicillium, Geotrichum, Humicola, Fusarium, Rhizomucor, Thermomyces, Metarhizium, Eurotrium,* and *Ophiostoma*,[113,115,126]

Since the 1950's, fungal lipases have been investigated for strains with different catalytic characteristics such as higher conversions, selectivity, thermal and pH stability and substrate specificity in order to be able to fulfil the growing industrial demands.[113,119,126,127] *Candida rugosa* has had great significance in enzyme biotechnology and it is labelled the organism of choice for lipase synthesis.[113,118] Lipases from *Mucor pusillus*, *Rhizopus homothallicus*, and *Aspergillus terreus* are well known for their thermal stability. On the other hand, *Mucor* sp. produces lipases that are stable under both alkaline conditions and high temperatures (alkalophilic and thermostable lipase).[113]

BACTERIAL LIPASES

Bacterial lipases were initially introduced by Genencor International in 1995: Lumafast™ from *Pseudomonas mendocina* and Lipomax™ from *Pseudomonas alcaligenes*.[117] Bacterial lipases are glycoproteins and lipoproteins.[127] Most of the bacterial lipases are extracellular and their production is affected by fermentation conditions such as temperature, pH, agitation, nitrogen and carbon sources, availability of lipids around the bacterial cell and oxygen concentration.[114] Among bacterial lipases being exploited, only a limited number is studied in literature and commercially utilized.[114,115] Those from *Achromobacter*, *Alcaligenes, Arthrobacter, Bacillus, Burkholderia, Chromobacterium* and *Pseudomonas* etc.[114] Of these, *Bacillus* and *Pseudomonas* sp. are the most commonly used for a range of biotechnological applications.[126]

LIPASE STRUCTURES

The first two, three dimensional (3D) structures of lipase were reported in 1990—a human pancreatic lipase and a fungal lipase from *Rhizomucor miehei*.[117] Since then, many lipase structures from diverse sources were reported in the literature, most of which were from microbes (fungi, yeast and bacteria) and the rest from mammalian enzymes.[128] Over the years, the solved three-dimensional structures of multiple lipases have helped with the understanding of the molecular structure and properties of these enzymes; particularly how they breakdown fatty acids .[128,129] All of the reported structures appeared to have catalytic triads closely reminiscent of serine proteases.[129]

Lipases weigh between 19 to 60 kDa, and 3D structure comparisons have shown that lipases exhibit a folding pattern known as α/β hydrolase fold.[117] **Figure 6** shows a ribbon diagram of the overall fold of *Pseudomonas cepacia* lipase.[128] The lipase core is made up of a central β sheet consisting of up to eight different β strands (β1—β8) and they are all connected by up to six α helices.[117] The central β sheet in the core conforms to strands β3—β8 of the α/β hydrolase fold and catalytically active serine residue at the active site is located at the C-terminal end of strand β5 in the strand-turn-helix motif called the 'nucleophilic elbow'.[117,128]

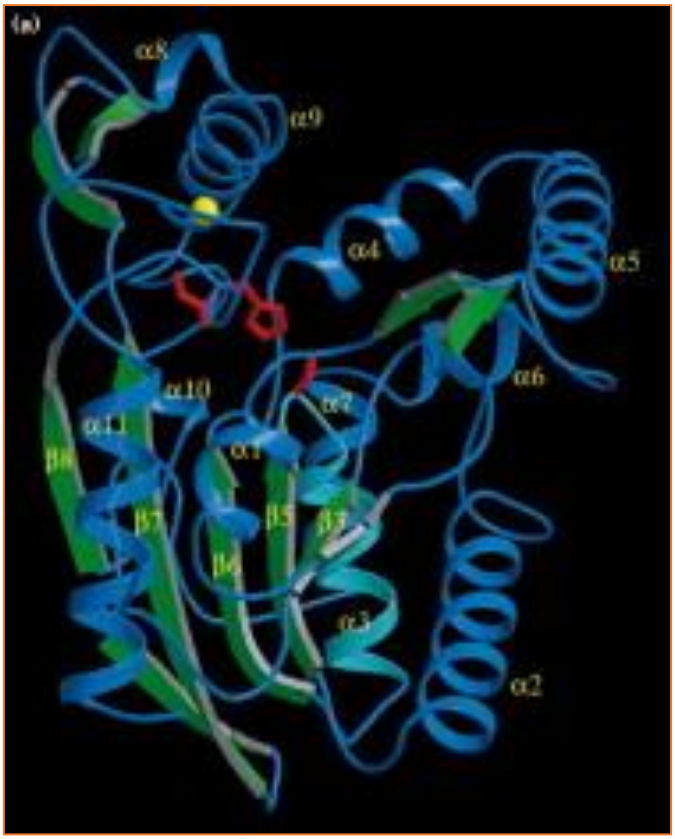


Figure 6: A ribbon representation of Pseudomonas cepacia lipase.[128]

The active site is made of a catalytic triad consisting of the amino acids serine, aspartic (or glutamic) acid and histidine.[117] The Ser-His-Asp/Glu catalytic triad is responsible for the catalytic reaction of the enzyme.[129,130] Hydrolysis of the substrate occurs through a nucleophilic attack on the ester carbonyl carbon by the catalytic-site- Ser oxygen atom, leading to the formation of a tetrahedral intermediate which is stabilized by hydrogen bonding (**Figure 7**).[117,130] An alcohol is released, leaving behind an acyl-enzyme intermediate, which is consequently hydrolysed and thereby releasing the fatty acid and the free the enzyme to repeat the cycle.[117]

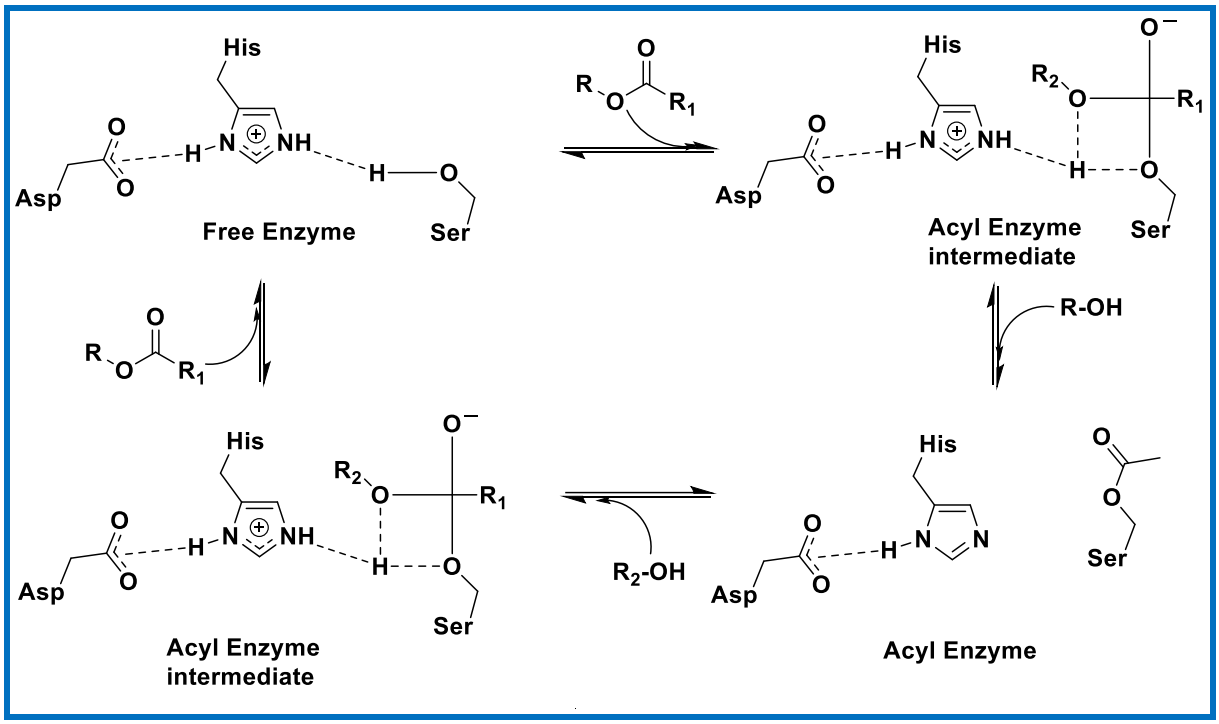


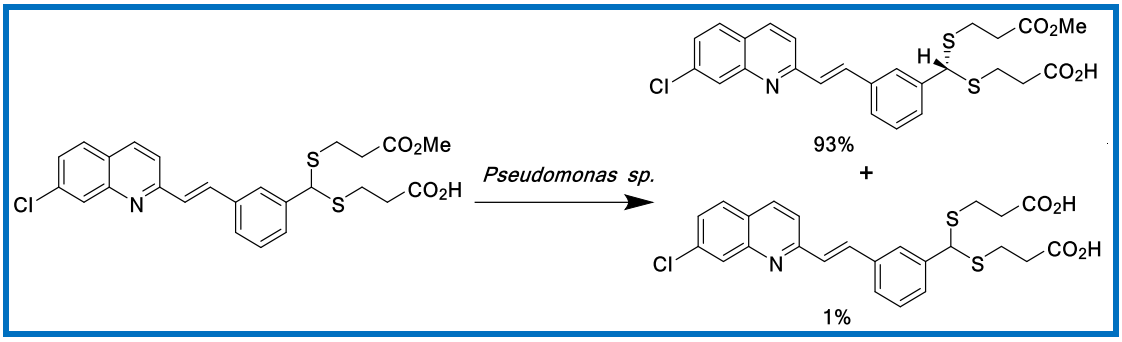
Figure 7: catalytic mechanism of CALB based on a Ser-His-Asp triad.[130]

APPLICATION OF LIPASES IN ORGANIC SYNTHESIS

For many years, lipases have been used by organic chemists to perform various organic transformations due to their regio-, chemo- and enantioselectivity.[117,127] As mentioned before, microbial lipases are the most popular amongst the lipolytic enzymes in organic chemistry.[117] Besides being able to perform reactions under mild conditions, lipases can perform selective reactions and they exhibit a very high level of substrate-selectivity for enantio-selective hydrolysis, and trans-esterification/amidation reactions.[117,127,131]

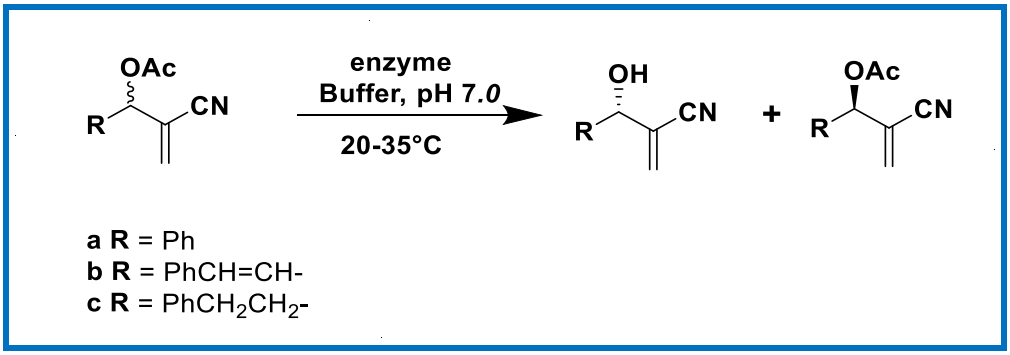
▪ ENANTIO-SELECTIVE HYDROLYSIS

In general, few catalysts are effective at more than one type of asymmetric reaction, however, lipases are capable of catalysing the reaction of prochiral substrates and also perform kinetic resolution of racemates.[117] Hughes, *et al* (1990) reported the hydrolysis of prochiral and racemic dithioacetal esters using commercially available lipases and achieved up to 98% enantiomeric excess (ee) (**Scheme 14**).[132]



Scheme 14: Lipase-catalysed Hydrolysis of Prochiral Diesters.

Here at Wits, Juma, *et al.* (2017) reported the lipase catalysed enzymatic kinetic resolution of Morita-Baylis-Hillman acetates under mild reaction conditions. A series of lipases were screened for activity against the enantioselective hydrolysis of Morita-Baylis-Hillman adducts and up to 94–97% ee were obtained (**Scheme 15**).[133]

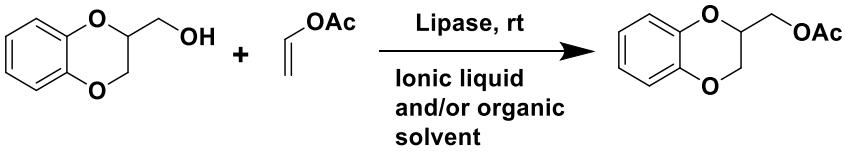


Scheme 15: Lipase-catalysed hydrolysis of Morita-Baylis-Hillman acetates.[133]

▪ TRANS-ESTERIFICATION/AMINATIONS

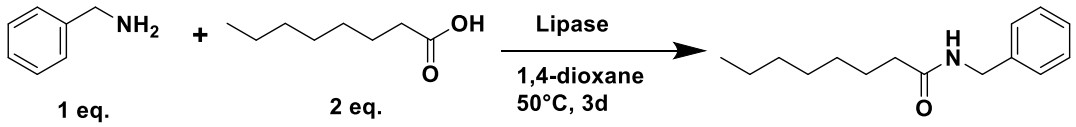
As the search for alternative fuels continues to grow, bio-based fuels produced from the transesterification of vegetable oils or animal fats with methanol have gained a lot of traction in recent years.[134] On the other hand, amides represent an important class of organic compounds found in a wide range of applications. They form part of important natural biological compounds as well as key intermediates in the synthesis of pharmaceuticals and organic heterocycles.[135] Commonly used approaches for trans-esterification/amidations suffer from many drawbacks, and as a result, immobilized lipases have been extensively investigated to perform these reactions as they can be more selective and they are easy to recover from the reaction mixture.[131,134]

Recently, a Lipase-catalysed transesterification in ionic liquids was reported. The transesterifications of 2-hydroxymethyl-1,4- benzodioxane catalysed by lipases using vinyl acetate was conducted in two different ionic liquids, 1-butyl-3- methylimidazolium hexafluorophosphate, [bmim]PF_6 and 1-butyl-3-methylimidazolium tetrafluoroborate, [bmim]$BF_4$and organic solvents (**Scheme 16**).[136]



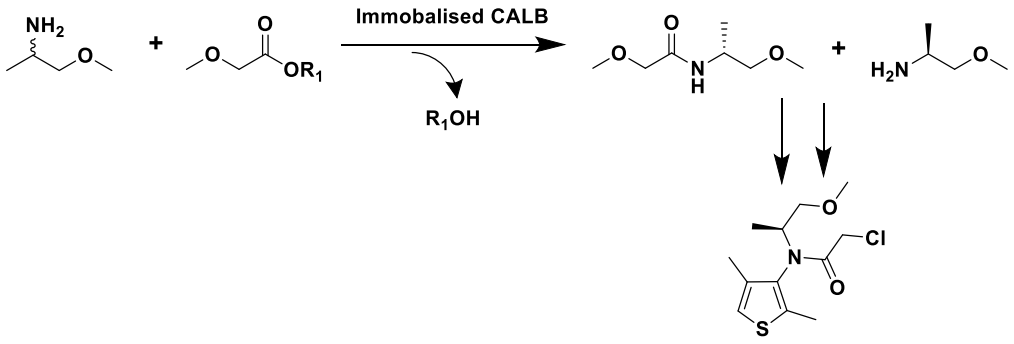
Scheme 16: Lipase catalysed transesterifications in ionic liquids and organic solvents.[136]

Manova *et al* (2018) described a simple and convenient lipase-catalysed direct amidation of free carboxylic acids and amines. The reactions were conducted using lipases from various origins including *Candida antarctica* Lipase B immobilized and up to 94% yields were obtained (**Scheme 17**). After the first reaction, the immobilized enzyme was recycled and used in the next run, and it was discovered that the enzyme was able to maintain a stable activity for about four cycles.[131]



Scheme 17: Lipase-catalysed amidation of carboxylic acid and amines.[131]

Apart from the above-described applications, CALB has been utilized in several reactions; including a selective acylation of (*S*)-1-methoxy-2-aminopropane with ethyl 2- methoxyacetate for the synthesis of Dimethenamide-P (**Scheme 18**).[127]



Scheme 18: Synthesis of Dimethenamide-P by lipase-catalysed enantioselective transamination.[127]

TUBERCULOSIS (TB)

INTRODUCTION

Communicable diseases are a grave and ever-present threat to human well-being.[137] Tuberculosis (**TB**) is one of the oldest known communicable diseases, caused by the bacterial pathogen *Mycobacterium tuberculosis* (**MTB**).[138] Throughout history, the disease tuberculosis has co-existed with humans for over 70 000 years, dating back before human emergence from Africa.[139] TB was declared a global health pandemic by the World Health Organization (WHO) almost 20 years ago, and it remains one of the most successful bacterial pathogens in the world to this day.[139,140] Worldwide over 2 billion people are infected with MTB and it accounts for approximately 10.4 million new infections and 1.4 million deaths each year.[139,141]

The WHO identified 22 countries as TB high-burden countries; which cumulatively accounts for 80% of all TB cases.[142] The African region accounted for 26% of the global TB cases in 2015, with South Africa holding the highest prevalence among the TB and HIV-burdened countries in the world.[143] It is estimated that one in 100 South Africans ends up developing active TB disease every year and of all the reported cases, over 60% were co-infected with HIV.[144] TB is the leading cause of HIV-related mortality among individuals living with HIV/AIDS and remains a primary risk factor among patients commencing antiretroviral therapy (ART) programmes—causing poor virological outcomes and early mortalities.[145]

LIFE CYCLE OF TB

The life cycle of MTB infection starts with the inhalation of an infectious bacterium (*M. tuberculosis*) through tiny droplets by a close contact, which penetrates the alveolar tissue of the lungs through alveolar macrophages (**Figure 8**).[146,147] Thereafter, the pathogen is transported to the lymph nodes by antigen-presenting cells (APCs) where an immune response is triggered.[139] Inflammatory response causes mononuclear cells from neighbouring blood vessels to provide fresh host cells to the growing bacterial

population, resulting in granuloma formation in the lungs.[140,147] The initial granuloma may appear as an out of shape mass of macrophages, monocytes, and neutrophils. However, the macrophages dissociate into cells such as foamy macrophages, and epithelioid macrophages.[147] When the disease progresses, there is an accretion of caseum in the granuloma centre, and the granuloma cavitates and disintegrates into the lungs; releasing the bacteria into the airways.[147] An indication of the initial infection is demonstrated by the remaining calcified Ghon focus lung tissue.[140]

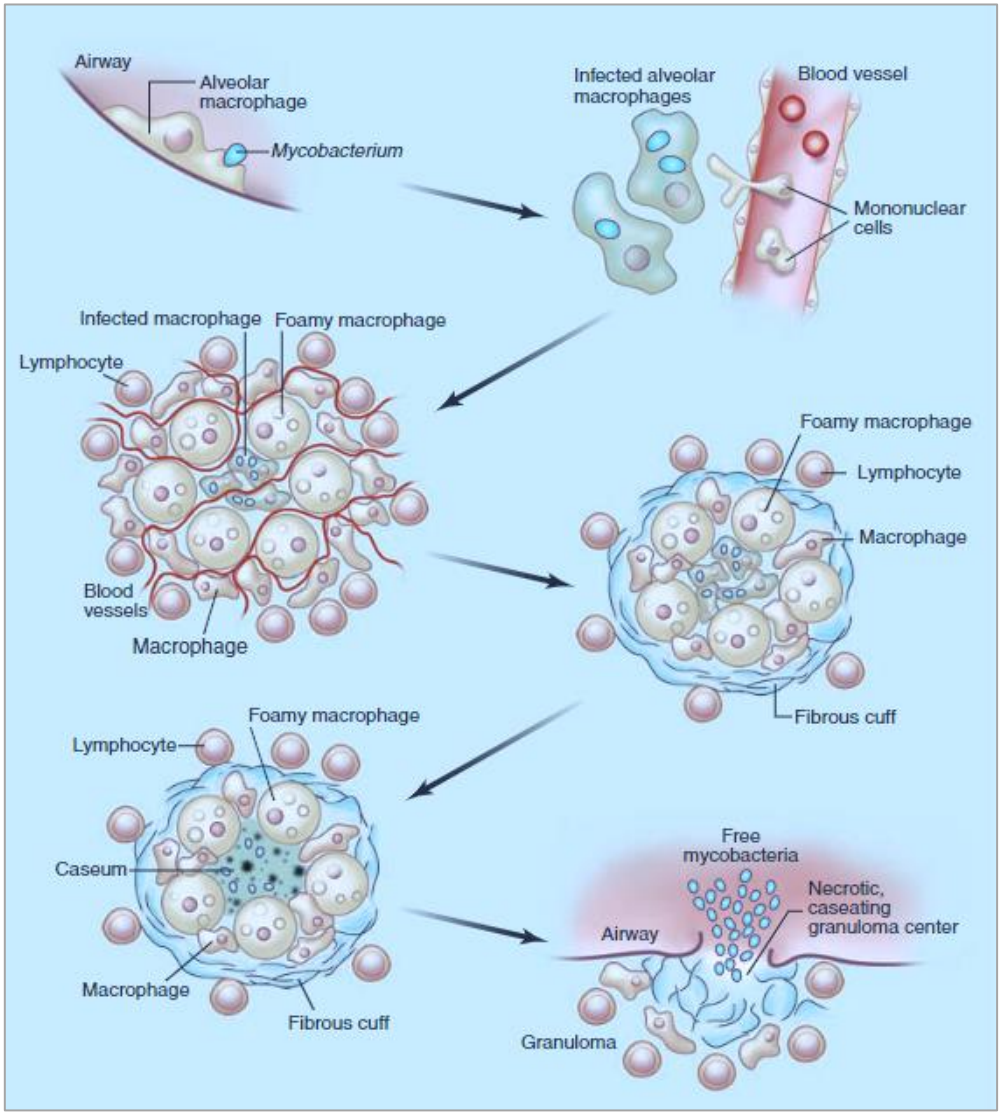


Figure 8: The lifecycle of *M. tuberculosis*.[147]

Most people (estimated over 90%) infected with *M. tuberculosis* never develop the active disease and the infection remains in a 'latent' form.[146] The latent MTB does not cause illness, but it is difficult to eradicate the bacteria from the host.[139] Should the host's immune system weaken, the infection may re-activate at a later stage and the

patient may develop active TB. The bacteria may escape out of containment and begin to replicate extracellularly, this may occur months after initial infection (often as miliary TB), or after decades, usually as apical pulmonary TB.[140,146]

DRUG-RESISTANT TB

Regardless of the availability of treatment drugs for drug-susceptible TB, multidrug-resistant tuberculosis (**MDR-TB**) remains a problem due to bacterial resistance to highly potent first-line anti-TB drugs such as rifampicin[148], streptomycin[149], ofloxacin[150] and isoniazid (**Figure 9**).[138,151,152] Although there are several promising anti-bacterial drugs such as rifamycins, fluoroquinolones, oxazolidinones, and nitroimidazopyrans, there are also looming drug-resistant forms of TB that have recently been reported.[138,153]

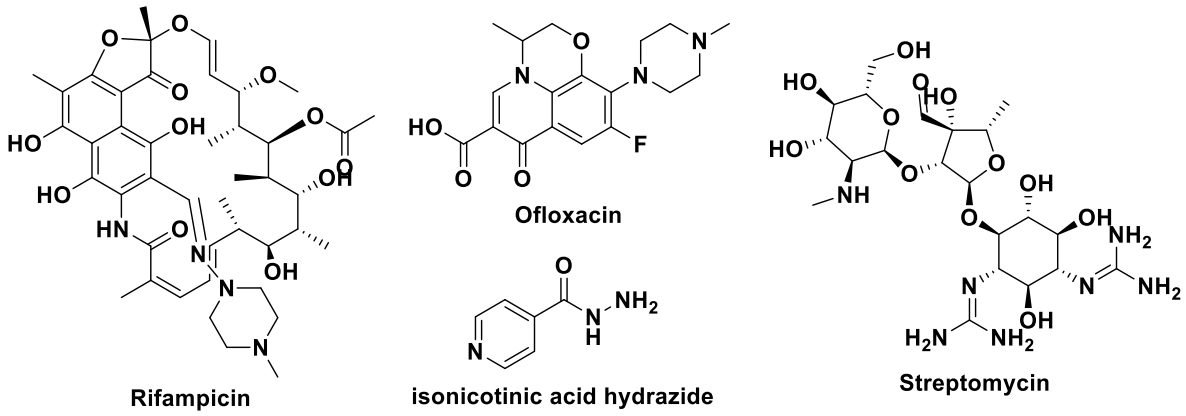


Figure 9: First-line antituberculosis drugs.

In 2016 the WHO estimated a global emergence of about 600 000 new cases of **MDR-TB**, with 240 000 deaths, predominately in Asia.[154] Promising novel drugs are being discovered; the FDA recently approved a new drug, bedaquiline, for MDR-TB therapy; it is to be administered concurrently with other TB medications.[138,155] South Africa now leads the world in rolling out bedaquiline with almost 2,000 patients treated with the drug since 2012.[155] On the other hand, Delamanid, a derivative of a nitro-dihydro-imidazooxazole is primarily used for treating multidrug-resistant pulmonary tuberculosis (**Figure 10**).[156] It has been shown to improve outcomes, reduces mortality and also displays potency against the MDR-TB in pre-clinical in *vivo* and *in vitro* assays, by inhibiting the synthesis of mycolic acids in the cell walls.[156,157]

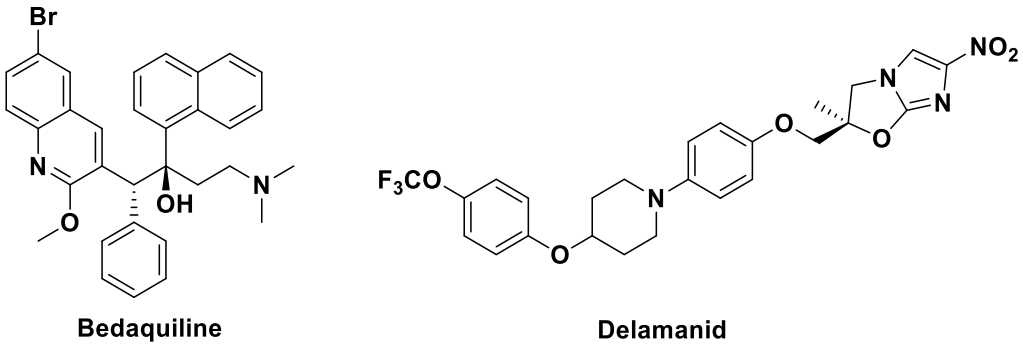


Figure 10: Promising novel drugs for MDR-TB therapy.

Currently, there are several treatment regimens recommended for drug-susceptible forms of TB, with the standard long-course treatments lasting for at least six months.[146] The need for finding effective new drugs remains a challenge, in particular drugs that are effective against the growing number of drug-resistant strains and with a shorter duration of treatments.[158] In the development of new drugs, researchers utilise concepts such as molecular hybridization, where a combination of pharmacophoric moieties of different bioactive compounds are used to generate new hybrid compounds with improved activities.[159,160] Potential multi-drug resistant agents with shorter treatment programmes include compounds bearing the benzimidazole and benzothiazole moieties (**Figure 11**).[161,162]

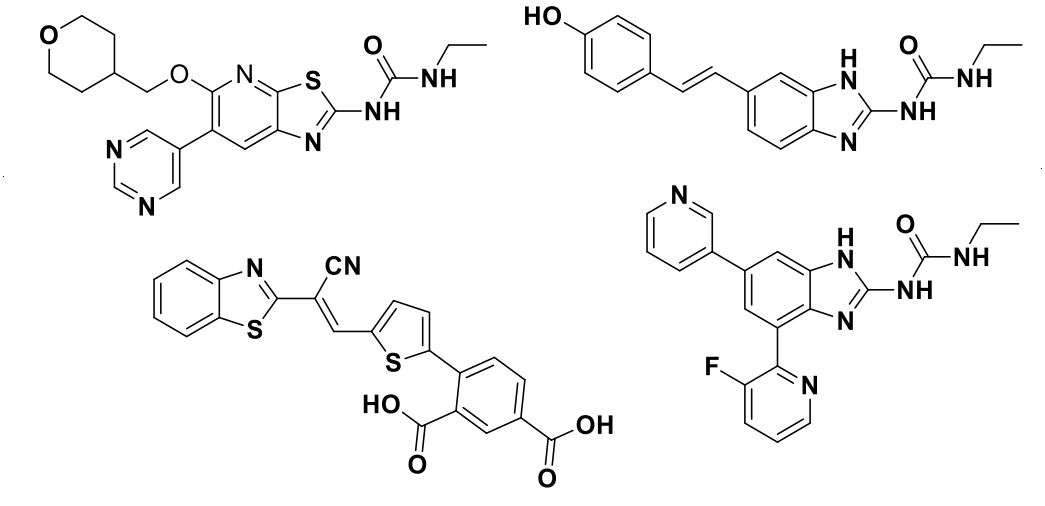
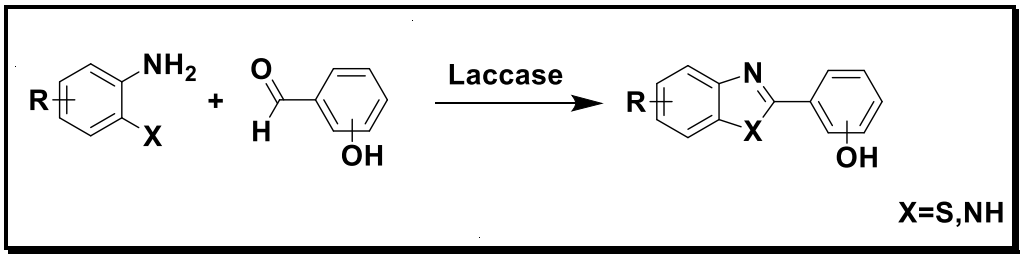


Figure 11: Bioactive anti-TB drugs with a benzothiazole and benzimidazole core.[161,162]

Aims and objectives

The overall aims and objectives of this project are to effectivity investigate and develop biocatalysis methods for the synthesis of target analogues with anti-tuberculosis properties. Our main goal is to develop methods in which biocatalysts are utilized for the key step in the synthesis and are also used to produce the important key intermediates (primarily by functional group interconversion). The target molecules in this project are **Triazoles** (Fused with **Benzimidazoles** and **Benzothiazoles**) and **Quinazolines**. Thus, the project was divided into three sections:

(i) In the first section of this project we further explore our published method for synthesizing **Benzimidazoles** and **Benzothiazoles**. We extend our methodology to benzimidazole and benzothiazole derivatives bearing a hydroxy group on the aromatic ring (**Scheme 19**). The obtained heterocycles will then be used as key intermediates towards the synthesis of triazole analogues in the next section.

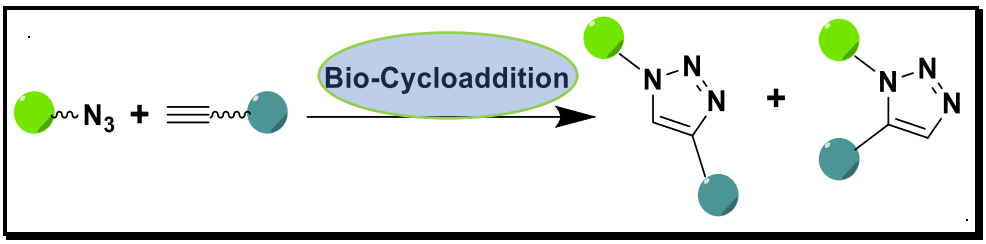


Scheme 19: Synthesis of benzimidazole and benzothiazole derivatives bearing a hydroxy group.

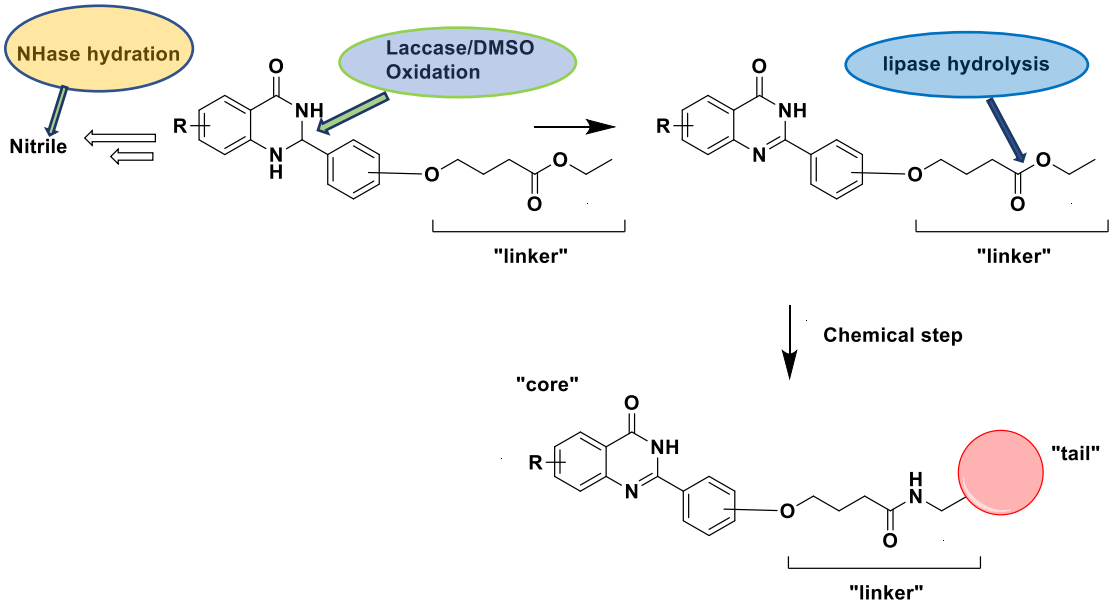
We wanted to better understand the scope and limitations of the laccase-catalysed cyclization reaction when applied to a variety of aryl hydroxy aldehydes. The obtained 2-arylbenzothiazole and 2-aryl-1*H*-benzimidazoles derivatives containing a hydroxy group will then be alkylated to form ether alkynes (Important for **Chapter 3**). Reaction parameters such as temperature, solvent effects and enzyme origin will be investigated.

(ii) The second aim is to investigate and develop a synthetic route for obtaining 2-aryl-1*H*-benzimidazoles and 2-arylbenzothiazole fused triazole analogues. The methodology is based on the combination of pharmacophoric moieties of different compounds to produce derivatives with improved pharmacological properties. As this is a green

project, we ensured that the method involves the incorporation of an enzyme catalyst in the key step of the synthesis. We investigate oxidoreductase enzymes such as laccase as alternatives to chemocatalysts for the synthesis of the triazole ring.



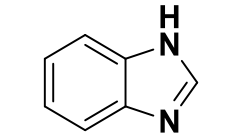
(iii) The third aim is to investigate and develop a synthetic method for obtaining 2,3-dihydro quinazolin-4(3*H*)-ones. We consider the development of a synthetic method in which biocatalysts are utilized for the key step through functional group interconversion. Our method makes use of the following enzymes: Nitrile hydratases (EC 4.2.1.84) for the hydrolysis of nitriles, Laccases (EC1.10.3.2) oxidative dehydrogenation reactions and lipases (EC 3.1) for the hydrolysis/amidation reactions. We aimed at an approach which would warrant the application of multiple enzymes in the synthesis of important pharmaceuticals. And finally, to evaluate and identify the current and potential limitations of the mentioned enzymes for their use in biocatalytic organic reactions.



Chapter Two

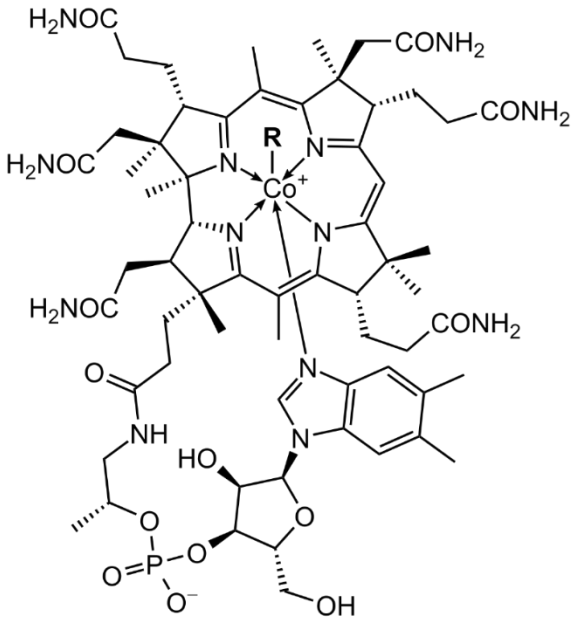
SYNTHESIS OF PROPARGYL BENZIMIDAZOLE AND BENZOTHIAZOLE DERIVATIVES

BENZIMIDAZOLES



Benzimidazole

Benzimidazole is a bicyclic nitrogen-containing heterocyclic aromatic compound made up of an imidazole ring fused with a benzene ring.[81,137] Benzimidazole compounds have continuously remained significant pharmacophores in natural products, pharmaceuticals and synthetic chemistry.[163] Their scaffold can be isolated from many naturally occurring compounds such as vitamin B12 (where *N*-ribosyl-dimethyl benzimidazole serves to provide an axial ligand for cobalt), and they have a structure related to that of purines (**Figure 12**).[81,164,165]



R = 5'-deoxyadenosyl, CH_3, OH, CN

Figure 12:: Structure of Vitamin B12.[165]

Because of their ability to form hydrogen bonds with many biological enzymes and protein receptors, the benzimidazole scaffold has been identified to display an extensive range of biological activities.[137,163] They are effective against human cytomegalovirus (HCMV)[137] and have been demonstrated as the common scaffold that inhibits kinases

through various binding modes.[166] They have also shown anti-microbial; anti-viral; anti-neoplastic; anti-tubercular; anti-Parkinson's, anti-diabetic and anti-depressant activities.[81,164,167] Some of the benzimidazole derivatives found in active pharmaceuticals include Maribavir (anti-cancer)[168]; Hoechst-33258 (antitumour activity)[164]; Astemizole (antihistamine)[165], Bendamustine (anti-cancer)[169] (**Figure 13**). Benzimidazoles fused with triazoles have displayed anti-candidal activity[163], anti-cancer agents[164,166], mushroom tyrosinase inhibitors[170] and anti-proliferative agents[167].

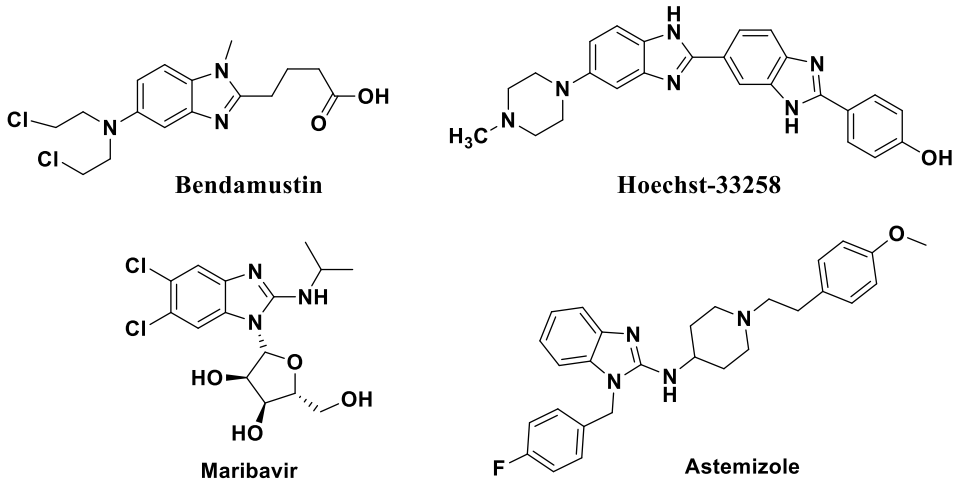
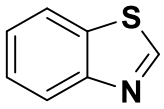


Figure 13: Selected bioactive compounds comprising benzimidazole core.

Compounds bearing the benzimidazole scaffold have also been useful in many fields including material science applications; for example, they are used as light-emitting diodes and as membranes for fuel cells.[81,171] Benzimidazoles can also be employed as organic solderability preservatives to prevent copper oxidation in circuit board manufacturing.[172] Moreover, numerous dyes derived from benzimidazoles are used in photosensitizer and fluorescence probes for interaction with double-stranded DNA and cells.[173]

The first benzimidazole was synthesised by Hoebrecker in 1872 through the reduction of 2-nitro-4-methyl acetanilide in acidic conditions yielding 2,5-dimethyl benzimidazole or 2,6-dimethyl benzimidazole regioisomers.[174] Currently the primary method for preparing benzimidazole derivatives is the condensation—dehydration reaction of 1,2-phenylenediamines with carbonyl compounds such as aldehydes or acid derivatives in the presence of strong oxidants.[81]

BENZOTHIAZOLES



Benzo[*d*]thiazole

Benzothiazole derivatives are an important class of bicyclic ring systems consisting of a benzene attached to a five-membered ring containing sulfur and nitrogen atoms (thiazole ring).[81,175] Compounds baring the benzothiazole scaffold are found in several marine and terrestrial natural compounds.[175,176] Benzothiazole was first extracted from the volatile components of the juice of the American cranberries (*Vaccinium macrocarpon* Ait. *var*. Early Black) in 1967.[177] Benzothiazole have appeared in various elements of the environment, including plants, animals, and bacteria. They have been discovered in the tail-gland of the red deer (*Cervus elaphus*), identified as one of the sulfur volatiles in wines, and also part of French oak wood extracts.[176,178] A Benzothiazole derivative (6-hydroxybenzothiazole-5-acetic acid) was isolated from bacterium *Actinosynnema* sp (**Figure 14**).[176]

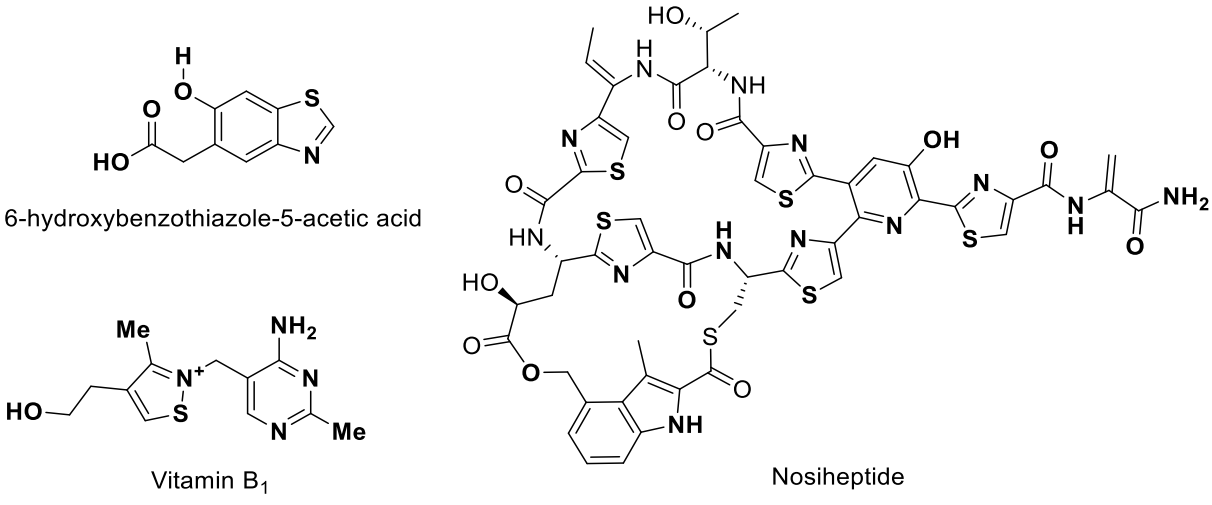


Figure 14: Natural occurring thiazole derivatives.[176]

However, benzothiazoles are somewhat rare as natural products, conceivably because the biosynthetic route towards benzothiazole ring systems is relatively difficult when compared to simple thiazoles rings. The thiazole ring occurs quite often in nature; it is

essential for the function of thiamine (vitamin B_1) and is also part of the scaffold of antibiotics such as nosiheptide as shown in **Figure 14** above.[176]

Benzothiazoles tailor to a wide range of essential roles both in synthetic and naturally occurring substances. They are widely used as plant growth regulators, vulcanization accelerators, antioxidants, imaging reagents, and fluorescence materials.[179] Benzothiazole derivatives form an important subset of interesting products widely used as precursors and intermediates in medicinal chemistry (**Figure 15**).[81,176,179] They provide an extensive range of therapeutic activities including, anti-nociceptive[175]; anti-inflammatory[180]; antitumor[181]; anti-cancer[182]; anti-microbial[183]; antitubercular[184]; anti-Moraxella catarrhalis[185]; anti-HIV[186]; anti-bacterial[187] and anthelmintic activity.[188] Naturally occurring benzothiazole derivatives such erythrazoles A and B have been reported to exhibit anti-cancer activity[189]; fluoro benzothiazole fused quinazolinyl oxadiazoles derivatives, compound A showed anti-bacterial activity[190]; and fatty acid oxidation inhibitor (R)-CVT-3501.[191]

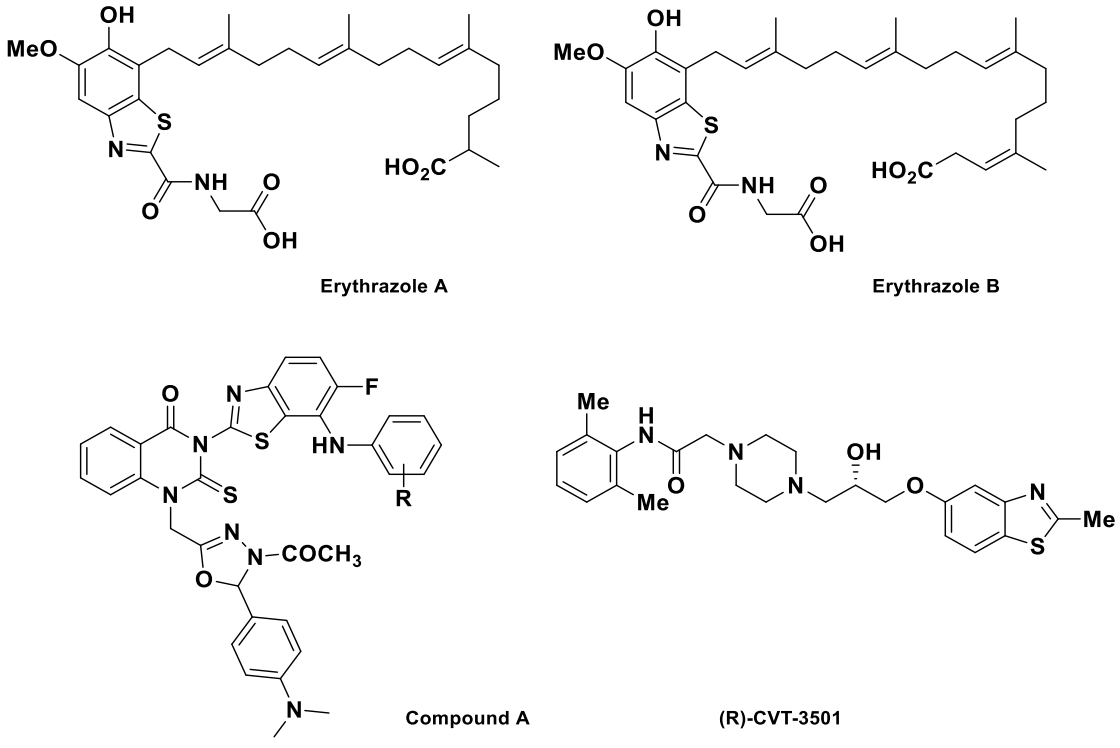


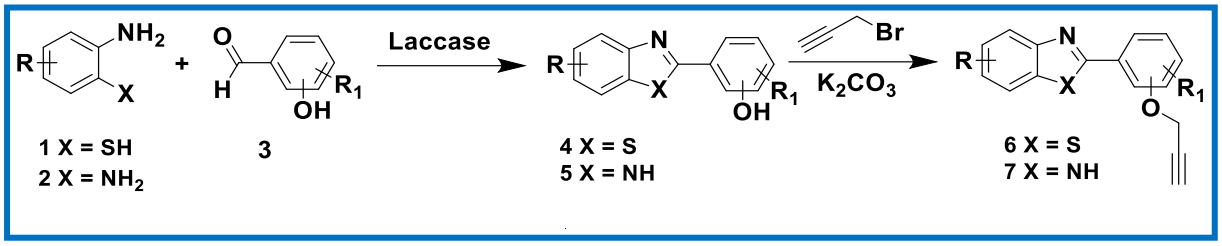
Figure 15: Bioactive benzothiazole derivatives.

There is an extensive scope of existing methodologies available for producing the benzothiazole nucleus.[192] Benzothiazole derivatives are prepared via two major routes: the common method adopted by many involves the oxidative dehydration synthesis

reaction between ortho-phenylamines and aldehydes or with carboxylic acids, acyl chloride or nitrile under high-temperature conditions.[81,192] Another effective method for benzothiazole synthesis is the Jacobsen cyclization; making use of potassium ferricyanide with sodium hydroxide to achieve radical based cyclization of thiobenzanilides yielding a single product.[192]

This chapter aims to give an overview of some of the investigations performed by our group on the synthesis of benzimidazole and benzothiazole heterocycles. We have previously published an effective method for the synthesis of benzothiazoles and benzimidazoles in very good yields. This was accomplished by treating *o*-arylamines with various arylaldehyde derivatives using commercial laccases, Novoprime and Suberase® under mild conditions.[81] In this chapter, we look at some more reactions of 2-aminoaromatics with benzaldehydes under laccase aerobic conditions. The general utility of our previously published method was extended to the synthesis of hydroxy-containing benzimidazole and benzothiazole heterocycles. The obtained heterocycles will be alkylated using the Williamson ether synthesis method and applied as intermediates in the synthesis of triazoles in **Chapter 3**.

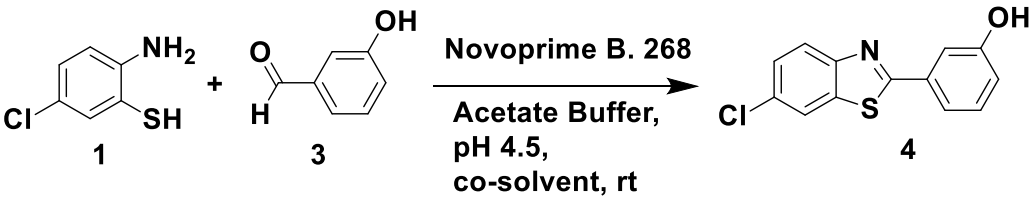
We have previously demonstrated the novel application of laccase in the synthesis of 2-aryl-1*H*-benzimidazoles and 2-arylbenzothiazoles, achieving a wide range of novel compounds (Maphupha *et al*. -2018).[81] We now want to build on these scaffolds, introducing a hydroxyl group to create novel compounds that would potentially have anti-tubercular properties.The obtained heterocycles will then be alkylated using the Williamson ether synthesis to form alkynes (**Scheme 20**). The main goal was to attach the benzimidazole and benzothiazole cores to another structural entity (triazoles) in **chapter 3** and assess the range and limitations of our synthetic method.



Scheme 20: preparation of 2-aryl-*1H*-benzimidazoles and 2-arylbenzothiazole alkyne derivatives.[81]

SYNTHESIS OF HYDROXY 2-ARYLBENZOTHIAZOLES

Previously, we reported acetate buffer (0.1 M, pH 4.5) and acetonitrile (co-solvent) as the optimal reaction medium for obtaining 2-arylbenzothiazoles under laccase conditions.[81] As a control, we began our study by investigating solvent effects using organic solvents such as ethanol, acetonitrile, methanol, acetone, DMF and DMSO as co-solvents. The condensation reaction between 2-amino-4-chlorobenzenethiol (**1**) and 3-hydroxybenzaldehyde (**3**) at room temperature was chosen as a model reaction for the solvent study (**Scheme 21**).



Scheme 21: Laccase-catalysed synthesis of 3-(6-chlorobenzo[d]thiazol-2-yl)phenol (4b).

The role of co-solvent on the condensation reaction between **1** and **3** was studied in detail by varying organic solvents and the results are summarised in **Table 2**. We were pleased to observe similar trends as we did previously. As shown the enzyme was found to be inactive in highly polar solvents such as DMF and DMSO (Table 2, entries 2;4). Like before, the reaction did not reach completion after 24 h while using methanol and ethanol as co-solvents. Acetonitrile and acetone were the best solvents for the condensation reaction with 90% and 83% yield of benzothiazole **4** respectively.

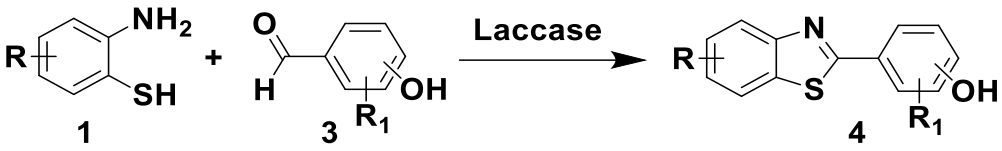
Table 2: Effect of co-solvent on Novoprime B.268-catalysed synthesis of 3-(6-chlorobenzo*[d]*thiazol-2-yl)phenol (**4**) by reaction of 2-Amino-4-chlorobenzenethiol (**1**) and 3-hydroxybenzaldehyde (**3**) in acetate buffer (0.1 M, pH 4.0).

Entry	Co-solvent	Time	Yield %
1	Acetonitrile	24h	90
2	DMF	24h	70[a]
3	Methanol	24h	–
4	DMSO	24h	–[a]
5	Ethanol	48h	–
6	Acetone	24h	83

[a]The crude product is a mixture of **4** and starting material **1**.

Having in hand the optimized conditions for the synthesis of 3-(6-chlorobenzo*[d]*thiazol-2-yl)phenol (**4**), various derivatives of 2-aminothiophenol (**1**) were reacted with a selection of aromatic hydroxy aldehydes (**3**) utilising laccase as a catalyst to yield hydroxy benzothiazoles (**4**) (**Scheme 22**). The synthesis of hydroxy-containing 2-arylbenzothiazoles was performed using a laccase-catalysed condensation reaction between 2-aminothiophenol derivatives (**1**) and hydroxybenzaldehyde (**3**) under aerobic conditions at room temperature afforded 2-phenyl-benzothiazoles **4(a-f)** in moderate to excellent yields (**Table 3**). To our surprise, our current method appeared to be problematic. Hydroxybenzaldehyde derivatives were chemically unstable, with many of the substrates decomposing during the reaction. For most of the entries, reactions with hydroxybenzaldehydes did not reach completion and purification of products was a challenge, predominantly due to insolubility of the products in organic solvents.

Table 3: Novoprime Base 268-catalysed synthesis of hydroxy-containing 2-arylbenzothiazoles via reaction of 2-aminothiophenol **1** with hydroxy-benzaldehyde **3** in acetate buffer (0.1 M, pH 4.0)/acetonitrile (50:50) for 24 h.



Scheme 22: Laccase-catalysed synthesis of hydroxybenzothiazoles.

Entry	Amino thiophenol	Benzaldehyde	Product	Yield %
1	R= H	R_1= 3-OH	4a[a]	72
2	R= 4-Cl	R_1= 2-OH,5-OMe	4e	66
3	R= 4-CF_3	R_1= 4-OH	4f	47
4	R= H	R_1= 2-OH, 5-Br	4d	39
5	R= 4-Cl	R_1= 3-OH	4b	90
6	R= H	R_1= 2-OH, 5-OMe	-	-
7	R= 4-CF_3	R_1= 3-OH	4c	86
8	R= 4-Cl	R_1= 2-OH, 5-Br	-	-

[a]The crude product is a mixture of **4**, starting material **1** and a polymerised side product of unknown structure.

Polymerized side products from the self-coupling reaction of 2-aminothiophenols under oxidative conditions were also observed, which suggests that the condensation between aminothiophenol and the aryl-aldehyde is relatively slow. With a combination of slow reaction rates, competition between condensation and self-coupling reactions and co-elution, selective generation of hydroxy 2-Arylbenzothiazoles was quite difficult and the yields were slightly worse than those obtained in the published method.[81]

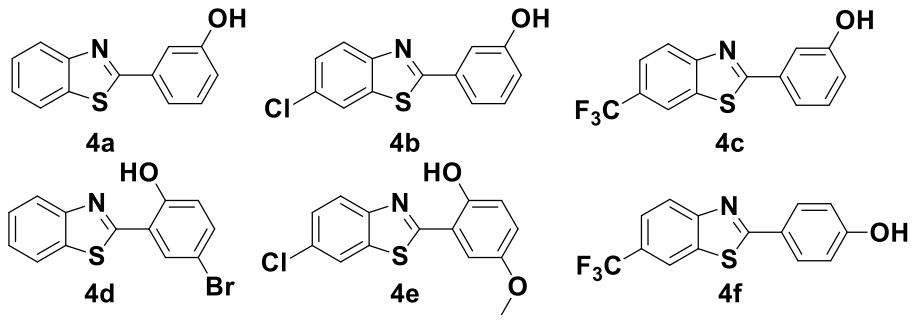


Figure 16:Hydroxy-benzothiazole derivatives.

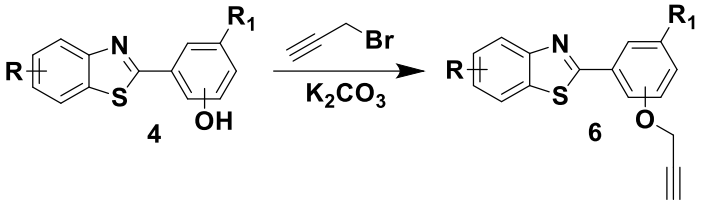
It is evident that the electronic nature and position of the functionalised hydroxybenzaldehydes and aminothiophenol have a strong influence on the dynamics of the reaction. Having electron-donating and withdrawing groups yielded the

subsequent products in moderate to good yields respectively (**Table 3, entries 2; 5; 7**). We noticed that meta hydroxybenzaldehydes were relatively stable during the reaction and as a result, better yields were obtained with hydroxy groups in the *meta* position (**Table 3, entries 1; 5; 7**), compared to the *ortho* and *para* positions (**Table 3, entries: 2; 3; 4**).

SYNTHESIS OF PROPARGYL 2-ARYLBENZOTHIAZOLES

The purified hydroxy 2-arylbenzothiazoles **4** were further treated with potassium carbonate and propargyl bromide to obtain the propargylated benzothiazoles (**6a-f**) in excellent yields (**Table 4**).

Table 4: Reaction of hydroxybenzothiazole (**4a-f**) with propargyl bromide under an inert atmosphere in acetone.



Entry	Benzothiazole	Time	6 (Yield %)[a]
1	4a	24 h	80[b]
2	4b	4 h	97
3	4c	6 h	88
4	4d	24 h	77[b]
5	4e	18 h	76
6	4f	24 h	82[b]

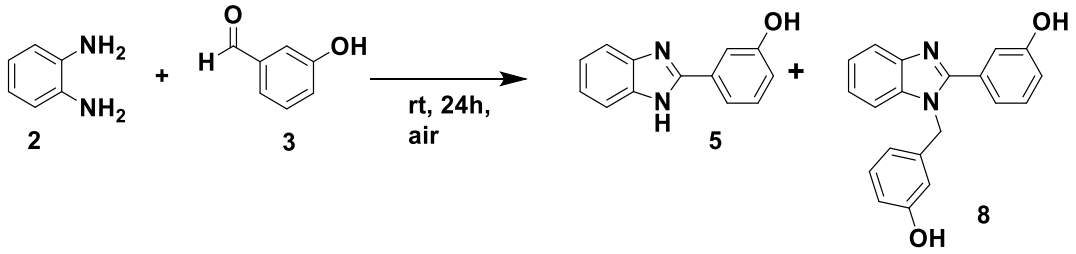
a- Reactions were conducted in acetone at 65°C under nitrogen
b- The crude product is a mixture of **6** and starting material **4**

The rapid alkylations of benzothiazoles were performed in anhydrous acetone at 65°C under nitrogen overnight and the reaction displayed high efficiency overall. However, purification challenges were encountered with a few substrates due to co-elution (**Table 4, entries 1; 4; 6**).

SYNTHESIS OF HYDROXY 2-ARYL-*1H*-Benzimidazoles

It has been extensively reported that the reaction between *o*-phenylenediamine and aldehydes is not selective. The reaction typically results in the formation of a 2-substituted benzimidazole concurrently with a 2,3-disubstituted side-product or sometimes as the principal product.[81,193] In our previous study, we demonstrated how to selectively control regioselectivity for a series of substrates using laccase enzymes.[81] In this study, our interest was in both the mono- and di-substituted benzimidazole. We, therefore, conducted reactions in the absence of laccase during our initial investigations and with the enzyme present in the second run (**Table 5**, **Scheme 23**). The reactions were carried out using *o*-phenylenediamine and 3-hydroxybenzaldehyde as the model reaction.

Table 5: Comparison of laccase and laccase-free methods in the synthesis of mono- and disubstituted hydroxy benzimidazoles through the reaction of *o*-phenylenediamine and 3-hydroxybenzaldehyde in acetate buffer (0.1 M, pH 4.5) and acetonitrile for 24h.



Scheme 23: synthesis of mono- and disubstituted hydroxy benzimidazoles.

Entry	Laccase	(5) Yield %	(8) Yield %
1	Catalyst free control	polymerisation	polymerisation
2	Novoprime B 268	a	62
3	Denilite II B.	-	-
4	*T. versicolor* laccase[a]	a	11
5	Suberase	a	a
6	*Denimax*[e]	polymerisation	polymerisation

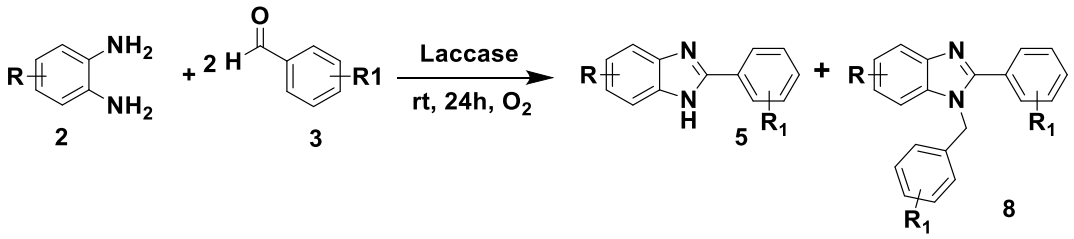
a- The crude product is a mixture of **5** and **11** with starting material **3.**

Similar to that of benzothiazoles, both enzyme and enzyme-free methods were inefficient for the reaction of *o*-phenylenediamine and 3-hydroxybenzaldehyde. For both the mono- and di-substituted benzimidazoles, the enzyme-free procedure was overall not as effective as the enzyme-catalysed method. The direct comparison of the two methods revealed that selectivity and isolated yields were slightly better with the

enzyme-catalysed method. On investigating the enzyme-free method, we found that multiple products were being generated and among these were the imine intermediate together with the mono- and di-substituted benzimidazoles. And in many of the cases, a series of insoluble polymer-like side products were formed. Additionally, decomposition of the hydroxy benzaldehyde substrates was much more common, often yielding a dark-brown or black tar-like substance.

Having identified the enzyme-catalysed method (Novoprime B. 268) as promising, *o*-phenylenediamine derivatives were treated with various hydroxy benzaldehyde derivatives and the reactions yielded both the mono- and disubstituted products. Benzimidazoles **5** and 2,3-disubstituted benzimidazoles **8** were synthesised through the reaction of *o*-phenylenediamine derivatives **2** and hydroxybenzaldehyde derivatives **3** using Novoprime B.268 as an oxidant at room temperature (**Table 6, Scheme 24**).

Table 6: Laccase-catalysed synthesis of hydroxy-benzimidazoles. The reaction of 1 equiv *o*-phenylenediamine derivative (**2**) with 2 equiv of hydroxybenzaldehyde (**3**), open vessel in acetate buffer (0.1 M, pH 4.0)/acetonitrile (50:50) in the presence of laccase.



Scheme 24: Laccase-catalysed synthesis of hydroxybenzimidazoles

Entry	Diamine	Aldehyde	(5) Yield %	(8) Yield %
1	R= H	R_1= 3-OH	a	62
2	R= 4-Cl	R_1= 2-OH, 5-OMe	-	-
3	R= H	R_1= 4-OH	a	a
4	R= 4-Br	R_1= 3-OH	54	-
5	R= 4-CH_3	R_1= 3-OH	24[b]	-
6	R= 4-Br	R_1= 2-OH, 5-OMe	-	-
7	R= 4-Cl	R_1= 3-OH	59	36[a]

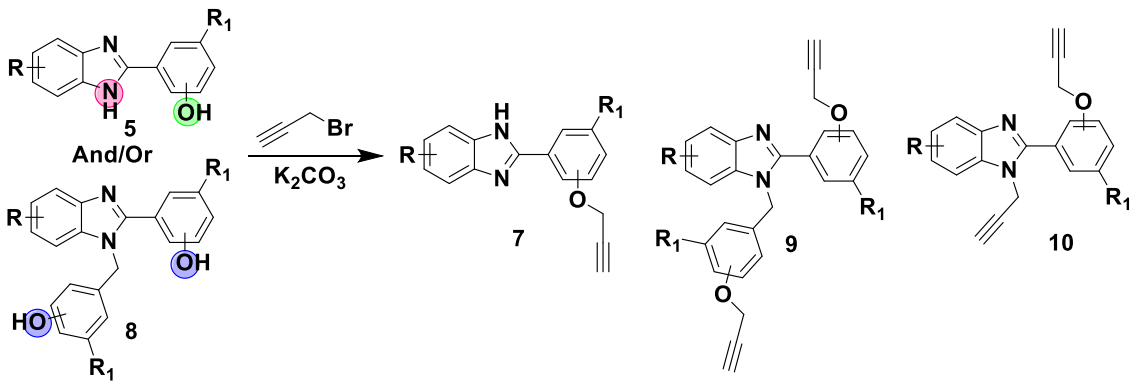
a-The crude product is a mixture of **5** and **8** with starting material **3.**

b- The crude product also contains a polymerised side product of an unknown structure.

The chemistry of benzimidazoles shows similarities to that of benzothiazoles—the synthetic utility of our method appears to be limited to hydroxy benzaldehydes. The overall trend observed in these reactions was the apparent decomposition of the hydroxy benzaldehyde substrates upon the addition of the diamine substrate. The imine failed to form from the initial condensation, presumably due to unstable benzaldehydes, but it was soon found that the yields improved upon the addition of two equivalents of the hydroxybenzaldehyde (**Table 6**). Consistent with the benzothiazoles, better results were obtained with hydroxybenzaldehyde derivatives functionalised at *meta* position (**Table 6, entries 1; 4; 8**), compared to *ortho* and *para* counterparts. Unlike benzothiazoles, the functionalised hydroxybenzaldehydes had limited reactivity and chemoselectivity, with frequent decompositions and polymerisations. For most of the substrates, an inseparable mixture consisting of multiple compounds was formed containing both the mono- and di-substituted benzimidazoles in the mixture.

SYNTHESIS OF PROPARGYL 2-ARYL-*1H*-BENZIMIDAZOLES

The reaction of *o*-phenylenediamine derivatives **2** and hydroxybenzaldehyde derivatives **3** produces a considerably large amount of side products. So, without purification, the products were further treated with a base and propargyl bromide under inert conditions to obtain propargylated benzimidazole derivatives (**Scheme 25**).

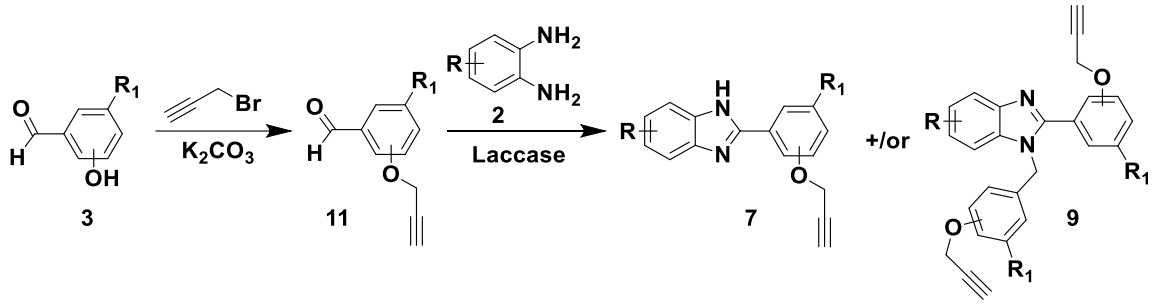


Scheme 25: Alkylation of benzimidazole derivatives with propargyl bromide.

Chemo-selectivity was a major challenge in this step, as shown in **Scheme 25** above, alkylation could occur in any of the highlighted positions; thereby making isolation of the target product difficult. To overcome this problem, it was decided to change the

reaction sequence and alkylate the hydroxybenzyladehyde derivatives first, and then subsequently perform the oxidative condensation reaction (**Table 7, Scheme 26**).

Table 7: Synthesis of alkyne-benzimidazole derivatives. The reaction of 1 equiv *o*-phenylenediamine derivatives (**2**) with 2 equiv of propargyl benzaldehydes (**11**), open vessel in acetate buffer (pH 4.0) in the presence of laccase.



Scheme 26: Synthesis of alkyne-benzimidazole derivatives.

Entry	Diamine	Propargylaldehyde	(7) Yield%	(9) Yield %
1	R= H	R_1= H, 3-propargyl	67	-
2	R= 4-CH_3	R_1= H, 3-propargyl	28[a]	-
3	R= H	R_1= H, 4-propargyl	36	a
4	R= 4-Br	R_1= H, 3-propargyl	56[a]	-
5	R= 4-CH_3	R_1= 5-OMe, 2-propargyl	-	-
6	R= 4-Cl	R_1= H, 3-propargyl	47[a]	-

a- The crude product is a mixture of **7** and **9** with starting material **11.**

Despite the minimisation of side reactions, some challenges remained. Regioselectivity was still a problem and the introduction of an electron excessive alkyne reduced electrophilicity of the carbonyl carbon of the aldehyde (**11**), which consequently resulted in slower reaction rates. We were able to isolate some of the target compounds (**Table 7, entries: 1; 3; 4**). However, due to solubility and co-elution, others were difficult to separate (**Table 7, entries: 2; 6**) and were carried on to the next step (**Chapter 3**) without purification.

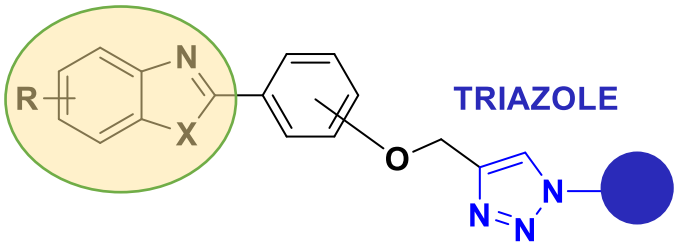
CONCLUSION

A critical review of our current protocol shows that the condensation reaction is dependent on the nature of the benzaldehyde derivative. The method works best with aryl aldehydes without a hydroxy group substituent, as these are relatively unstable under laccase conditions. Although we faced multiple challenges, as a consequence of aryl aldehyde decomposition, polymerisation, poor product solubility and co-elution, the work has provided many insights on the utility and limitations of our current method. Far better outcomes were accomplished with hydroxybenzothiazole derivatives with yields of up to 90%, although hydroxybenzimidazole reactions yielded limited results. Nonetheless, using our published method, we have achieved a series of hydroxy 2-aryl-1*H*-benzimidazoles and 2-arylbenzothiazoles, some of which are novel. Perhaps a different approach, like making use of stable substrates such as carboxylic acids or alcohols instead of aldehydes would yield better results. With purified hydroxy benzothiazoles or benzimidazoles in hand, performing the alkylation of the preceding substrates in anhydrous acetone carried on effectively. Relatively, we have shown that conducting the alkylation of the hydroxy benzaldehyde before the condensation reaction provides a useful and selective approach to the introduction of the alkyne group on arylbenzaldehyde derivatives.

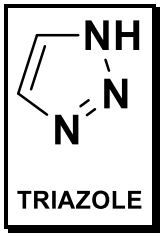
In summary, we believe that our approach to the preparation of propargyl benzothiazoles and benzimidazoles has great potential. We are confident that if we limit or eliminate the decomposition of the hydroxy benzaldehyde substrates during the reaction, our reaction outcomes will improve drastically.

Chapter Three

A BIOCATALYTIC SYNTHESIS OF NOVEL 2-ARYL-*1H*-BENZIMIDAZOLES AND 2-ARYLBENZOTHIAZOLES FUZED TRIAZOLES



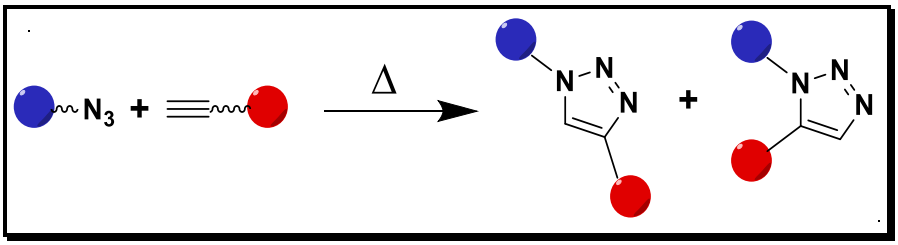
X = S, NH



TRIAZOLES

The concept "click chemistry" was independently introduced by Sharpless and Meldal groups in 2001.[194,195] It describes a copper-catalysed alkyne-azide cycloaddition reaction (CuAAC) that allows the effective and selective synthesis of 1,2,3-triazole rings.[196] The history of triazoles dates back to the 1800s when Michael discovered a synthesis of 1,2,3-triazoles through a reaction between phenyl azide and dimethyl acetylenedicarboxylate. This was then later recognized by Rolf Huisgen as the (3+2) 1,3-dipolar cycloaddition (reaction between an azide and a terminal/internal alkyne), which led to the discovery of the famous Huisgen 1,3-dipolar cycloaddition reaction.[195,197]

The conventional Huisgen cycloaddition reaction results in the formation of a mixture of 1,4- and 1,5-disubstituted 1,2,3-triazole regioisomers, but with a rather slow reaction rate (**Scheme 27**).[198,199] The reaction rates and regioselectivity issues were later explained by Sustmann *et al.* and Houk *et al.* using a frontier molecular orbital (FMO) model.[197] Concisely, the model explains how the reaction between the 1,3-dipole and the 1,3-dipolarophile progresses through the interaction between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of the reactants, where the reaction rate is dependent on the energy gap between them .[197,200]



Scheme 27: Thermal synthesis of triazole ring.

Both regioselectivity and rate of reaction depend on the electron-donating and withdrawing effects of the 1,3-dipole and the 1,3-dipolarophile; with the electron-poor and electron-rich alkynes yielding the 1,4-regioisomer and the 1,5-regioisomer respectively.[197] The reaction rate subsequently increases with an electron-withdrawing group lowering the LUMO together with the electron-donating group raising HOMO energy levels (**Figure 17**).[197,201]

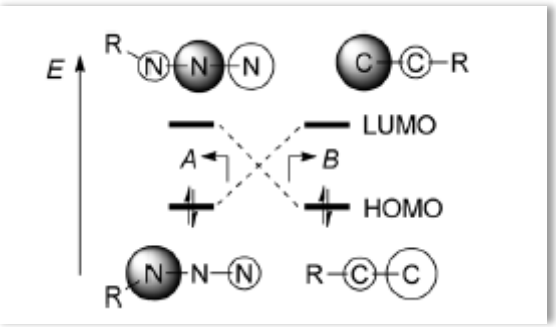


Figure 17: FMO interactions between azide and alkyne (R = Ph).[197]

Over the years, several methods were explored to gain control over both efficiency and selectivity of the reaction for the formation of the triazole ring.[195] The copper(I)-catalysed azide-alkyne cycloaddition reaction (CuAAC) remains the most prominent and well recognized, yielding exclusively the 1,4-regioisomer with great efficiency.[195,202] McNulty *et al*.,[203] explored the silver acetate protocol; silver(I)-catalysed cycloaddition of azides onto terminal alkynes (Ag-AAC) reaction to exclusively obtain the 1,4-triazole regioisomer.[195,203] Zhang, *et al.* (2005)[204] described the ruthenium-catalysed azide-alkyne cycloaddition reaction (Ru-AAC) for the synthesis of 1,5-disubstituted-triazole regioisomer, employing cyclopentadienyl (Cp) ligands.[195,197,204]

Several non-classical methodologies developed for the synthesis of 1,2,3-triazoles derivatives include microwave-assisted click-chemistry[137,166,205,206], ultrasound irradiation[166,207,208], flow chemistry[195,209,210], click-chemistry on polymer support[195,211], room temperature ionic liquids (RTIL)[212,213] and *in situ* click-chemistry (using enzyme targets to synthesise inhibitors from corresponding building block reagents).

Triazoles are unexpectedly stable relative to other organic compounds containing three nitrogens adjacent to one another. Because of hydrogen bonding properties, triazoles

have excellent solubility properties and can easily bind to biomolecular targets.[214] Compounds bearing the triazole skeleton have been shown to exhibit pharmacological properties such as anti-tubercular, anti-bacterial, anti-allergic, and anti-fungal activity.[138,215] Triazoles exhibit anti-TB and antifungal activity by inhibiting cell wall synthesis and some of the examples of well known antifungal drugs are Albaconazole, Ravuconazole, Voriconazole, Itraconazole, Posaconazole and Fluconazole.[138,216] A series of important biological activities 1,2,3-triazole pharmacophores fused to benzimidazoles and benzothiazoles heterocycles, this include antibacterial[137], anti-tuberculosis[160], antimicrobial and anti-tb[185] (**Figure 18**). Because of this, several synthetic methods have been developed for the synthesis of triazole incorporated derivatives.

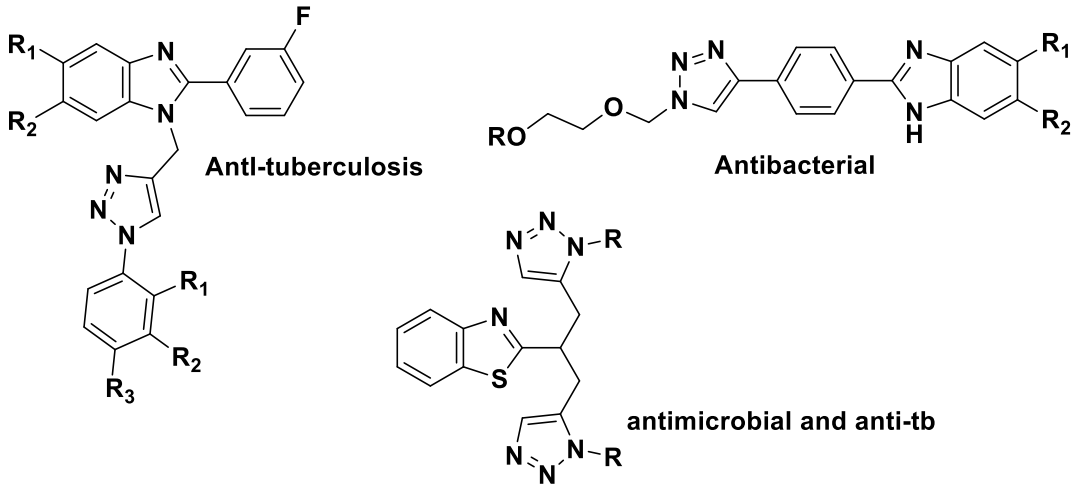
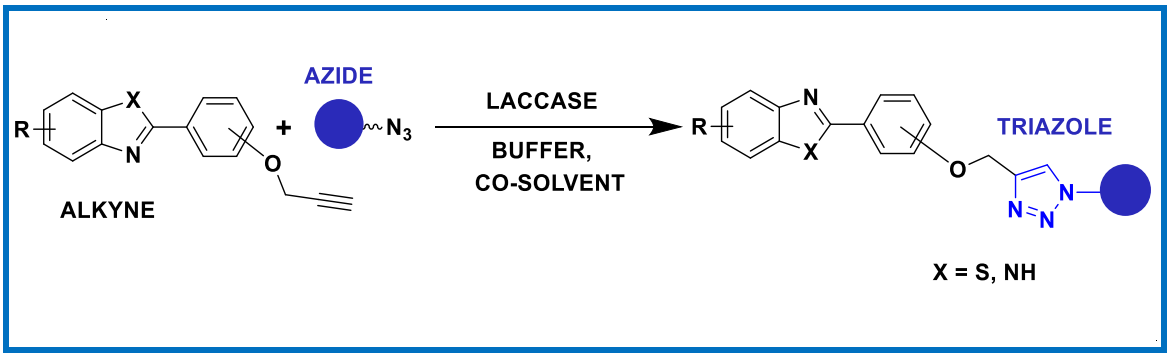


Figure 18: Bioactive clubbed 1,2,3-triazoles by benzimidazole and benzothiazoles.

Herein, we report for the first time, a laccase catalysed synthesis of triazole ring fused with benzimidazole and benzothiazole derivatives for their use as anti-tuberculosis agents. The methodology is based on the combination of pharmacophoric moieties of different compounds to produce derivatives with improved pharmacological properties. To the best of our knowledge, this is the first example of the use of laccase enzymes as coupling reagents in the alkyne-azide cycloaddition reaction.

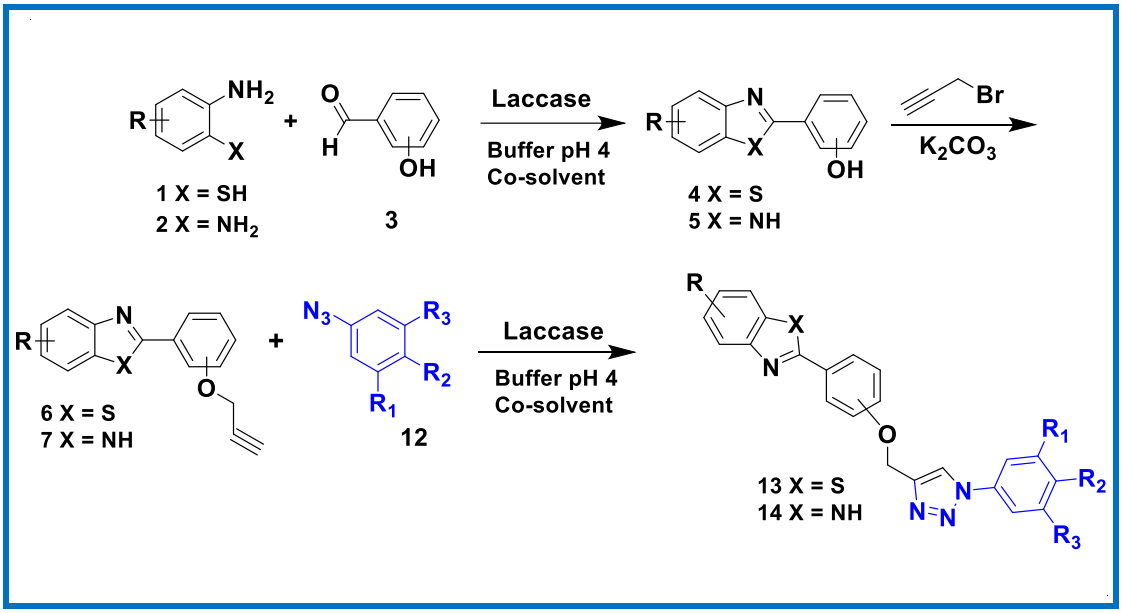
We introduce a new approach for synthesizing triazole rings through the application of copper-containing oxidoreductase enzymes. A series of benzothiazole and benzimidazole-1,2,3-triazoles were synthesized through a laccase catalysed 1,3-dipolar cycloaddition (**Scheme 28**).



Scheme 28: Laccase-catalysed synthesis of benzimidazole/benzothiazole fused triazoles.

Most of the research has been driven into understanding the mechanistic characteristics of the CuAAC reaction and finding alternative and improved catalytic systems. It has been reported that reaction parameters such as sources of copper(I), electron-donating and withdrawing effects of azides, solvents effects and the use of additives for CuAAC systems, play a significant role in the selectivity and progression of the CuAAC reaction.[217] Based on the premise that laccases are copper-containing enzymes, we explored their potential as catalysts for the alkyne-azide cycloaddition reaction. Our synthetic route follows a simple and atom economy friendly procedure.

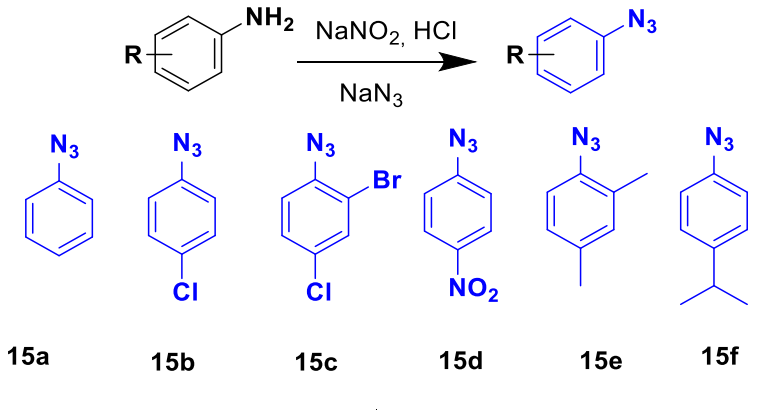
In **Chapter Two** we synthesised a series of hydroxy-based benzothiazole and benzimidazole derivatives**,** following our previous laccase protocol for obtaining benzothiazoles and benzimidazoles through reacting 2-aminoaromatics with various hydroxybenzaldehyde derivatives.[81] This was then followed by alkylation of the obtained hydroxy-benzothiazole/benzimidazole derivatives with propargyl bromide under basic conditions. In continuation, the resultant propargyl derivatives were treated with phenyl-azides under laccase conditions to obtain the target benzothiazole and benzimidazole-1,2,3-triazole derivatives (**Scheme 29**).



Scheme 29: The synthetic route implemented for the synthesis of triazole hybrids.

SYNTHESIS OF PHENYL AZIDES

Having our target benzimidazole and benzothiazole alkynes ready from **Chapter two**, our next step was to prepare phenyl azides. Following the standard azide synthesis approach, several aromatic amines were successively converted into azides from the reaction of sodium nitrite with *in situ* generated diazonium salts (**Scheme 30**).[218]



Scheme 30: Synthesis of phenyl azides from anilines.

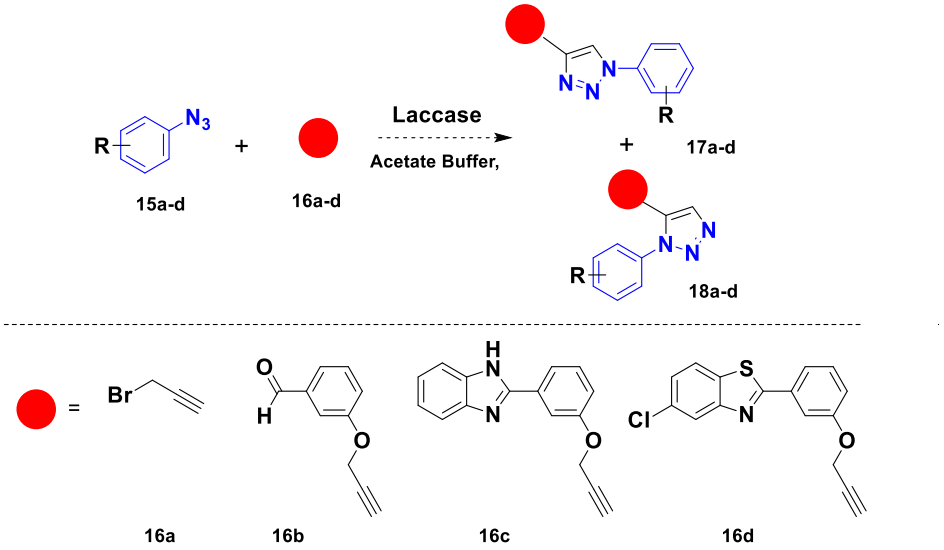
SYNTHESIS OF TRIAZOLES

In the first step towards the laccase catalysed synthesis of triazole rings, we conducted an activity study on an arbitrary selection of azides to identify whether the enzyme

exhibits activity towards the 1, 3-dipolar cycloaddition reaction. We investigated the laccase catalysed reaction between phenyl azides (**15a-d**) and propargyl derivatives (**16a-d**), using Novoprime base 268 from Novozymes (**Table 8, Scheme 31**). The reactions were carried out at room temperature using equimolar amounts of phenyl azides (**15**) and propargyl derivatives (**16**) in acetate buffer (pH 4.5). We were pleased to discover that the enzyme displayed activity for the 1,3-dipolar cycloaddition reaction of propargyl derivatives (**16a**, **b** and **d**) with azides (**15a** and **d**), with a formation of **17** and **18** regioisomers.

- **ENZYME ACTIVITY STUDY**

Table 8: Laccase-catalysed synthesis of substituted 1,2,3-triazoles. Novoprime Base 268-catalysed the formation of substituted 1,2,3-triazoles through the reaction of phenyl azides (**15a-d**) and propargyl derivatives (**16a-d**) in acetate buffer (0.1 M, pH 4.5).



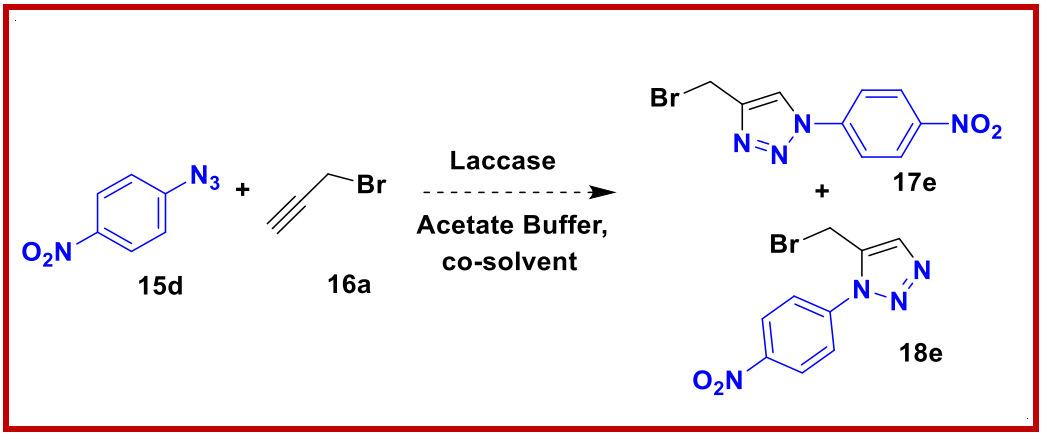
Scheme 31: Novoprime Base 268 activity assay.

Entry	Azide	Alkyne	Time	(17) Yield %	(18) Yield %
1	15a	16a	24 h	5	3
2	15b	16b	24 h	Trace amounts	Trace amounts
3	15c	16c	24 h	-	-
4	15d	16d	24 h	12	7
5	15d	16a	24 h	14	6

However, the reaction produced exceedingly low product yields; with the highest estimated conversion of only 20% (**Table 8**). We observed that the various substituted aromatic substrates were insoluble in the aqueous system, which possibly limited their interaction with the enzyme. Therefore, to improve substrate solubility in our systems, we employed the use of co-solvents together with a buffer. A selection of 'green' solvents compatible with laccases used includes methanol, ethanol, acetone, acetonitrile and 2-methyl-THF.

- **SOLVENT SCREENING**

From our results, propargyl bromide **16a** gave the best results, it was therefore chosen as the model reaction for the solvents effects study (**Scheme 32, Table 9**).



Scheme 32: Laccase-catalysed synthesis of substituted 1,2,3-triazoles in different solvents.

Table 9: Novoprime Base 268-catalysed formation of benzothiazole-1,2,3-triazoles through the reaction of phenyl azides (**15d**) and propargyl bromide (**16a**) in acetate buffer (0.1 M, pH 4.5) and various co-solvents.

Entry	Azide	Solvent	Time	(17) Yield %	(18) Yield %
1	15d	Methanol	24 h	-	-
2	15d	Ethanol	24 h	Trace amounts	Trace amounts
3	15d	Acetone	24 h	24	9
4	15d	Acetonitrile	24 h	20	11
5	15d	2-Methyl-THF	24 h	Trance amounts	-

No significant improvement in the yield was observed, however, the reaction rate increased with an increase in solubility of the substrate. Although no significant conversion was achieved while using methanol, ethanol and 2-methyl-THF as co-solvents, improved activity was observed with acetone and acetonitrile.

Through tracking the reaction using thin-layer chromatography (TLC), it was observed that the products formed shortly after enzyme addition, suggesting that the reaction might have stopped due to enzyme deactivation. Several factors could lead to laccase denaturation; including prolonged exposure to radicals,[219] conformation changes and unfolding[23,220] and dissociation of the copper ions from the active site of the enzyme.[221] Therefore, the reaction in **Scheme 32** was repeated using various quantities of laccase. However, the amount of enzyme used had no significant effect on the outcome of the reaction. Laccase activity varies according to the amino acid sequence, hence molecular weight, coordination ligands; and ultimately redox potential leading to substrate specificity.[23,24] Therefore we investigated the role of enzyme type by comparing various laccase preparations (Suberase®, Denilite® II Base, Denimax, *Trametes versicolor* and Novoprime Base 268) (**Table 10**).

- **ENZYME SCREENING**

Table 10: Evaluation of commercial laccase preparations in the synthesis of substituted 1,2,3-triazoles through the reaction of phenyl azides (**15d**) and propargyl bromide (**16a**) in acetate buffer (0.1 M, pH 4.5) and acetone for 24h.

Entry	Laccase	(17) Yield %	(18) Yield %
1	Suberase	Trace amounts	-
2	Novoprime B 268	f	Trace amounts
3	Denilite II B.	-	-
4	*T. versicolor* laccase[a]	24	11
5	*T. versicolor* laccase *denatured control*[b]	-	-
6	*T. versicolor* laccase oxygen-free control	-	-
7	Catalyst free control	-	-
8	*Denimax*[e]	-	-

a-Also known as *Coriolus versicolor*

Negative control reactions:

[b] Enzyme was purposefully denatured through heating before the reaction (repeated 6 times)

[c] The reaction mixture was purged with nitrogen gas before enzyme addition.

[d] No enzyme added (repeated 3 times).

[e] Enzyme preparation without laccase activity.

[f] Product repeatedly formed, but unrepeatable with subsequent enzyme preparation batches

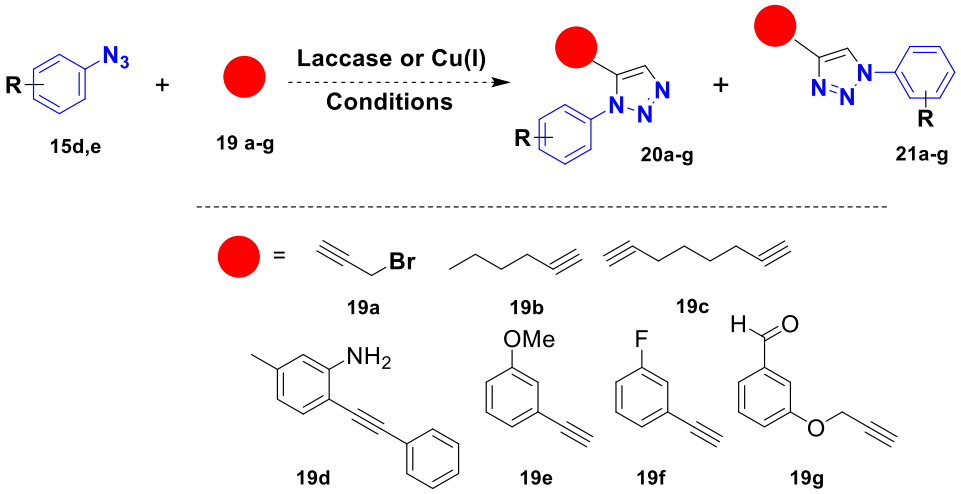
Most of the enzyme preparations tested showed negligible activity overall. Novoprime Base 268 provided some encouraging results, however, it exhibited activity that varied with enzyme batch. One batch of Novoprime Base 268 convincingly catalysed the reaction; however, subsequent batches of Novoprime Base 268 were not active, possibly due to the presence of secondary enzymes in commercial enzyme products.[222] Laccases preparations from *Myceliophthora thermophile* (Suberase® and Denilite® II Base) and from *genus Thielavia* (Denimax) displayed minimal to no activity. With further testing of various laccase preparations, we found that a *Trametes versicolor* laccase preparation successfully catalysed the reaction and activity was present in both enzyme batches tested. The reaction conducted using *Trametes versicolor* afforded a yield of 24% of the 1,2,3-triazole (**17e**) and 11% of the 1,2,4-triazole (**18e**). The reaction did not proceed without enzyme addition.

To confirm the reaction was enzyme catalysed (rather than leaching metal ion from enzyme preparation) we heated the enzyme preparation (*Trametes versicolor*) to 120°C prior to use and found it was deactivated. This also proved that any trace amounts of copper ions associated with the laccase active site or were not capable of catalysing the reaction without the undenatured protein component of the enzyme (apoenzyme). Moreover, we purged the reaction medium with nitrogen gas to prove it was an oxygen-dependent reaction due to an oxidase (as opposed to promiscuous enzyme activity) and no activity was observed under these conditions.

- **SUBSTRATE SCREENING**

Using *Trametes versicolor* laccase, we catalysed the reaction between phenyl azide and a selection of electron-withdrawing and donating alkyne substrates. Control reactions were conducted using $CuSO_4.5H_2O$ based Click reaction (**Table 11**).

Table 11: Synthesis of substituted 1,2,3-triazoles. Evaluation of electron-donating and withdrawing effects of substrates in the synthesis of substituted 1,2,3-triazoles through the reaction of phenyl azides (**15d, e**) and propargyl derivatives (**19a-g**) under laccase and copper conditions.



Entry	Azide	Alkyne	Catalyst	Time	(20) Yield	(21) Yield
1	15d	19a	*T. versicolor*	24h	24%	11%
			$CuSO_4.5H_2O$	4h	a	a
2	15d	19b	*T. versicolor*	24h	-	-
			$CuSO_4.5H_2O$	4h	54%	-
3	15d	19c	*T. versicolor*	24h	-	-
			$CuSO_4.5H_2O$	4h	62%	-
4	15d	19d	*T. versicolor*	24h	Trace amounts	Trace amounts
			$CuSO_4.5H_2O$	24h	Trace amounts	Trace amounts
5	15d	19e	*T. versicolor*	24h	14%	6%
			$CuSO_4.5H_2O$	24h	27%	-
6	15d	19f	*T. versicolor*	24h	26%	16%
			$CuSO_4.5H_2O$	4h	39%	-
7	15d	19g	*T. versicolor*	24h	23%	19%
			$CuSO_4.5H_2O$	24h	37%	26%
8	15e	19g	*T. versicolor*	24h	-	-
			$CuSO_4.5H_2O$	24h	41%	-

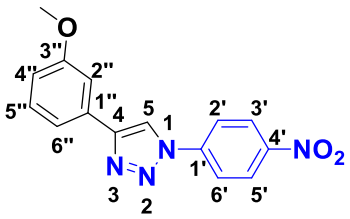
a- The crude product also contains a mixture of side products of unknown structure.

Accordingly, the cycloaddition of phenyl azide derivatives (**15d;15e**) and the alkyne substrates was expected to yield the 1,4- and 1,5-substituted triazoles under laccase conditions. The electron-deficient alkynes **19b** and **19c** did not display activity over the 24 h reaction period. However, good activity and regioselectivity were observed while using $CuSO_4.5H_2O$ as a catalyst, with the highest yield of 62%. Mild activity was observed with both *Trametes versicolor* and $CuSO_4.5H_2O$ towards internal alkyne **19d**, where only trace amounts of product were detected (TLC). For sterically challenging alkynes, the reaction often requires elevated temperatures and prolonged reaction times, which is unfavourable for enzyme catalysts.[197] The remaining aryl-alkynes (**19e**; **19f**; **19g**) gave fairly decent yields with both catalysts, with laccase yielding both regio-isomers. The substrates containing electron-withdrawing atoms favoured the 1,4-substituted triazoles with the highest combined isomer yield of 42% (**Table 11, Entry**

7). This is slightly a better overall conversion than the copper catalyst, but with poorer regioselectivity.

In establishing the structures of the derivatives IR, mass spectrometry and multidimensional **NMR** (**COSY**, **HSQC**) techniques were employed. To distinguish between the 1,4- or 1,5-disubstituted-*1H*-1,2,3-triazoles isomeric triazoles, compounds **20e** and **21e** were chosen as model structures. The assignments of the proton and carbon resonances were made based on the characteristic chemical shifts, splitting patterns and correlation experiments from **2D NMR** techniques **HSQC** and **HMBC**. The structural elucidation of compounds **20e** and **21e** was further confirmed by comparison with the chemical shift values of resembling structures in literature.[223,224]

Table 12. **^{1}H**-Chemical shift assignments of triazole **20e**

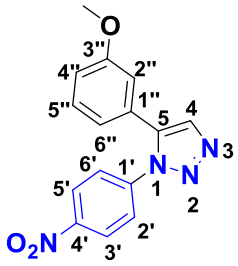


Assignment	**H3'&H5'**	**H5**	**H2'&H6'**	**H2''**	**H6''**	**H5''**	**H4''**	**OMe**
Chemical Shift (δ in ppm)	8.46	8.29	8.05	7.53-7.52	7.46	7.40	6.96	3.91
Integration	2	1	2	1	1	1	1	3
Multiplicity	d	s	d	m	d	t	dd	s
Coupling constants (*J* value in Hz)	9.0 Hz	-	9.0 Hz	-	7.7 Hz	7.9 Hz	8.1, 2.1 Hz	-

As expected, the triazole proton gives out a singlet signal **H5** at 8.29 ppm. The aromatic protons **H3'&H5'** are the most deshielded as they experience a large shielding effect from the electron-withdrawing adjacent nitro group. Coupled over three bonds (*J* = 9.0 Hz) with **H2'&H6'** protons, they yield a doublet signal at 8.46 ppm. A doublet signal **H2'&H6'** at 8.05 (d, *J* = 9.0 Hz, 2H) further confirms our assignment. On the methoxy ring, what appears to be a multiplet signal is observed for the **H2''** proton between 7.53-7.52 ppm. Upon examining the **COSY** spectrum, a positive correlation is observed between **H4''** and **H2''**. The broadening of the **H2''**signal is interpreted as being due to the inductive effect imparted by the methoxy oxygen atom to the **H2''** nuclei. A doublet

of doublets signal is observed for the **H4''** proton, coupled to proton **H2''** and **H4''** yielding a 6.96 (dd, *J* = 8.1, 2.1 Hz, 1H) signal. This assignment was supported by **COSY** and **HSQC NMR** correlation experiments.

Table 13. **^{1}H**-Chemical shift assignments of triazole **21e**



Assignment	H3'&H5'	H4	H2'&H6'	H5''	H6''	H2''&H4''	OMe
Chemical Shift (δ in ppm)	8.29	7.87	7.59	7.31	6.97	6.86 – 6.71	3.76
Integration	2	1	2	1	1	2	3
Multiplicity	d	s	d	t	d	m	s
Coupling constants (*J* value in Hz)	9.0 Hz	-	9.0 Hz	7.9 Hz	7.9 Hz	-	-

The triazole proton resonates around 7.87 ppm as a singlet signal **H4**, an upfield shift from the 1,4- isomer. As expected, the aromatic protons **H3'&H5'** are the most deshielded due to the electron-withdrawing effects of the nitro group, appearing as a doublet signal at 8.29 ppm. **H2'&H6'** protons were assigned based on the characteristic coupling constant 7.59 (d, *J* = 9.0 Hz, 2H). The signal from **H6''** appears to be now shielded, due to the electron-dense aryl ring now closer to it. It was assigned 6.97 (d, *J* = 7.9 Hz, 1H) based on the splitting pattern and this assignment was corroborated by the **COSY** spectrum (**Figure 19**). Signals from protons **H2''** and **H4''** are overlapping, yielding a multiplet signal between 6.86 – 6.71 ppm.

Figure 19:COSY NMR spectrum for 21e.

▪ DISTINGUISHING BETWEEN TRIAZOLE 20e AND 21e

Because a number of triazoles structures have been confirmed and proven by Sharpless/Fokin and many other studies.[223–225] It is now unlikely that the structure of triazoles is proven in literature, but it is rather assumed that Copper catalysis gives the 1,4-substituted isomer, while ruthenium catalysis gives the 1,5- isomer.[223]

The 1,4-substituted-*1H*-1,2,3-triazole **20e** was distinguished from the 1,5-substituted-*1H*-1,2,3- triazole **21e** using one-dimensional **^{13}C NMR** spectroscopy along with gradient-selected 2D NMR experiments (**HSQC** and **HMBC**). Assignments to the peaks have been made by comparison of the **^{13}C NMR** chemical shift data reported by Creary *et al.* (2012).[223] Using gated decoupled **^{13}C NMR** spectrum and GIAO computational studies, Creary *et al.* (2012) showed that C-5 carbon from the 1,4- isomer appears upfield on the carbon spectrum when compared to the C-4 carbon from the 1,5-isomer.[223]

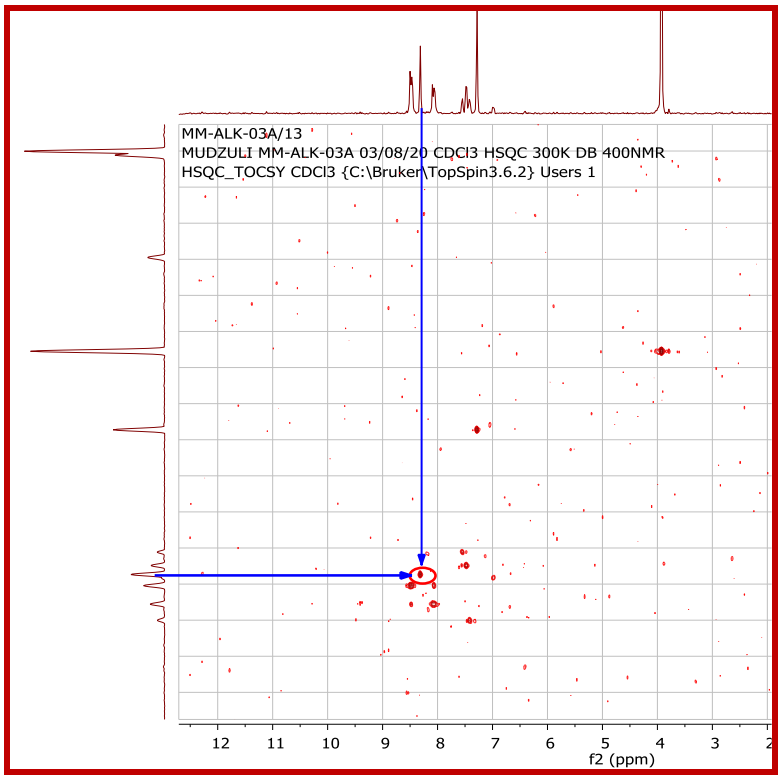


Figure 20:HSQC NMR spectrum for 20e

Using **HSQC**, we observed a positive correlation between the triazole singlet proton **H-5** and carbon **C-5** from the 1,4- isomer **20e** around 117.3 ppm (**Figure 20**). Correlation between the triazole singlet **H-4** and **C-4** was observed around 134.2 ppm for the 1,5-isomer **21e** (**Figure 21**). These results are consistent with the literature findings, confirming the upfield shift of **C-5** of **20e** relative to **C-4** in **21e**.[223] For the rest of the **^{13}C NMR** spectra, the assignment was in exact agreement with the expected number of carbon atoms present.

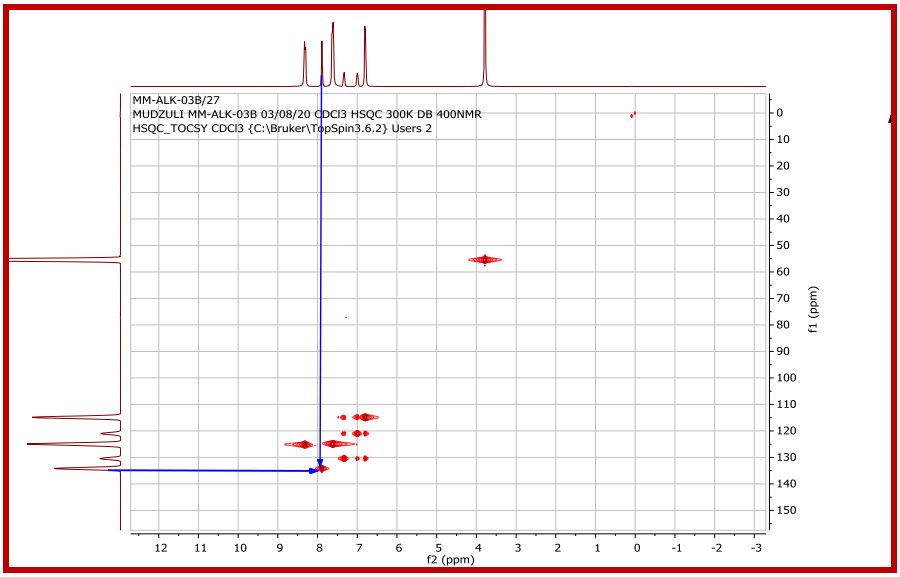


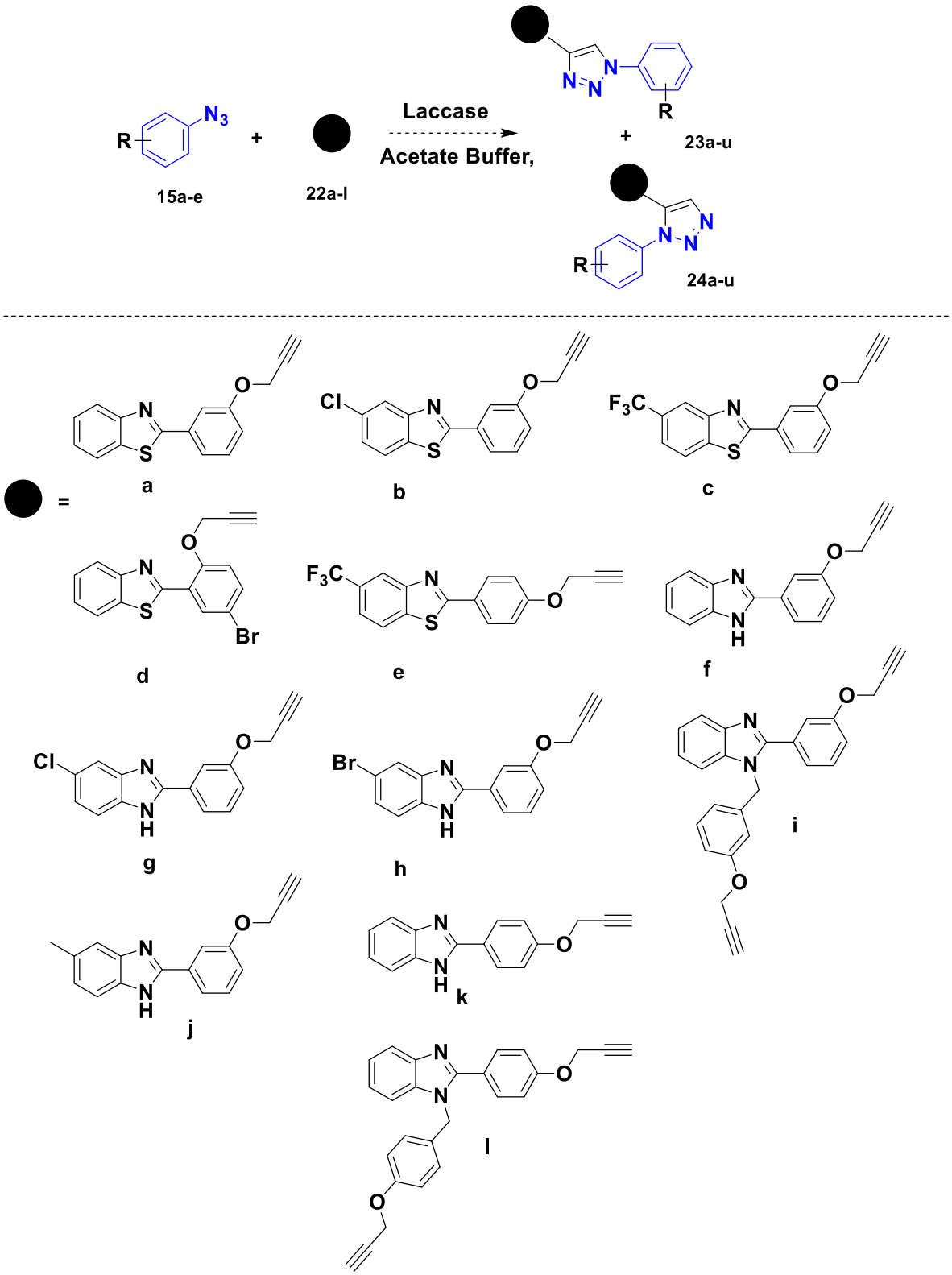
Figure 21: HSQC NMR spectrum for 21e.

NOVEL TRIAZOLE FUZED DERIVATIVES

The optimized reaction conditions were applied to a library of propargyl benzothiazole and benzimidazole derivatives. A series of pure and unpurified benzothiazole and benzimidazole alkyne derivatives were treated with aryl-azides under laccase and copper (I) 1,3-dipolar cycloaddition conditions.

A click reaction of phenyl-azide (**15a-e**) and propargyl-benzimidazole/benzothiazole (**22a-l**) was comprehensively investigated in the presence of laccase enzyme (*T. versicolor*) as compared to a copper salt ($CuSO_4.5H_2O$) and the results are shown in **Table 14**. As expected, our results showed that using $CuSO_4.5H_2O$ catalyst yields the 1,2,3-triazoles efficiently and with extremely high regioselectivity for the 1,4-regioisomer. The laccase provided similar trends in activity to the copper catalyst, but typically with a lower yield. Moreover, the laccase enzyme in many cases yielded both regioisomers with low product yield. Better activity and selectivity were observed with benzimidazole units with a highest yield of 43% (**Table 14**, **entry 12**), which also yielded exclusively the 1,4-regioisomer. Unfortunately, neither the regioselectivity nor the yield of the laccase catalysed reaction could be optimised to compete with that of the copper catalysed reactions in the current study, but perhaps the huge variation possible with enzymes will enable this achievement.

Table 14: Synthesis of novel benzimidazole and benzothiazole fused triazole derivatives.



Entry	Azide	Alkyne	Catalyst	Time	(23) Yield	(24) Yield
1	**15c**	**22a**	*T. versicolor*	24h	Polymerisation	-
			$CuSO_4.5H_2O$	4h	Polymerisation	-
2	**15f**	**22b**	*T. versicolor*	24h	34%	7%
			$CuSO_4.5H_2O$	16h	43%	-
3	**15c**	**22c**	*T. versicolor*	24h	16%	14%
			$CuSO_4.5H_2O$	4h	67%	-
4	**15f**	**22d**	*T. versicolor*	24h	polymerisation	-
			$CuSO_4.5H_2O$	24h	polymerisation	-
5	**15d**	**22e**	*T. versicolor*	24h	polymerisation	-
			$CuSO_4.5H_2O$	4h	polymerisation	-
6	**15b**	**22f**	*T. versicolor*	24h	9%	a
			$CuSO_4.5H_2O$	24h	23%	-
7	**15d**	**22g**	*T. versicolor*	24h	5%	3%
			$CuSO_4.5H_2O$	2h	31%	-
8	**15d**	**22h**	*T. versicolor*	24h	7%	-
			$CuSO_4.5H_2O$	4h	21%	-
9	**15f**	**22i**	*T. versicolor*	24h	-	-
			$CuSO_4.5H_2O$	4h	-	-
10	**15e**	**22j**	*T. versicolor*	24h	13%[b]	a
			$CuSO_4.5H_2O$	4h	a	-
11	**15a**	**22k**	*T. versicolor*	24h	43%	-
			$CuSO_4.5H_2O$	4h	71%	-
12	**15a**	**22l**	*T. versicolor*	24h	4%	-
			$CuSO_4.5H_2O$	4h	a	a
13	**15d**	**22b**	*T. versicolor*	24h	a	a
			$CuSO_4.5H_2O$	4h	a	-
14	**15d**	**22c**	*T. versicolor*	24h	28%	21%
			$CuSO_4.5H_2O$	4h	62%	-
15	**15a**	**22c**	*T. versicolor*	24h	2.4%	a
			$CuSO_4.5H_2O$	4h	11%	-
16	**15b**	**22d**	*T. versicolor*	24h	-	-
			$CuSO_4.5H_2O$	4h	-	-
17	**15b**	**22c**	*T. versicolor*	24h	Polymerisation	-
			$CuSO_4.5H_2O$	4h	Polymerisation	-
18	**15c**	**22f**	*T. versicolor*	24h	6%	a
			$CuSO_4.5H_2O$	4h	55%	a
19	**15e**	**22k**	*T. versicolor*	24h	22%	-
			$CuSO_4.5H_2O$	4h	48%	-
20	**15e**	**22l**	*T. versicolor*	24h	5.3%	-
			$CuSO_4.5H_2O$	4h	13%	-
21	**15d**	**22d**	*T. versicolor*	24h	Polymerisation	-
			$CuSO_4.5H_2O$	4h	Polymerisation	-

a- The crude product contained starting materials and an insoluble polymerised product of unknown structure.

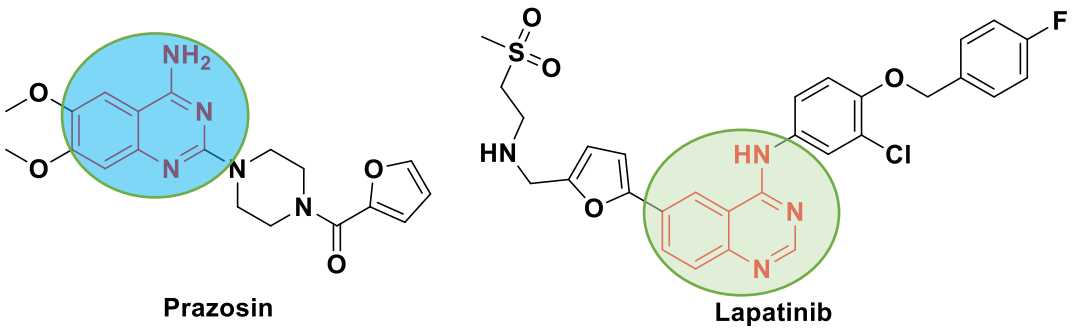
b- The crude product contained an insoluble polymerised product of an unknown structure.

CONCLUSION

An extensive library of novel benzimidazole and benzothiazole derivatives containing 1,4- and 1,5-substituted triazoles units were synthesised using the sequential application of two laccase enzymes. The laccase-based click chemistry approach, demonstrated here for the first time, employs a biocatalytic [1,3]-dipolar cycloaddition mechanism between azides and alkynes. Performing click reactions under copper-free conditions could prove useful by eliminating toxic copper catalysts and ligands; click chemistry can be applied to biorthogonal reactions in living cells and whole organisms. This is important for bioimaging, drug discovery technology as well as other applications like activity-based protein profiling.

Chapter FOUR

A BIOCATALYTIC SYNTHESIS OF NOVEL QUINAZOLINONES



Commercial quinazolines

QUINAZOLINES

Quinazoline (1,3-diazanaphthalene) is a nitrogen-containing aromatic heterocycle with a bicyclic structure composed of a benzene ring fused to a pyrimidine (**Figure 21**).[226–228] Quinazoline chemistry has been investigated for centuries; first synthesised by Griess et al. in 1869 through a condensation reaction.[226,229,230] This was followed by a synthesis of analogues by Bischler and Lang (1895) and Gabriel and Colman (1902).[226,231,232] The quinazoline moiety appears in more than 100 alkaloids, commonly found in the form of quinazolin-4(*3H*)-one (**Figure 21**).[233,234] Quinazoline alkaloids remain a small class of secondary natural compounds, where the first natural product (-)/(+)-peganine (also known as vasicine) was discovered in 1888.[234]

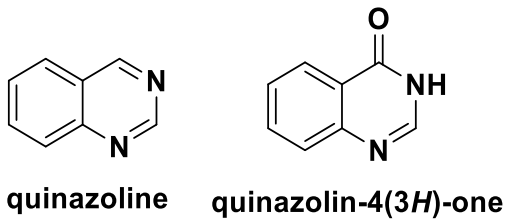


Figure 22: Structures of quinazoline derivatives

The quinazoline scaffold is an important pharmacophore and substituted derivatives have been synthesized for a range of pharmaceutical and agrochemical applications.[233] There are many commercially available drugs bearing the quinazolinone core (**Figure 22**).[235] Huggan evaluated 3-substituted quinazolin-2,4-dione for activity against Human African Trypanosomiasis (HAT).[236] Ahmed *et al*. (2013) reported that novel series of 6,8-dibromo-4(*3H*)quinazolinone derivatives exhibit potent antiproliferative activity against the human breast carcinoma cell lines.[237] Leivers *et al*. (2014) determined that 2-amino-4,6-disubstituted-4(*3H*)quinazolinone displays high selectivity for type III phosphatidylinositol 4-kinase alpha (PI4KIIIα) and potently inhibits Hepatitis C virus (HCV) replication in vitro.[238] Hucke discovered that 1*H*-quinazolin-4-one (QAZ) are allosteric HCV NS5B thumb pocket 2 (TP-2) inhibitors.[239] Chern designed and synthesized novel quinazolin-4-one derivatives as potent Histone deacetylase 6 (HDAC6) inhibitors.[240] Relatedly, other quinazoline derivatives display properties such as antitumour[241], antituberculosis[242], analgesic[243], anti-inflammatory[244],

antihypertensive[245], anticonvulsants[246], antimicrobial[247], antimalarial[248], antifungal[248,249], anti-viral[250], and antibiotic.[251] Similarly, due to their fungicidal[252], insecticidal[253] and herbicidal[254] activities, quinazolinone have gained a lot of attention as pesticides.[253]

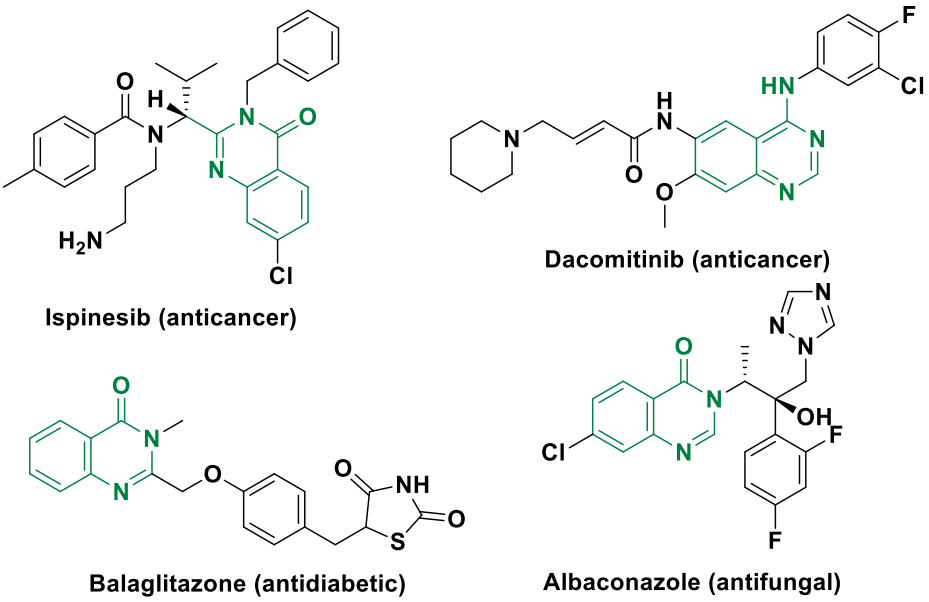


Figure 23: commercially available quinazolinone drugs.[235]

Because of their vast wealth of biologic activities, the need to develop new and improved methods for the production and biological investigation of compounds derived from quinazoline is ever-increasing.[255] In recent years, substantial progress has been made in the synthetic procedures, while demonstrating both efficiency and sustainability.[256] The conventional synthetic approaches for quinazoline derivatives often make use of 2-aminobenzoic acid derivatives[233], anthranilonitrile derivatives[257], isotonic anhydride[258], halobenzimidamides[257], 2-carbomethoxyphenyl isocyanate[259] and *N*-arylnitrilium salts and *4H*-3,1-benzoxazinone as starting materials.[260] Where the classical method is via a three-component reaction of isotonic anhydride, 2-aminobenzamides, and aldehydes, followed by the oxidation of the aminal intermediate.[258] Several oxidants have been used to catalyse these reactions; often toxic and non-renewable oxidants such as MnO_2[261], TBHP[262], $FeCl_3$[263], $Cu(OAc)_2$[264], I_2[265], $NaHSO_3$[266], and KMnO4.[267]

The various techniques for synthesizing quinazolines are categorized into five different classes; Aza-reaction, Microwave-assisted reaction, Metal-catalysed reaction, Ultrasound-promoted reaction and Phase-transfer catalysis.[255] Unfortunately, most of

these synthetic methods are disadvantageous because they make use of hazardous substances, volatile solvents, toxic and expensive metal catalysts and often abrasive reaction conditions (high temperatures and pressures).[258,265] Greener and environmentally friendly approaches have been reported recently; Shariati, *et al* (2019) described the Laccase/ 2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) aerobic oxidative synthesis of 2-substituted quinazolines from 2-aminobenzylamine and aldehydes.[268] Ghorashi, *et al* (2020) reported another laccase/DDQ aerobic oxidative synthesis of quinazolinones under mild conditions.[269] However, these methods deploy DDQ as a laccase mediator, which happens to be a stoichiometric catalyst, costly, highly toxic and environmentally unfriendly.[269] Marjan *et al*. (2014) reported the synthesis of quinazolinones *via* a tandem reaction using the laccase-HBT mediator systems under mild conditions.[258] Even though great yields were obtained in this study, the method works best in a citrate buffer and not in organic (co-)/solvents, and this limits substrate scope for many organic insoluble substrates.

The conjugation of quinazolinone derivatives to other bioactive cores to produce compounds with improved selectivity and potency have become increasingly popular in the literature.[238,240,270] The various linkers and/or cores have been evaluated for activities such as antioxidant, antimicrobial[270], anti-Alzheimer's disease[240], and anti-tubercular (**Figure 24**).[271]

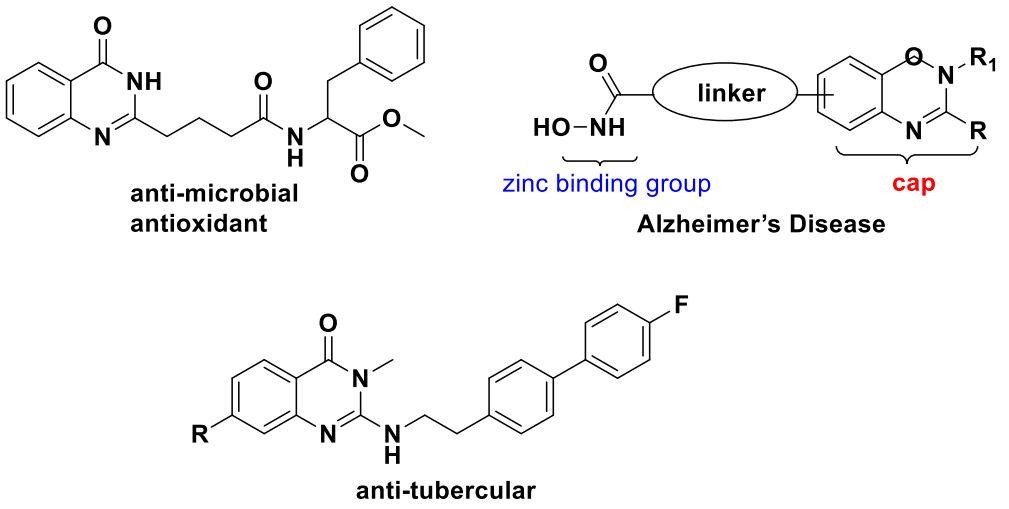


Figure 24: Bioactive dual quinazilinone derivatives.

In this chapter, we synthesised a series of quinazolinone derivatives containing a "linker" and a "tail" conjugated to the core of the molecule (**Figure 25**). The obtained heterocycles will be evaluated for their anti-tuberculosis activity at a later state.

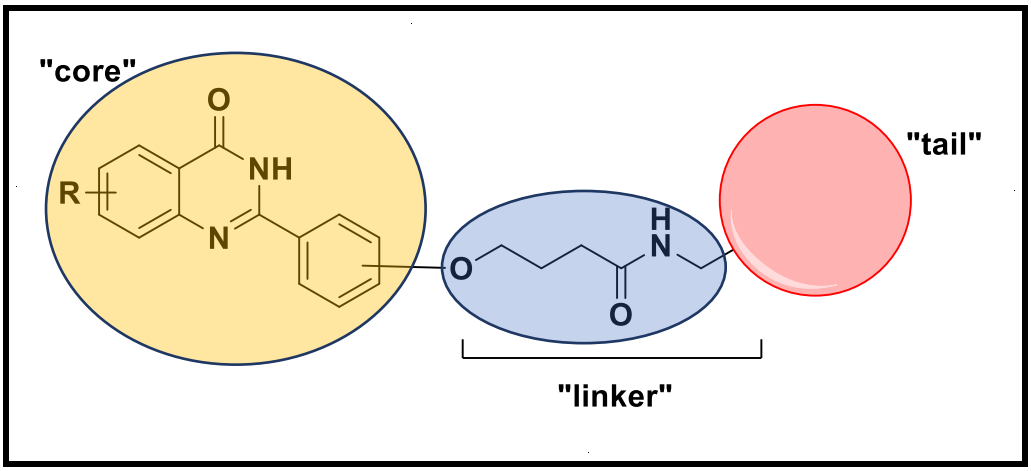
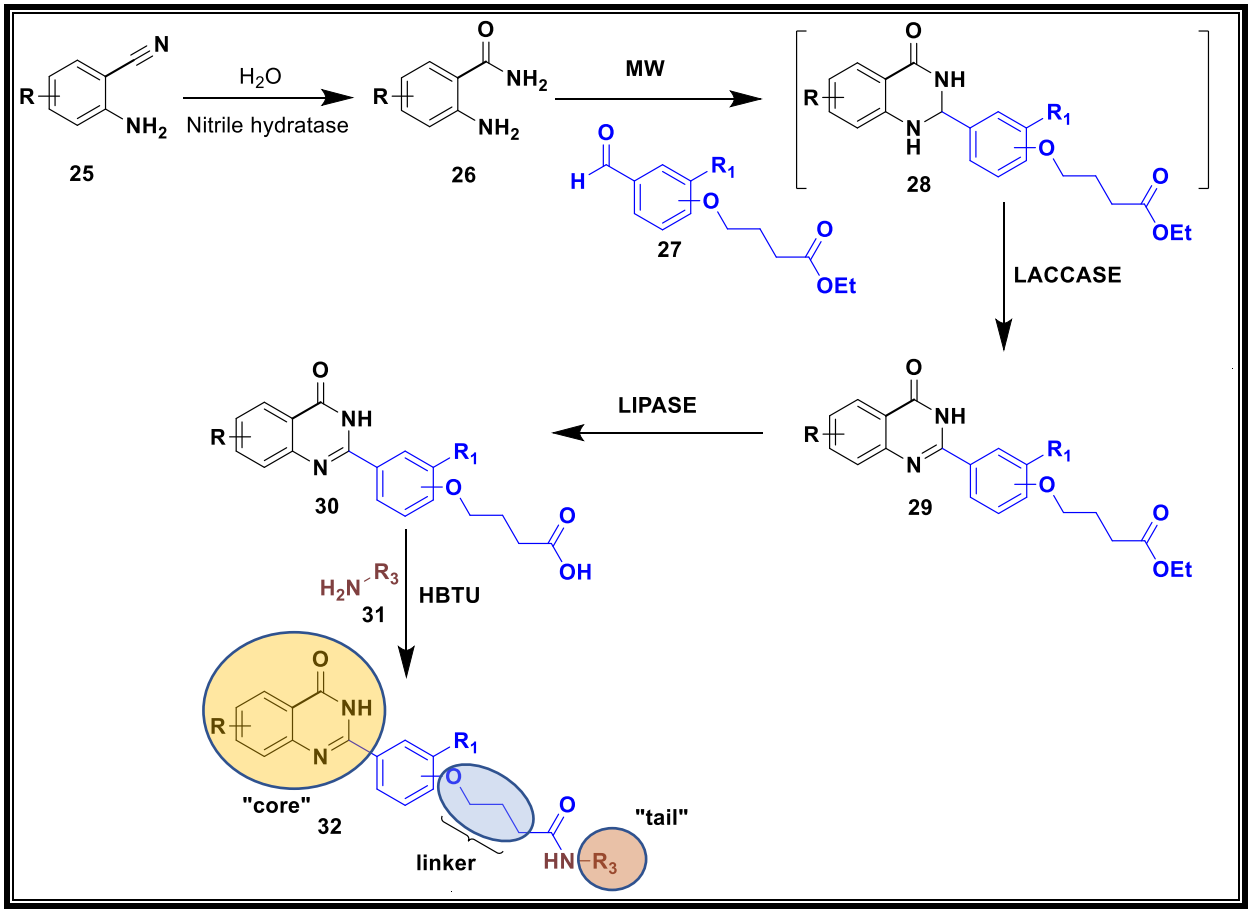


Figure 25: Target quinazoline derivatives.

We also investigate the reactions between 2-aminobenzamides and aldehydes, for the synthesis of quinazolinone derivatives. The major downside of this reaction is the chemically unstable aldehydes and their derivatives compared to their corresponding alcohols.[258,264] However, we solve this problem through a simple modification of the reaction conditions. We also investigate the incorporation of biocatalytic steps into reaction routes, while considering the limitations for each class of enzymes. A novel series of quinazolin-4(3*H*)-one derivative was synthesized from nitriles through a chemo-enzymatic stepwise process. The green process describes the nitrile hydratases (EC 4.2.1.84) mediated hydrolysis of nitriles, oxidative dehydrogenation with laccases (EC1.10.3.2) and hydrolysis/amidation with lipases (EC 3.1).

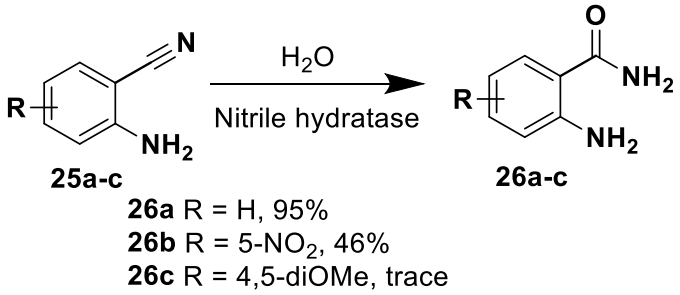
Our preliminary objective was to explore an organic synthesis route of novel quinazoline derivatives comprising of functional group interconversions catalysed by enzymes. A cascade involving a combination of these enzymes (laccase, lipase and nitrile hydratase) to synthesise quinazolines has not been explored before. We highlight functional group interconversions catalysed by enzymes through a series of steps; (i) We make use of nitrile hydratase for the hydrolysis of nitriles to their corresponding amides. (ii) This is then followed by a laccase catalysed oxidative dehydrogenation of aminal intermediate. (iii) Hydrolysis of the subsequent quinazoline esters mediated by lipases (**Scheme 33**).



Scheme 33:Chemoenzymatic route for the synthesis of quinazolin-4(*3H*)-one derivative containing an amide moiety.

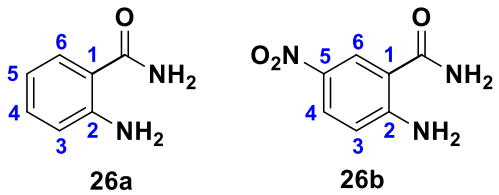
SYNTHESIS OF 2-AMINOBENZAMIDES

The synthesis of quinazolin-4(*3H*)-one derivatives was carried out from the reaction of 2-aminobenzamides and aldehydes under aerobic conditions. Initially, 2-aminobenzamide derivatives were prepared by treating nitriles **25a-c** with nitrile hydratases according to the published dissertation by M. Soobben (2020) (**Scheme 34**).[272] A series of benzonitrile derivatives were treated with 50 mL of cell lysate containing a nitrile hydratase in 20 mL of Tris buffer (7.6 pH). The enzyme showed higher affinity for 2-aminobenzonitrile (up to 100% conversion), but no activity was observed for 2-amino-4,5-dimethoxybenzonitrile **25c**. Although the enzyme displayed low affinity for substrate **25c**, the overall activity was pleasing, and no further optimisation of the reaction conditions was conducted.



Scheme 34:Synthesis of amide derivatives catalysed by Nitrile hydratase.

The structures of compounds **26a-b** were assigned by **IR, ^{1}H** and **^{13}C NMR** spectroscopy. The spectroscopic data indicated the absence of the –CN group and the presence of the amide NH_2.



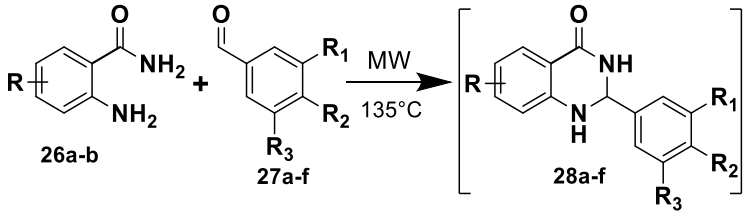
The successful conversion of the nitrile (**25a**) to amide (**26a**) was clear in the **^{1}H NMR** spectrum, which showed two distinct broad signals (amide NH_2) at δ 7.73 and δ 7.07. The **^{13}C NMR** spectrum confirmed the expected carbonyl signal at 171.7 ppm for the amide carbonyl. Another broad signal was observed at 6.57 ppm for the amine (NH_2) signal. The rest of the aromatic signals were assigned by integration, coupling constants,

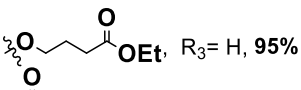
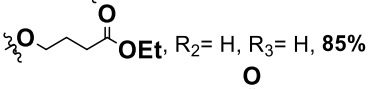
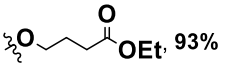
COSY, **HSQC** and **HMBC** experiments. Proton **6** is the most deshielded at 7.53 ppm, coupling with proton **H5** (*ortho* coupling *J* = 8.0 Hz) and **H4** (*meta* coupling *J* = 1.6 Hz) to give a doublet of doublets. Proton **H4** appears at 7.13 ppm as a doublet of doublets of doublets, coupling with proton **H5 & H3** (*ortho* coupling *J* = 8.4, 7.0 Hz), and **6** (*meta* coupling *J* = 1.6 Hz). Proton **H3** appears at 6.68 ppm as a doublet of doublets, coupling with proton **H4** (*ortho* coupling *J* = 8.3 Hz) and **H5** (*meta* coupling *J* = 1.2 Hz). And finally, Proton **H5** appears at 6.48 ppm as a doublet of doublets of doublets, coupling with proton **H6 & H4** (*ortho* coupling *J* = 8.1, 7.0 Hz), and **6** (*meta* coupling *J* = 1.2 Hz).

IR spectroscopy further confirmed the successful conversion of the nitrile (**25a-b**) to amide (**26a-b**). The absence of the nitrile signal typically found as a sharp absorption at 2250 cm^{-1} was indicative of the successful nitrile conversion. The primary amides showed a broad C=O absorption in the range from 1660-1654 cm^{-1}. A pair of fairly sharp N-H stretching bands appear at about 3198-3191 cm^{-1} and 3412-3405 cm^{-1}.

SYNTHESIS OF QUINAZOLIN-4(*1H*)-ONE DERIVATIVES

The oxidative cyclization synthesis of quinazolin-4(3*H*)-one was carried out in a two-step process; firstly, the cyclization of the obtained 2-aminobenzamides **26a-b** with benzaldehyde derivatives under microwave irradiation to obtain aminal intermediates (**Scheme 35**). Secondly, laccase catalysed oxidation of the aminal intermediate under laccase aerobic conditions (**Scheme 36, Table 13**).



28a R = H, R_1= H R_2= H, R_3= H, **98%**
28b R = H, R_1= OMe, R_2= **OMe**, R_3= H **95%**
28c R = H, R_1= OMe, R_2= **OMe**, R_3= OMe **93%**
28d R = H, R_1= H R_2=  , R_3= H, **95%**
28e R = H, R_1 =  , R_2= H, R_3= H, **85%**
28f R = **5-NO_2**, R_1 = **OMe**, R_2=  , **93%**

Scheme 35: Microwave-assisted laccase oxidative cyclization synthesis of quinazolin-4(*3H*)-ones.

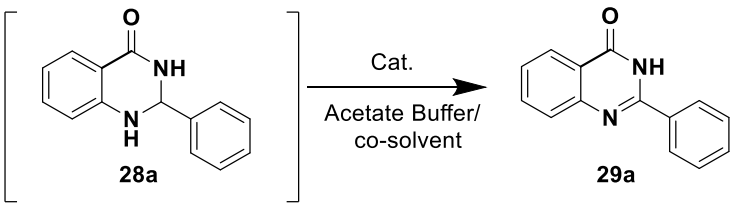
To begin with, we examined the general procedures for the condensation reaction between 2-aminobenzamides **26** and aldehydes **27** to form 2,3-dihydroquinazolin-4(*1H*)-one **28**. We planned to investigate the condensation reactions and thereafter explore the substrate scope of laccase oxidations with the ester chain on substrate **28(d-f)**. We realise that the reaction generally suffers from several disadvantages, this includes the use of elevated temperatures, acids and transition metal catalysts, and aldehyde derivatives are often reported to be chemically unstable (**Chapter 2**).[258,268,273] A microwave-assisted heterocyclization of anthranilamide and aldehydes has been reported to be highly efficient in the literature.[274] Bakavoli *et al.* (2018) reported a microwave-assisted synthesis of quinazolin-4(3*H*)-ones using potassium permanganate in dimethylacetamide as an oxidant, producing high yields of products.[275] Kang *et al.* (2018) reported another efficient microwave-assisted synthesis of quinazolin-4(3*H*)-ones using antimony(III) trichloride ($SbCl_3$) as a Lewis acid catalyst.[276]

We investigated the cyclocondensation reaction between benzamides **26a-b** and aldehydes **27a-f** under microwave and solvent-free conditions. We prepared benzaldehydes **27d-f** from hydroxy-benzaldehydes using a Williamson ether synthesis method in acetone.[277] Under conversional conditions, benzaldehydes **27d-f** would decompose during reaction (similar to results obtained in **Chapter two**). However, microwave irradiation resolved the setbacks usually associated with these reactions significantly and the cyclocondensation of **26** and **27** completed within 5 minutes. Trituration of the reaction mixture with ethanol afforded pure 2,3-dihydroquinazolin-4(1*H*)-ones **28** in quantitative yields (**Scheme 35**).

Having established an efficient microwave method for the cyclocondensation of benzamide **26** and aldehyde **27**, our next step was to optimize the reaction conditions for the laccase aerobic oxidation of 2,3-dihydroquinazolin-4(1*H*)-ones **28.** The oxidation of 2-phenylquinazolin-4(1*H*)-one **26a** to 2-phenylquinazolin-4(3*H*)-one **29a** was chosen as the model reaction and reaction conditions such as solvents, temperature, and mediators were investigated. The reactions were carried out using Novoprime Base 268 laccase in an acetate buffer (0.1 M, pH 4.0) with various co-solvents (**Scheme 36, Table 15).** Commercially available immobilized laccase Novoprime Base 268 was chosen for the model studies due to its tolerance of high temperatures.[278]

OXIDATION OF 2-PHENYLQUINAZOLIN-4(1*H*)-ONE

Table 15: Novoprime Base 268-catalysed oxidation of 2-phenylquinazolin-4(1*H*)-one **28a** in the presence of different mediators and co-solvents (75% v/v).



Scheme 36: Oxidation of 2-phenylquinazolin-4(1*H*)-one

Entry	Laccase	Mediator	Co-solvent	Temperature(°C)	Time	Yield %
1	–	–	–	100	24h	–
2	Novoprime Base 268	–	–	70	24h	35
3	Novoprime Base 268	ABTS	Acetonitrile	70	24h	55
4	Novoprime Base 268	–	Acetonitrile	70	24h	30
5	Novoprime Base 268	ABTS	DMF	70	24h	57
6	Novoprime Base 268	ABTS	Methanol	70	24h	–
7	–	–	DMSO	120	48h	96
8	Novoprime Base 268	TEMPO	Methanol	65	24h	–
9	Novoprime Base 268	–	DMSO	70	18h	95
10	Novoprime Base 268	TEMPO	DMSO	70	16h	97
11	Novoprime Base 268	ABTS	DMSO	70	24h	95
12	Novoprime Base 268	–	DMF	70	18h	31
13	Novoprime Base 268	TEMPO	Ethanol	65	18h	–
14	-	-	DMSO	70	36h	30

Initially, the reaction was carried out in the absence of laccase or mediator and no conversions of starting materials were detected (**Table 15, Entry 1**). When laccase was added to the reaction media, a low yield was observed using acetate buffer without a co-solvent (**Table 15, Entry 2**), moderate yields of the product were detected while using acetonitrile and DMF as co-solvents and excellent conversions were obtained while using DMSO as a co-solvent (**Table 15, Entries 7, 9, 10, 11**). These results demonstrated that DMSO is essential for an efficient oxidative dehydrogenation of 2-phenylquinazolin-4(1*H*)-one **28a** to 2-phenylquinazolin-4(3*H*)-one **29a** under aerobic conditions. Low conversion was observed with DMSO at 70 degrees, without the

catalyst present (**Table 15, Entry 14**). The crude product was precipitated out of solution by the addition of water to the reaction mixture and the pure product was obtained by recrystallisation of the crude product with ethanol. All the starting material was consumed as indicated by TLC and the formation of quinazolinone **29a** was confirmed by **^{1}H** and **^{13}C NMR** spectroscopy. The reactions with lower yields were difficult to work with, with more tedious reaction workups. Purification of the products from incomplete reactions was done through column chromatography (Ethyl acetate/Hexane 80:20)

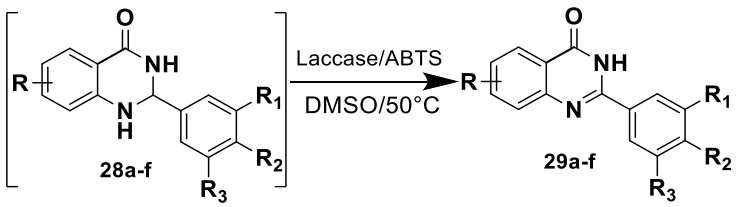
Shariati *et al.* (2019) reported no activity while using pure organic solvents including DMSO for the oxidative conversion of 2-phenyl-1,2-dihydroquinazoline to 2-phenylquinazoline using Laccase from *Trametes versicolor* due to enzyme denaturing.[268] Similar results were reported by Heidary *et al.* (2014) using THF, DMF, and MeCN for the oxidation of 2,3-dihydroquinazolin-4(1*H*)-one, also due to laccase denaturing.[258] However, this wasn't observed with the immobilized Novoprime Base 268, the complete conversion of 2,3-dihydroquinazolin-4(1*H*)-one to the desired product was observed in acetate buffer/DMSO (25:75 v/v). The efficiency of our laccase/DMSO catalyst system for the oxidation of 2-phenyl 2,3-dihydroquinazolin-4(1*H*)-one **28** was compared with the literature reported DMSO method (DMSO/100°C/36h/**98%** yield).[279] Addition of an oxidant such as laccase considerably sped up the reaction from 36h to 18h and lowered the reaction temperature from 100°C to 70°C (**Table 13, Entry 9**). The reaction mechanism the Laccase/DMSO oxidation of 2,3-dihydroquinazolin-4(1*H*)-one follows, was not investigated. We postulate the reaction might follow the Swern[280] or Kornblum[281] type oxidations, but this is yet to be confirmed.

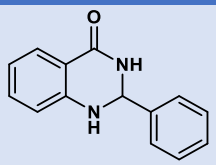
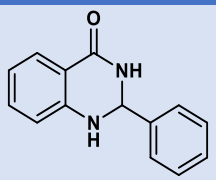
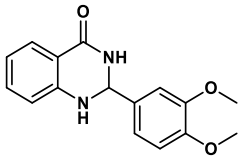
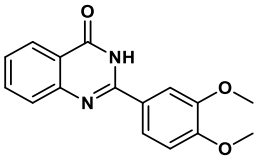
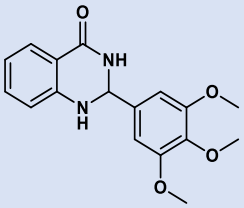
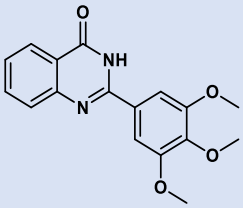
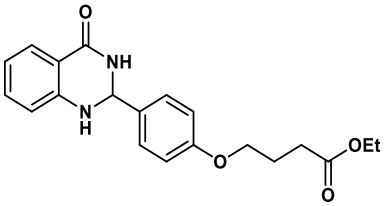
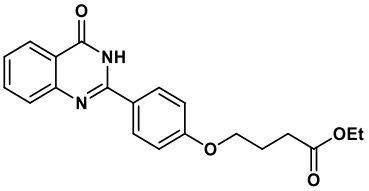
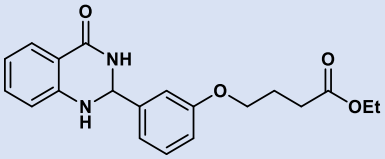
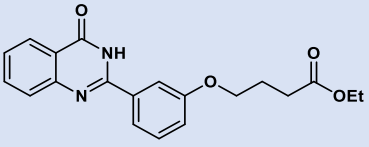
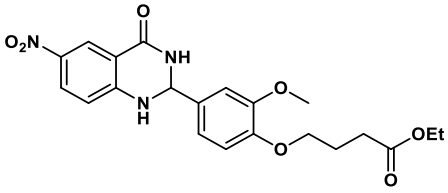
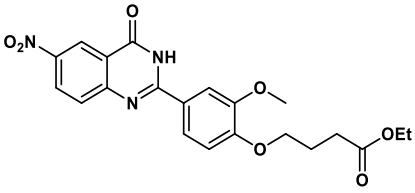
SYNTHESIS OF 2,3-DIHYDROQUINAZOLIN-4(3*H*)-ONES

Having successfully developed an effective method for the oxidative synthesis of 2,3-dihydroquinazolin-4(3*H*)-one, we expanded the application of the laccase/DMSO catalytic system for the aerobic oxidation of a range of substrates (**Table 16**). We tested the general applicability of this method for the synthesis of 2-substituted quinazolines from a range of substituted benzaldehydes of different sizes **28a-f**. We were interested in whether this procedure could be effectively useful in the preparation of 2,3-disubstituted quinazolinones with the ester chain attached (**28d-f**). Our main concern

was steric effects, whether the long chain would prevent the substrate from fitting into the enzyme's active site.

Table 16: A laccase/DMSO catalysed aerobic oxidative synthesis of 2-substituted quinazolin-4(3*H*)-one derivatives.

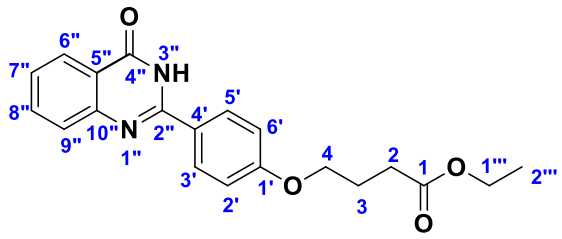


Entry	Substrate	Product	Time	Yield %
1			18h	95
2			16h	99
3			16h	98
4			18h	96
5			18h	90
6			18h	99

To our delight, the expected reaction progressed efficiently to afford 2,3-disubstituted quinazolinone esters in excellent yields (**Table 16, Entries 4-6**). Our results demonstrated that using DMSO in the presence of a catalytic amount of laccase (Novoprime Base 268) promotes the oxidative dehydrogenation of 2,3-dihydroquinazolin-4(*1H*)-one for a broad range of substrates. We were able to improve upon the currently existing methods by reducing reaction time and temperature, using microwave irradiation and molecular O_2 (air) as a green and abundant oxidant.

Characterization data for all the materials agreed with those reported in the literature.[279,282] The successful oxidation of 2,3-dihydroquinazolin-4(*1H*)-one was clear in the **^{1}H NMR** spectrum, which showed the distinct amide NH singlet at around 12 ppm. The **^{13}C NMR** spectrum displayed the expected signals around 160 ppm for the amide and 170 ppm for the ester carbonyls (**Table 16, entries 4-6**). We assigned the **^{1}H NMR** spectroscopic data for ethyl 4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanoate (**29d**) highlighting the key signals in the compound (**Table 17**).

Table 17: Assigning **^{1}H NMR** data for ethyl 4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanoate (**29d**).



Assignment	NH	H3'& H5'	H6''	H8''	H9''	H7''	H2'& H6'	H4& H1'''	H2	H3	CH_3
Chemical Shift (δ in ppm)	12.37	8.18	8.13	7.81	7.70	7.48	7.07	4.08	2.52–2.43	2.01	1.19
Integration	1	2	1	1	1	1	2	2	2	2	3
Multiplicity	s	d	d	t	d	t	d	m	m	*p*	t
Coupling constants (*J* value in Hz)	-	8.8 Hz	7.9 Hz	8.3 Hz	8.1 Hz	7.5 Hz	8.8 Hz	7.3 Hz	-	6.9 Hz	7.1 Hz

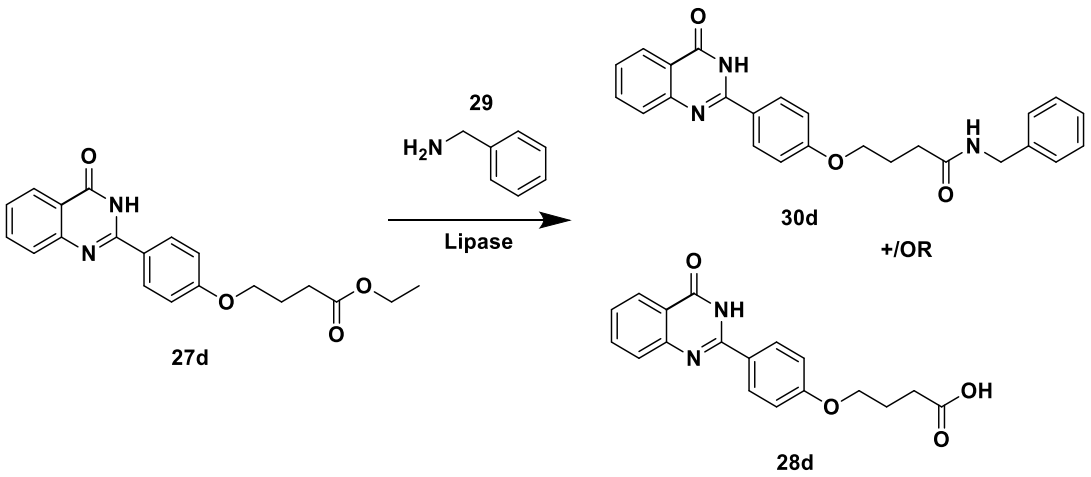
The successful coupling, cyclisation, and oxidation of the amide (**26**) with the ether benzaldehyde (**27**) was confirmed by the typical NH signal (quinazolinone amide

proton) at 12.37 ppm. The absence of the amide protons and the broad amine (NH_2) signal at 6.57 ppm was another indication that coupling took place. The remainder of the aromatic signals were assigned from characteristic chemical shift values, integration and splitting patterns. Based on their proximity to the carbonyl group, protons **H3'&H5'** are the most deshielded, coupling with protons **H2'&H6'** (*ortho* coupling J = 8.8 Hz) to give an intense doublet signal at 8.18 ppm. Coupling with two triplets (**H7'' & H8''**), protons **H6''** and **H9''** appear as doublets (*ortho* coupling J = 7.9, 8.1 Hz respectively). It has been reported that for benzo-fused heterocyclic rings, the typical J values for ortho interactions ($^3J_{1H,1H}$) are dependent on the heteroatoms. This effect is apparent in the quinazolinone compounds; where the coupling constants for protons closer to the heteroatom (Nitrogen in this case) are greater than those farther.[283] The observed value of $^3J_{H8'', H9''}$ (*ortho* coupling J = 8.1 Hz) which is greater than $^3J_{H6'', H7''}$ (*ortho* coupling J = 7.9 Hz) further confirms our assignments (**Table 17**). On the aliphatic chain, signals from CH_2 protons **4** (expected triplet) and the ethyl CH_2 protons **H1'''** (expected quartet) are overlapping at 4.08 ppm giving a multiplet signal. The CH_2 signal from protons **H2** (expected as a triplet) is overlapping with the signal from the solvent (DMSO-d_6), thereby giving a multiplet signal between 2.52–2.43 ppm. As expected, a quintet (p, J = 6.9 Hz, 2H) signal was observed for the CH_2 protons **H3** at 2.01 ppm.

SOLVENT SCREENING FOR THE DIRECT AMIDATION OF ESTERS (29d-f)

Subsequently, we investigated a simple and convenient method for the direct amidation of the obtained quinazolinone esters **29** and amines using lipase enzymes under mild conditions. The direct amidation reaction of ethyl 4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanoate **29d** with benzyl amine in the presence of lipase under mild conditions was investigated in various solvents (**Table 18, Scheme 36**). Immobilized Candida Antarctica Lipase B (CAL-B) was selected for the model reaction because of its wide substrate range and for its compatibility with many organic solvents.[130] The various solvents screened include organic solvents such as toluene, DMF, DMSO, 2-Methyl-THF, 1,4-dioxane, acetone, acetonitrile and diethyl ether.

Table 18: CAL-B catalysed amidation of ethyl 4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanoate **27d** with benzyl amine.



Scheme 36:The amidation of quinazoline esters with amines under lipase conditions.

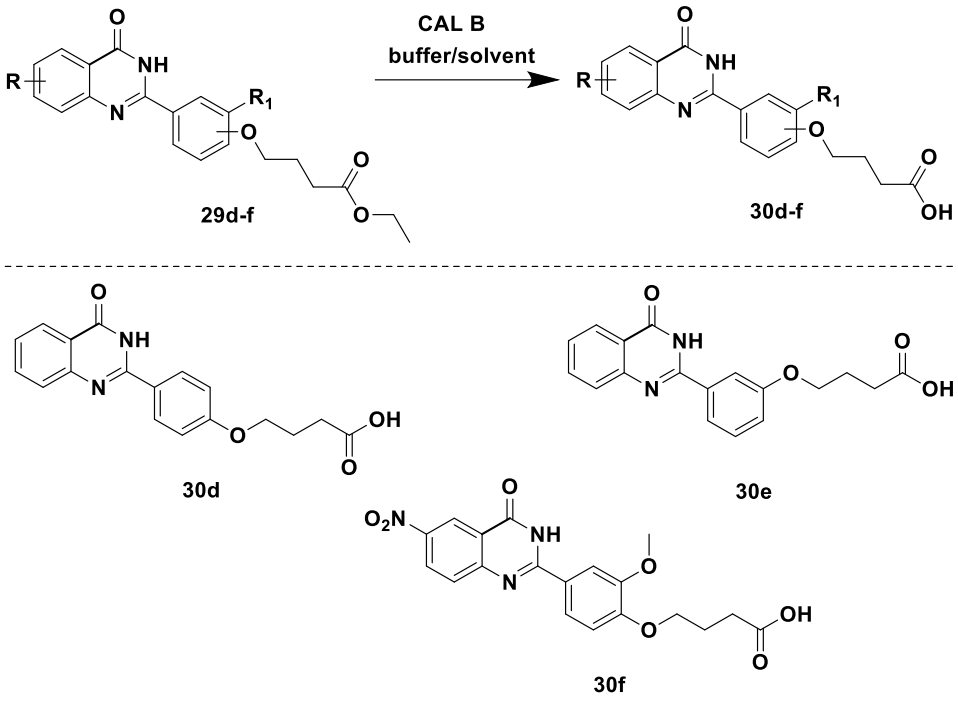
Entry	Ester	Solvent	Time	30	28[a]
1	**27d**	Toluene	3 days	-	-
2	**27d**	DMF	3 days	-	40%
3	**27d**	DMSO	3 days	-	30%
4	**27d**	Acetonitrile	5 days	-	-
5	**27d**	2-Methyl-THF	5 days	-	-
6	**27d**	1,4-dioxane	5 days	-	Trace amounts
7	**27d**	Acetone	5 days	-	Trace amounts
8	**27d**	Diethyl ether	5 days	-	-

a-Estimated conversion from crude ^{1}H NMR.

For most of the solvents screened, solubilization of the reactants was a challenge, particularly ethyl 4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanoate **29d.** However**,** good solubilities were observed with DMF and DMSO as solvents. The reactions were conducted in the presence of a small amount of water (10% v/v) and the results obtained were unsatisfactory. We noticed that the amidation did not take place for any of the solvents screened, instead, a carboxylic acid was formed (**Table 18, Entries 2;3**).

SOLVENT SCREENING FOR THE HYDROLYSIS OF ESTER 29

Because the Lipase-catalysed amidation of the quinazoline ester **29d** resulted in the cleavage of the ester group, we tested the hydrolysis activity on the three quinazoline ester derivatives (**29d-f**) to form carboxylic acids **(30d-f)** (**Scheme 37**).



Scheme 37: Enzymatic hydrolysis of quinazoline ester 29.

The enzymatic reactions were conducted in a phosphate buffer (0.2M, pH 7.4) and 70% DMSO (v/v) at 30°C. CALB Lipase was found to be effective to cleave the quinazoline esters **29d-f** to release the corresponding carboxylic acids and DMSO was chosen as a co-solvent to promote substrate dissolution. We have also screened greener or safer solvent alternatives (acetone, 2-Methyl-THF). However, low conversions were observed due to their inability to dissolve the substrates. The overall trend observed was; yields over 80% were accomplished with DMSO and DMF as co-solvents, reasonable yields were attained using acetone and poor yields were obtained using solvents such as 2-Methyl-THF, 1,4-dioxane, and acetonitrile (**Table 19**).

Table 19: Solvent screening for the hydrolysis of the three quinazoline ester derivatives (**29d-f**).

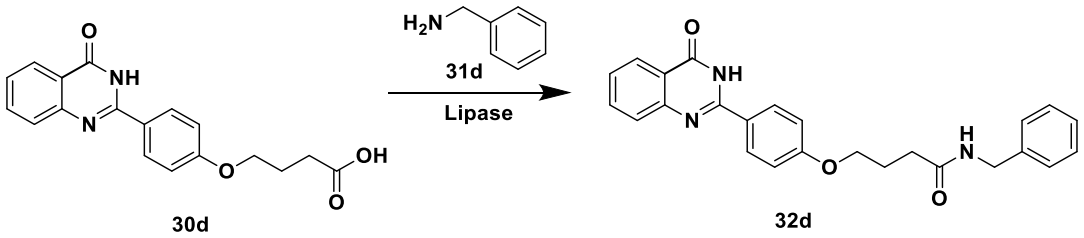
Entry	Ester	Solvent	Time	%Yield 30
1	**29d**	DMF	24 h	82
2	**29e**	DMF	24 h	66
3	**29f**	DMF	24 h	52
4	**29d**	DMSO	24 h	80
5	**29e**	DMSO	24 h	65
6	**29f**	DMSO	24 h	68
7	**29d**	Acetone	5 days	56
8	**29e**	Acetone	5 days	48
9	**29f**	Acetone	5 days	51
10	**29d**	2-MeTHF	5 days	-
11	**29e**	2-MeTHF	5 days	-
12	**29f**	2-MeTHF	5 days	-
13	**29d**	1.4-dioxane	5 days	-
14	**29e**	1.4-dioxane	5 days	-
15	**29f**	1.4-dioxane	5 days	-
16	**29d**	DMSO	72 h	86
17	**29e**	DMF	72 h	76
18	**29f**	DMSO	72 h	70

It seems that the dissolution of the substrate plays a great role in the outcome of the reaction. We also monitored the effect of temperature, pH value of the buffer and reaction time. We found that the overall optimal temperature was 30°C, with a buffer pH of 7.5 and longer reaction times (72h) led to a favourable ester hydrolysis (86%). The conversion of an ester to the corresponding acid is expected to show a relatively small number of electronic changes in the molecule. The hydrolysis of the parent ester **29** into the subsequent acid **30** results in two main spectroscopic changes in the **^{1}H NMR** spectrum. The distinct CH_2 quartet of the ethyl group typically found overlapping with the phenoxy ether CH_2 (see **Table 19**, structure **29d**), disappears together with the CH_3 triplet. There is an emergence of a hydroxyl hydrogen from the carboxylic O-H group, typically not observed as hydroxyl protons tend to exchange with one another or with the solvent. The O-H signal was observed for acid **30d**, giving a sharp singlet at 12.40 ppm. However, this was not the case for acids **30e** and **30f** under the same conditions.

LIPASE SCREENING

Lipases are well-known to exert a range of specificity towards substrates, we have then investigated catalyst type and enzyme preparation on the amidation of quinazoline acids **30**. The reaction between butanoic acid (**30d**) with benzylamine **31a** was chosen as a model reaction and it was performed in DMSO, DMF and acetone using 3Å molecular sieve (50 mg) for anhydrous conditions (**Table 20**).

Table 20: Lipase screening.



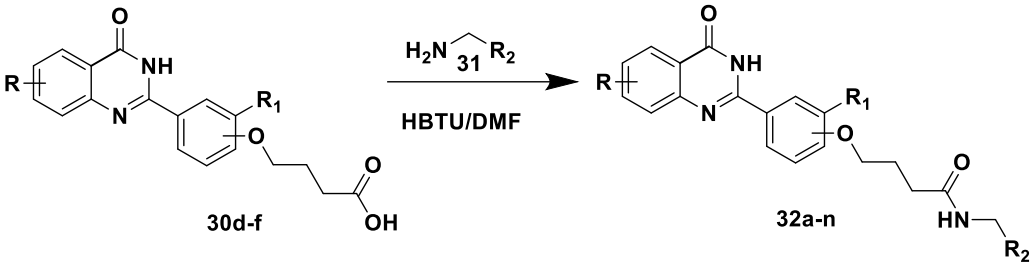
Entry	Catalyst	Solvent	Temperature (°C)	Time	Yield %
1	–	DMF	100	72h	–
2	Novozyme 435	Acetone	30	72h	trace
3	Amano lipase from *P. fluorescens* (534730)	Acetone	30	72h	–
4	Lipozyme_ CALB L (Novozymes)	Acetone	50	72h	65
5	Novozyme 435	DMSO	35	72h	–
6	Amano lipase from *P. fluorescens* (534730)	DMSO	30	72h	–
7	Lipozyme_ CALB L (Novozymes)	DMSO	50	72h	trace
8	Novozyme 435	DMF	30	72h	–
9	Amano lipase from *P. fluorescens* (534730)	DMF	35	72h	–
10	Lipozyme_ CALB L (Novozymes)	DMF	50	72h	trace
11	HBTU	DMF	25	2h	95

As a positive control, we conducted the reaction without a catalyst using DMF as a solvent and we did not detect any amide formation. It appears that the lipases from Amano lipase from *P. fluorescens* (534730) and Novozyme 435 were almost entirely inactive (**Table 20, Entry 2, 3, 5, 8, 9**). Using acetone as a solvent, *Candida antarctica* Lipase B (CALB) was found to be effective to catalyse the amidation of **30d** to the corresponding carboxylic amide **32d** (**Table 20, Entry 4**). Surprisingly, this was not the case while using DMF and DMSO as solvents (**Table 20, Entry 7, 10**), given that good solubility and activity was observed for the hydrolysis of the previous esters (**29**). Excellent results were achieved using a coupling reagent Hexafluorophosphate

Benzotriazole Tetramethyl Uronium (HBTU) in DMF (**Table 20, Entry 11**). HBTU had proven to be an ideal catalyst for mediating direct amidation of butanoic acid (**30d**) under basic conditions.

Having optimized the conditions for the direct amidation reactions of quinazoline acid, we then explored the scope of the reaction on a series of amines (**Table 21**). We were able to efficiently couple the 2-substituted quinazoline acids with a range of substituted amines using HBTU under basic conditions in DMF.

Table 21: HBTU catalysed direct amidation of quinazoline carboxylic acids.

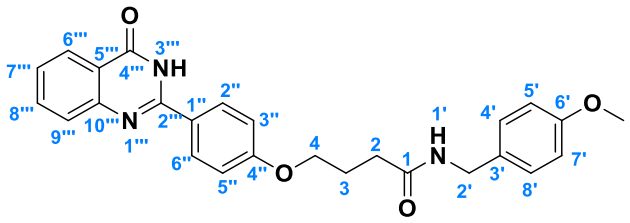


Entry	Quinazoline Acid	Amine	Time	Product	Yield %
1	30d	Benzylamine	2 h	32a	95
2	30e	4-Methoxybenzylamine	24 h	32b	96
3	30f	4-Methoxybenzylamine	24 h	32c	98
4	30f	Benzylamine	24 h	32d	88
5	30d	2-Aminobenzimidazole	2 h	32e	–
6	30d	Isopropylamine	2 h	32f	91
7	30e	Benzylamine	4 h	32g	78
8	30e	Isobutylamine	8 h	32h	66
9	30f	2-Amino-6-nitrobenzothiazole	2 h	32i	–
10	30d	Isobutylamine	4 h	32j	85
11	30e	2-Aminobenzothiazole	8 h	32k	–
12	30d	4-Methoxyphenethylamine	2 h	32l	74
13	30f	4-Chloroaniline	24 h	32m	–
14	30e	Isopropylamine	24 h	32n	60
15	30d	4-Methoxybenzylamine	2h	32o	92
16	30d	2-ethylhexan-1-amine	2h	32p	91
17	30e	2-(3,4-dimethoxyphenyl)ethan-1-amine	2h	32q	58
18	30d	2-(3,4-dimethoxyphenyl)ethan-1-amine	2h	32r	89
19	30d	2-(4-chlorophenyl)ethan-1-amine	2h	32s	93
20	30f	2-(4-chlorophenyl)ethan-1-amine	2h	32t	77

The general methods used for amide synthesis in both small and large-scale processes still suffer from many problems. The typical methods make use of stoichiometric amounts of activating reagents such as EDC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide), thionyl chloride, HATU (Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium), CDI (1,1'-Carbonyldiimidazole) etc. and often generating large sums of waste.[135]

In efforts to shift towards a more sustainable approach, we conducted the above amidation reactions using *Candida antarctica* Lipase B (CALB) in acetone. Unfortunately, this approach proved unsuccessful for the entire library except for one (referring to **Table 21, Entry 15**, yield 60%). On the other hand, the HBTU-catalysed condensation of quinazoline carboxylic acid **30** and amine **31** to give amide **32** gave excellent results (**Table 21**). Interestingly, no amide formations were detected with aromatic amines 2-aminobenzimidazole, 2-Aminobenzothiazole, 2-Amino-6-nitrobenzothiazole and 4-Chloroaniline (**Table 21, Entry 5, 9, 11 and 13**). We observed that the amidation is efficient with aliphatic and benzyl–amines but fails to couple to aniline derivatives. **^{1}H** and **^{13}C NMR** spectroscopy measurements were used to confirm the identity and structure for each compound. The data consistently showed chemical shift changes in going from the carboxylic acid **30** to the corresponding amide **32**. The assignments of the well-resolved **^{1}H** and **^{13}C NMR** signals of quinazolinone amide **32o** are discussed below.

Table 22: KEY SIGNALS IN THE NMR SPECTRA OF QUINAZOLINE 32o



Assignment	NH 3'''	NH 1'	H2''& H6''	H6'''	H8'''	H9'''	H7'''	H4'& H8'	H3''& H5''	H5'& H7'
Chemical Shift (δ in ppm)	12.43	8.36	8.19	8.14	7.82	7.71	7.49	7.16	7.06	6.85
Integration	1	1	2	1	1	1	1	2	2	2
Multiplicity	s	t	d	dd	ddd	m	ddd	d	d	d
Coupling constants (*J* value in Hz)	-	5.9 Hz	9.0 Hz	8.0, 1.5 Hz	8.5, 7.1, 1.6 Hz	-	8.1, 7.1, 1.2 Hz	8.6 Hz	9.0 Hz	8.7 Hz

The NMR assignments of quinazolinone amide **32o** (**Table 22**) was based on simple **^{1}H** and **^{13}C NMR** measurements and corroborated by correlated spectroscopy **COSY**, heteronuclear single quantum correlation experiment **HSQC** and Heteronuclear Multiple Bond Correlation **HMBC** experiments. Like before, the highly deshielded signal (12.43 ppm) was expected to be from the cyclic quinazoline amide proton (broad peak). The successful installation of the amid group was evident in the **^{1}H NMR**, the amide proton signal is expected to be deshielded, coupled over three bonds with **H-2'** protons and appears as a triplet at 8.36 ppm. A doublet CH_2 signal at 4.21 (d, *J* = 5.9 Hz, 2H) further confirms our assignment, as proven by the positive **COSY** correlation spectrum (**Figure 26**). On the basis of chemical shifts, the most deshielded aromatic protons would be the symmetrical protons **H-2''** and **H-6''**, and the observed coupling constants (d, *J* = 9.0 Hz, 2H) with **H-3''** and **H-5''**confirmed this assignment.

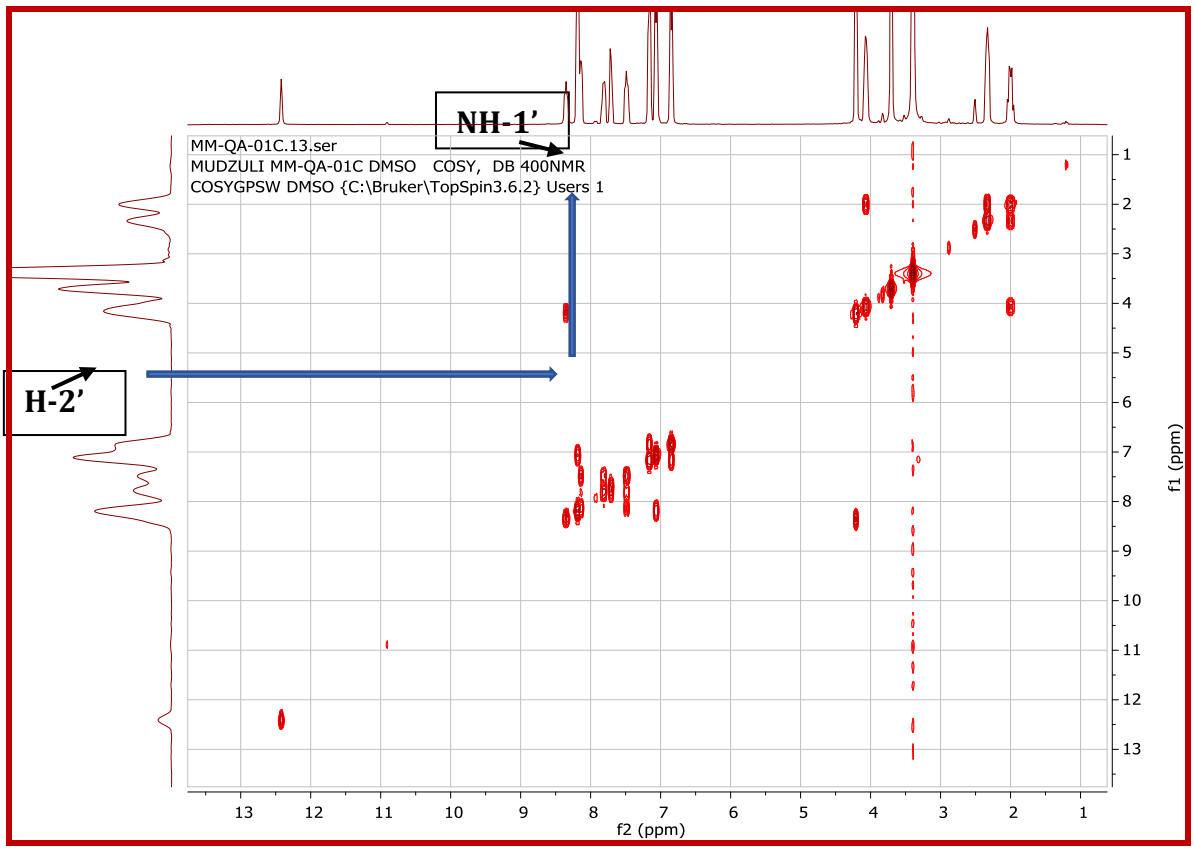
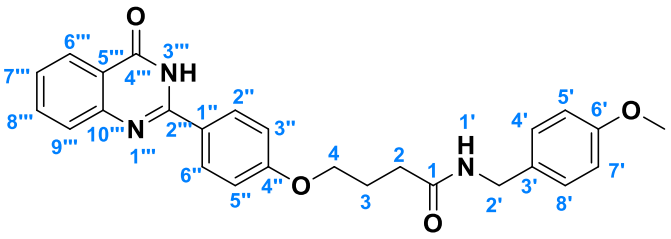


Figure 26: COSY NMR spectrum for quinazolinone amide 30o.

The signals for protons **H-7'''** and **H-8'''** show two sets of ortho couplings, each yielding two sets of doublets of doublets of doublets (ddd). The assignment of each signal was based on splitting patterns, and the typical *J* values associated with benzo-fused heterocyclic rings described above (refer to compound **29d**). Another set of doublets is

observed for the protons on the 4-methoxybenzyl ring (**H-4'**&**H-8'** and **H-5'**&**H-7'**). We have assigned the **H-4'**&**H-8'** signal to the lower field peak 7.16 (d, *J* = 8.6 Hz, 2H), because of the concomitant increase in electron density around protons **H-5'**&**H-7'** due to the shielding effect imposed by the nearby electron-donating group (-**OMe**); yielding a doublet signal at 6.85 (d, *J* = 8.7 Hz, 2H). The chemical shifts and splitting patterns for the long chain were consistent with the typical aliphatic protons (4.21-1.99 ppm) and their assignment was further supported by **^{13}C NMR** and **HSQC**.

The **^{13}C NMR** chemical shift values are shown in (**Figure 27**). All signals were assigned from the relative proton resonances using 2D **HSQC** and **HMBC** correlation experiments.



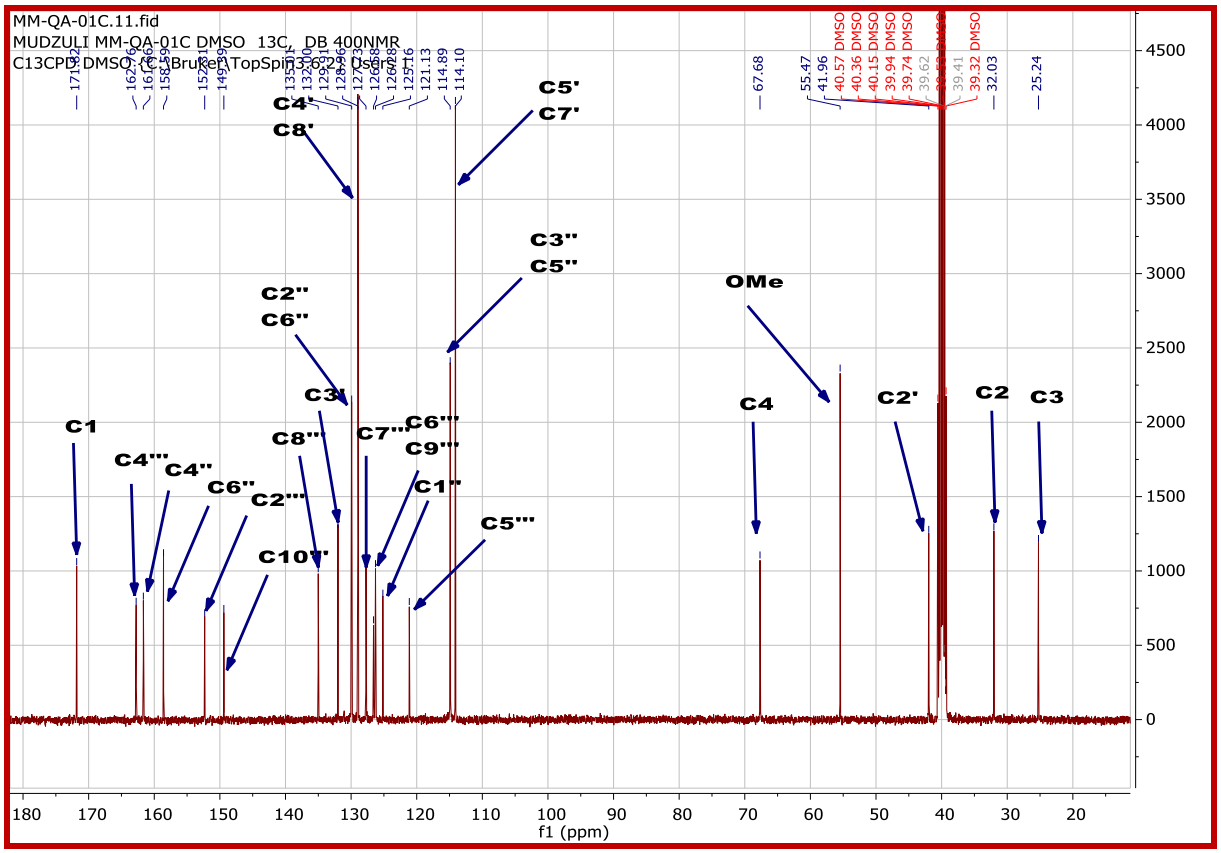


Figure 27: ^{13}C NMR of compound 30o.

The carbons were assigned based on the characteristic chemical shifts and the relative correlations caused by close and long-range **^{1}H-^{13}C NMR** interactions from correlation experiments **HSQC** and **HMBC.** The highly deshielded signal (171.8 ppm) was expected to be from the amide quaternary carbon (**C1**) and the **HMBC** correlations over two and three bonds with **NH-1'** and **H-2'** respectively corroborated this assignment (**Figure 28**). The second quaternary carbon **C4'''**, is the second most deshielded and the assignment was supported by **HMBC** correlations over three bonds with **H-6'''.**

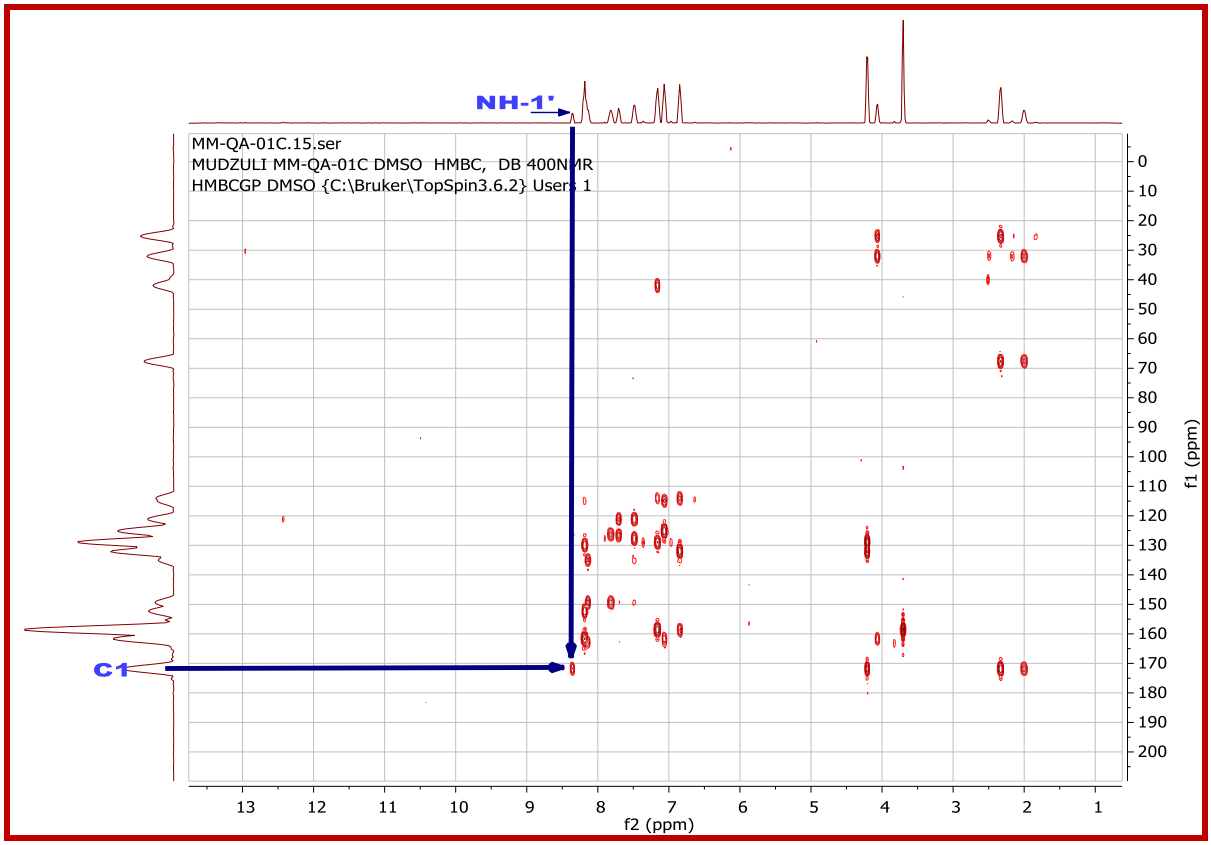


Figure 28:13C NMR of compound 30o.

For the aromatic quaternary carbons of the cyclized ring (**C-5'''**& **C-10'''**); it has been shown that the carbon directly attached to the nitrogen atom(**C-10'''**) resonates at a much lower field than (**C-5'''**).[283] Accordingly, **C-10'''** resonates close to 149.3 ppm and **C-5'''** around 121.1 ppm in our compound. This assignment was supported by **HSQC** and **HMBC** experiments. The **^{13}C NMR** chemical shifts for the long chain were strait forward, appearing in the range (67.6-25.2ppm).

CONCLUSION

In conclusion, the target compounds bearing a “linker” and “tail” conjugated to a quinazoline core were achieved. We were able to develop an efficient method for the total syntheses of novel 2-substituted quinazolinone amides. Our approach is based on a biocatalytic retrosynthetic analysis, where possible, intermediates and key chemical transformations were catalysed by enzymes. Various enzyme classes in combination with chemical steps were incorporated in the synthesis of a broad range of quinazolinone target molecules. Demonstrated here for the first time, our method employs commercial enzymes such as laccase, lipase and nitrile hydratase to obtain quinazolinone amides in excellent yields. Due to time constrains, the obtained target molecules were not evaluated for their anti-tubercular properties, however this will be done at a later stage.

Chapter FIVE

CONCLUSION AND FUTURE WORK

The principal goal of this PhD work was to evaluate the current applications of biocatalysts and explore various ways in which the well-established commercial enzymes may be utilised more productively in synthetic organic chemistry. We effectivity developed green synthetic methods for obtaining target analogues with anti-tuberculosis properties. The target molecules achieved in this work include important pharmaceutical scaffolds such as **benzimidazoles**, **benzothiazoles**, **triazoles** and **quinazolinones**. We underline recent contributions from our group regarding using Nitrile hydratases, Laccases and Lipases in the key steps and in performing functional group interconversions.

In **Chapter 2**, We extended our published methodology for obtaining 2-arylbenzimidazoles and benzothiazoles to derivatives bearing a hydroxy group. Subsequently, the obtained hydroxy heterocycles were alkylated to form benzimidazoles and benzothiazole ether alkynes. We discovered inherent challenges associated with the condensation of diamines with hydroxy benzaldehydes. This includes substrate decomposition, polymerisation, poor product solubility and co-elution. Despite our numerous advances, there remain major hurdles to this protocol. We hope to improve our reaction outcomes by eliminating substrate decomposition and this could be achieved by making use of microwave irradiation or employing carboxyl acids or alcohols instead of aldehydes.

In **Chapter 3**, We introduced for the first time, a laccase catalysed 1,3-dipolar cycloaddition reaction between alkynes and azide derivatives. The 2-aryl-*1H*-benzimidazoles and 2-arylbenzothiazole alkynes obtained in **Chapter 2** were treated with phenyl-azides under laccase conditions to obtain target benzothiazole and benzimidazole fused 1,2,3-triazole derivatives. To the best of our knowledge, the

application of oxidoreductase enzymes such as laccases in the construction of the triazole ring has not yet been explored. Our novel protocol proved not to be regiospecific, but not necessarily ineffective, yielding both the 1,4-Disubstituted-*1H*-1,2,3-triazoles and the 1,5-disubstituted-*1H*-1,2,3-triazoles in adequate to moderate yields. A total of 28 novel 1,2,3-triazoles were generated using this method. Future characterisation and directed evolution of the laccase isozymes of *T. versicolor*[284,285] using genetic engineering techniques may permit the optimization of the catalytic efficiency and regioselectivity of the enzyme.

In **Chapter 4**, We demonstrated how biocatalysts can be useful, practical, and environmentally friendly alternatives to conventional chemocatalysts. By conducting a multistep biocatalytic continuous-flow synthesis in combination with chemical steps, we were able to synthesise a series of novel quinazolinone derivatives in excellent yields. Our novel protocol uses a combination of enzymes such as Laccases (immobilised Novoprime Base 268), nitrile hydratase (*Rhodococcus rhodochrous*_whole cells), and lipase (immobilised Lipozyme_CALB L). We effectively catalysed the hydration of nitriles to amides using whole cells containing nitrile hydratase. Using microwave irradiation and Laccase as an oxidant, we were able to improve upon the currently existing methods for obtaining quinazolinones from aldehydes and 2-aminoamides. Subsequently, we hydrolysed the quinazolinone esters to acids using immobilised lipase. Using HBTU coupling reagent we effectively catalysed the direct amidation reaction of the quinazolinone acid to obtain the target quinazolinone amide derivatives in excellent yields. We were able to generate a total of 22 novel quinazolinone compounds using this method. Future work will include testing for biological activity against known tuberculosis strains. And also, the investigation of structure-activity relationship by molecular docking on target TB enzymes, to better understand the drug-receptor interactions of the mentioned compounds, with a probable outcome of producing derivatives with improved pharmacological properties.

Chapter SIX

EXPERIMENTAL SECTION

GENERAL

All reagents and solvents were commercially available and used as received without additional purification unless otherwise stated. Solvents were distilled before chromatographic separations were carried out. Thin-layer chromatography (TLC) was performed on a Merck Aluminium foil backed plates coated with silica gel (60 F254) and visualization was done under UV light. Silica gel column chromatography was performed using Macherey-Nagel silica gel 60 (particle size 0.063 mm to 0.20 mm) purchased from Merck. Melting points (mp) were determined on a Stuart SMP10 instrument. Bruker AVANCE 300 MHz, Bruker AVANCE 400 MHz and Bruker AVANCE III 500 MHz spectrometers were used to record both the ^{1}H and ^{13}C Nuclear Magnetic Resonance data in either DMSO-d_6 or $CDCl_3$. Data processing of the spectra was done using MestreNova Software under license from Mestrelab Research, CA, USA. Chemical shifts are expressed in ppm downfield against an internal standard, TMS (δ scale). Coupling constants are conveyed in Hertz. Note: For some of the structures, not all NMR active ^{13}C nuclei were detected. Bruker Tensor-27 Fourier Transform single-channel infrared spectrometer was used to record infrared spectra.

High-resolution mass spectra were recorded on an inked Bruker Compact Q-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) using an ESI positive source. A 10 µL of the sample was injected into the Dionex Ultimate 3000 UHPLC system (Thermo Scientific, Dionex, Sunnyvale, California, USA) and run through a loop for one minute at 50% Solvent A consisting of 0.1 % formic acid in H_2O (v/v) and 50% solvent B consisting of 0.1 % formic acid in acetonitrile (v/v) at a flowrate of 0.3 mL/min.

THE FOLLOWING METHODS WERE USED FOR THE PREPARATION OF 2-ARYLBENZOTHIAZOLES DERIVATIVES 4a–f

- **METHOD A**

A mixture of 2-aminothiophenol derivative (15.0 mmol) and hydroxybenzaldehyde derivative (10.0 mmol) in acetonitrile (10.0 mL) and acetate buffer (10.0 mL, pH 4.0) was stirred at room for 5 minutes. Novoprime Base 268 (0.105 g) was then added into the mixture and the contents were stirred overnight under aerobic conditions. After the reaction period, the contents of the reaction mixture were extracted with ethyl acetate (30.0 mL) and water (3 × 20.0 mL). The organic layer was separated, dried over $MgSO_4$, and the products were recrystallized from cold acetonitrile or purified by column chromatography (40% ethyl acetate/hexane) to afford the desired product **4**.

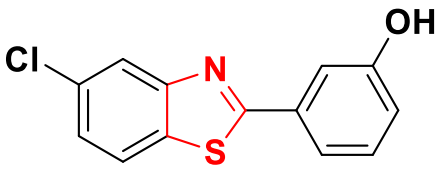
- **METHOD B**

The same as **Method A**, except that DMF (10.0 mL) was used instead of acetonitrile.

- **METHOD C**

The same as **Method A**, except that Acetone (10.0 mL) was used instead of acetonitrile.

- **Synthesis of 3-(5-chlorobenzo[*d*]thiazol-2-yl)phenol (4b)**



METHOD A.

Stirring time = 24 h. Purified through washing with cold acetonitrile (3 × 20.0 mL) to remove excess starting material (confirmed by TLC) to afford a cream solid (2.35 g, 90%). R_f (40% EtOAc/hexane) 0.78. Mp = 195–198 °C (lit. 198 °C)[286]. **^{1}H NMR (300 MHz, DMSO-*d*$_6$)** δ 10.24 (br s, 1H), 8.12 – 7.90 (m, 2H), 7.52 – 7.37 (m, 3H), 7.34 (t, *J* = 7.8 Hz, 1H), 6.96 (dd, *J* = 8.1, 2.4 Hz, 1H). **^{13}C NMR (75 MHz, DMSO-*d*$_6$)** δ 170.0, 157.4, 153.8, 133.3, 132.8, 131.4, 130.7, 125.6, 123.5, 121.8, 118.9, 118.4, 113.3.

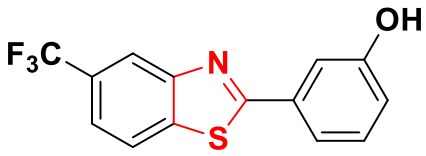
METHOD B.

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 × 20.0 mL) to remove any excess starting material was not successful (traces of starting material were present on TLC) to afford a yellow oil (1.83 g, 70%). R_f (40% EtOAc/hexane) 0.78.

METHOD C.

Stirring time = 24 h. Purified through washing with cold acetonitrile (3 × 20.0 mL) to remove excess starting material (confirmed by TLC) to afford a cream solid (2.16 g, 83%). R_f (40% EtOAc/hexane) 0.78.

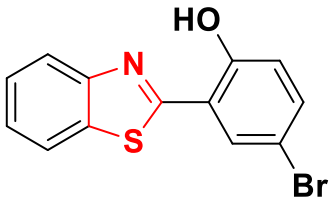
- **Synthesis of 3-(5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)phenol (4c)**



METHOD A.

Stirring time = 24 h. Purified through washing with cold acetonitrile (3 × 20.0 mL) to remove excess starting material (confirmed by TLC) to afford a white solid (2.54 g, 86%). R_f (40% EtOAc/hexane) 0.77. Mp = 186–188 °C. **IR (KBr) (cm^{-1}):** 3110, 2723, 1486, 1224. **^{1}H NMR (300 MHz, DMSO-*d*$_6$)** δ 6.41 (s, 1H), 6.35 (dd, *J* = 8.5, 3.4 Hz, 1H), 5.83 (d, *J* = 8.5 Hz, 1H), 5.71 (s, 1H), 5.62 (dd, *J* = 15.3, 7.8 Hz, 2H), 5.27 (dd, *J* = 7.8, 2.1 Hz, 1H). **^{13}C NMR (101 MHz, Chloroform-*d*)** δ 169.7, 156.2, 153.5, 134.4, 130.5, 122.2, 121.6, 121.6, 120.4, 118.7, 114.1. **HRMS** m/z calcd for $C_{14}H_8F_3NOS$: 295.0279, found $[M+H]^+$ 296.0203.

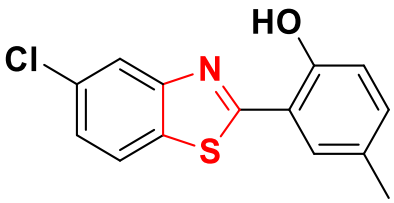
- **Synthesis of 2-(benzo[*d*]thiazol-2-yl)-4-bromophenol (4d)**



METHOD A.

Stirring time = 24 h. Purified by column chromatography (40% ethyl acetate/hexane) to afford a green solid (1.14 g, 39%). R_f (40% EtOAc/hexane) 0.84. Mp = 208–214°C (lit. 210°C)[286]. **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 12.50 (br s, 1H), 8.99 (s, 1H), 7.94 (d, *J* = 2.6 Hz, 1H), 7.63 – 7.56 (m, 1H), 7.47 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.39 (td, *J* = 7.6, 1.5 Hz, 1H), 7.32 (td, *J* = 7.5, 1.5 Hz, 1H), 7.00 (d, *J* = 8.8 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 162.2, 159.5, 146.5, 136.5, 134.3, 131.0, 128.8, 128.6, 127.0, 121.8, 119.6, 119.0, 110.6.

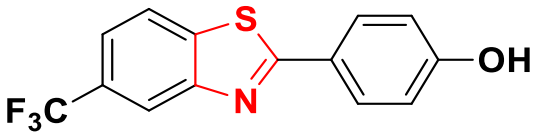
- **Synthesis of 2-(5-chlorobenzo[*d*]thiazol-2-yl)-4-methoxyphenol (3e)**



METHOD A.

Stirring time = 24 h. Purified by column chromatography (40% ethyl acetate/hexane) to afford a yellow solid (1.14 g, 39%). R_f (40% EtOAc/hexane) 0.76. Mp = 228–232 °C. **IR (KBr) (cm^{-1}):** 3077, 2085, 1474, 1214. **^{1}H NMR (400 MHz, Chloroform-*d*)** δ 13.15 (br s, 1H), 8.68 (d, *J* = 2.6 Hz, 1H), 8.49 – 8.26 (m, 2H), 8.12 (d, *J* = 8.4 Hz, 1H), 7.89 – 7.52 (m, 1H), 7.26 – 7.16 (m, 1H), 2.18 (s, 3H). **^{13}C NMR (101 MHz, Chloroform-*d*)** δ 168.9, 162.6, 150.4, 127.8, 124.2, 122.0, 119.2, 118.5, 115.4, 30.4.

- **Synthesis of 4-(5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)phenol (3f)**



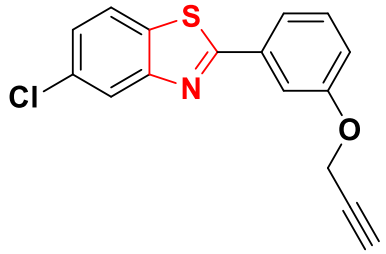
METHOD A.

Stirring time = 24 h. Purified by column chromatography (40% ethyl acetate/hexane) to afford brown crystals (1.40 g, 47%). R_f (40% EtOAc/hexane) 0.76. Mp = 199–202 °C. **IR (KBr) (cm^{-1}):** 3220, 2965, 1445, 1218. **^{1}H NMR (400 MHz, Chloroform-*d*)** δ 8.29 (s, 1H), 8.00 (t, *J* = 8.3 Hz, 3H), 7.60 (d, *J* = 9.0 Hz, 1H), 6.97 (d, *J* = 8.7 Hz, 2H), 5.44 (s, 1H). **^{13}C NMR (126 MHz, Chloroform-*d*)** δ 169.8, 158.6, 153.7, 138.2, 128.9 (q, $J_{C\text{-}F}$ = 32.7 Hz), 129.6, 126.0, 124.2 (q, $J_{C\text{-}F}$ = 272.1 Hz), 121.1 (q, $J_{C\text{-}F}$ = 3.6 Hz), 119.9 (q, $J_{C\text{-}F}$ = 4.1 Hz), 116.0. **HRMS m/z** calcd for $C_{14}H_8F_3NOS$: 295.0279, found $[M+H]^+$ 296.0203.

PREPARATION OF PROPARGYL BENZOTHIAZOLE DERIVATIVES 6a–f

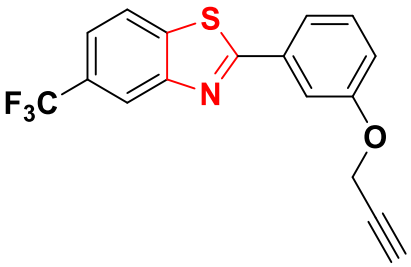
A mixture of hydroxybenzothiazole derivative (10.0 mmol) and potassium carbonate (2 g) in dry acetone was stirred for 10 minutes under an inert atmosphere. A solution of propargyl bromide (10.0 mmol, 1.17g) was slowly added and the resultant mixture was refluxed at 60℃. After completion, the mixture was filtered through cotton wool and the product was purified using column chromatography.

▪ Synthesis of 5-chloro-2-(3-(prop-2-yn-1-yloxy)phenyl)benzo[*d*]thiazole (6b)



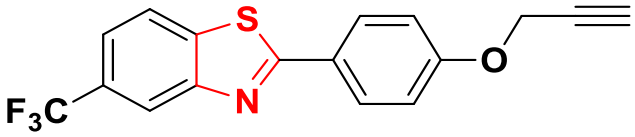
Stirring time = 4 h. Purified by column chromatography (40% ethyl acetate/hexane) to afford a colorless oil (2.90 g, 97%). R_f (40% EtOAc/hexane) 0.84. **IR (KBr) (cm^{-1}):** 3325, 2927, 1612, 1455, 1210. **^{1}H NMR (500 MHz, DMSO-d_6)** δ 8.44 (m, 2H), 7.82 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.78 – 7.74 (m, 1H), 7.72 (t, *J* = 2.1 Hz, 1H), 7.56 (t, *J* = 8.0 Hz, 1H), 7.27 (dd, *J* = 8.2, 2.6 Hz, 1H), 4.96 (d, *J* = 2.4 Hz, 2H), 3.64 (t, *J* = 2.4 Hz, 1H). **^{13}C NMR (126 MHz, DMSO-d_6)** δ 170.1, 158.2, 153.5, 134.0, 131.2, 124.4, 121.1, 119.3, 113.5, 79.3, 79.2, 56.2. **HRMS** m/z calcd for $C_{16}H_{10}ClNOS$: 299.0172, found $[M+H]^+$ 300.3092

- **Synthesis of 2-(3-(prop-2-yn-1-yloxy)phenyl)-5(trifluoromethyl) benzo[*d*] thiazole (6c)**



Stirring time = 6 h. Purified by column chromatography (40% ethyl acetate/hexane) to afford a cream solid (2.92 g, 88%). R_f (40% EtOAc/hexane) 0.8. Mp = 32 °C. **IR (KBr) (cm^{-1}):** 3320, 2933, 1466, 1213. **^{1}H NMR (300 MHz, Chloroform-*d*)** δ 8.06 (d, *J* = 2.0 Hz, 1H), 7.81 (d, *J* = 8.5 Hz, 1H), 7.77 – 7.62 (m, 2H), 7.49 – 7.29 (m, 2H), 7.14 (ddd, *J* = 8.3, 2.5, 0.9 Hz, 1H), 4.81 (s, 2H), 2.57 (s, 1H). **^{13}C NMR (75 MHz, Chloroform-*d*)** δ 158.0, 130.2, 125.7, 123.1, 122.3, 121.0, 118.2, 113.5, 75.9, 56.0. **HRMS** m/z calcd for $C_{17}H_{10}F_3NOS$: 333.0435, found $[M+H]^+$ 334.0337.

- **2-(4-(prop-2-yn-1-yloxy)phenyl)-5-(trifluoromethyl)benzo[*d*]thiazole (6f)**



Stirring time = 24 h. Purified by column chromatography (40% ethyl acetate/hexane) to afford a light brown solid (2.74 g, 82%). R_f (40% EtOAc/hexane) 0.78**.** Mp = 48 °C. **IR (KBr) (cm^{-1}):** 3320, 2971, 1465, 1217. **^{1}H NMR (400 MHz, Chloroform-*d*)** δ 8.29 (s, 1H), 8.06 (d, *J* = 8.7 Hz, 2H), 7.98 (d, *J* = 8.4 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 1H), 7.11 (d, *J* = 8.7 Hz, 2H), 4.79 (s, 2H), 2.57 (s, 1H). **^{13}C NMR (101 MHz, Chloroform-*d*)** δ 169.60, 160.22, 153.87, 138.35, 129.30, 128.9 (q, J_{C-F} = 32.7 Hz), 126.69, 124.2 (q, J_{C-F} = 272.3 Hz), 122.10, 121.1 (q, J_{C-F} = 3.5 Hz), 120.0 (q, J_{C-F} = 4.2 Hz), 115.47, 55.96. **HRMS** m/z calcd for $C_{17}H_{10}F_3NOS$: 333.0435, found $[M+H]^+$ 334.0342.

THE FOLLOWING METHODS WERE USED FOR THE PREPARATION OF 2-ARYL-1*H*-BENZIMIDAZOLES DERIVATIVES 5/8

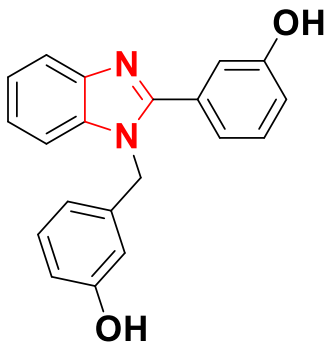
- **Method D.**

A mixture of *o*-phenylenediamine (10.0 mmol) and hydroxybenzaldehyde derivative (20.0 mmol) in acetonitrile (10.0 mL) and acetate buffer (10.0 mL, pH 4.0) was stirred at room for 5 minutes. Novoprime Base 268 (0.105 g) was then added into the mixture and the contents were stirred overnight under aerobic conditions. After the reaction period, the contents of the reaction mixture were extracted with ethyl acetate (30.0 mL) and water (3 × 20.0 mL). The organic layer was separated, dried over $MgSO_4$, and the products were recrystallized from cold acetonitrile or purified by column chromatography (40% ethyl acetate/hexane) to afford the desired product.

- **Method E.**

A repetition of **Method D** using *Trametes versicolor* (100 mg) laccase instead of Novoprime Base 268 (0.105 g).

- **Synthesis of 3-(1-(3-hydroxybenzyl)-1*H*-benzo[*d*]imidazol-2-yl)phenol (8a)**



Method D.

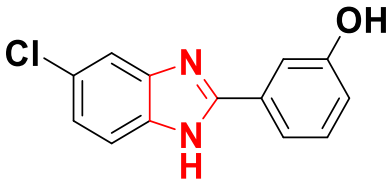
Stirring time = 24 h. Purification by washing with cold acetonitrile (3 × 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (2.58 g, 82%). R_f (40% EtOAc/hexane) 0.23. MP = 246 – 249 °C (Lit. 243-245 °C)[287]. ($[M + H]^+$ Found: 316 $C_{20}H_{16}N_2O_2$ requires $[M + H]^+$, 316.1286). **^{1}H NMR (500 MHz, DMSO-d_6)**: δ

9.77 (1H, d, *J* = 30.8 Hz), 9.43 (1H, s), 7.73 (1H, d, *J* = 7.7 Hz), 7.39 (1H, d, *J* = 7.5 Hz), 7.33 (1H, q, *J* = 7.7 Hz), 7.23 (3H, td, *J* = 17.7, 16.0, 8.7 Hz), 7.17 – 7.06 (2H, m), 6.95 (1H, d, *J* = 8.1 Hz), 6.65 (1H, d, *J* = 8.0 Hz), 6.50 (1H, d, *J* = 7.5 Hz), 6.40, 5.49. **^{13}C NMR (126 MHz, DMSO-*d*$_6$)**: δ 156.0, 155.9, 151.6, 140.9, 136.7, 134.2, 129.5, 128.1, 120.9, 120.5, 117.8, 117.5, 115.2, 114.9, 114.3, 112.8, 111.0, 109.4, 45.8.

Method E.

Stirring time = 24 h. Purified by column chromatography (60% ethyl acetate/hexane) to afford a brown solid (0.347 g, 11%).

- **Synthesis of 3-(6-Chloro-1*H*-benzo[*d*]imidazol-2-yl)phenol (5h)**



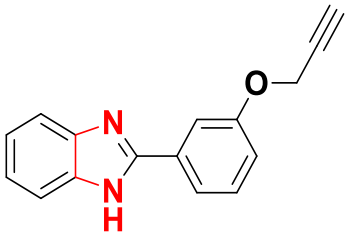
Method D.

Stirring time = 24 h. Purified by column chromatography (40% ethyl acetate/hexane) to afford a Dark brown solid (0.61g, 25%). R_f (40% EtOAc/hexane) 0.27. MP = 241 – 242 °C (Lit. 236 - 237°C).[288] **^{1}H NMR (300 MHz, DMSO-*d*$_6$)** δ 9.83 (d, *J* = 3.0 Hz, 1H), 9.45 (d, *J* = 4.7 Hz, 1H), 7.59 (d, *J* = 11.7 Hz, 1H), 6.94 (d, *J* = 15.7 Hz, 1H), 6.65 (d, *J* = 8.0 Hz, 1H), 6.47 (d, *J* = 11.0 Hz, 1H), 6.37 (s, 1H), 5.52 (s, 1H). **^{13}C NMR (400 MHz, DMSO-*d*$_6$)** δ 158.5, 155.2. 138.8, 130.7, 127.4, 121.3, 120.2, 118.1, 116.7, 115.1.

PREPARATION OF PROPARGYL BENZIMIDAZOLE DERIVATATIVES 7/9

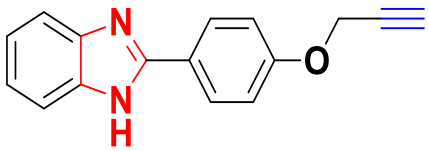
A mixture of hydroxybenzothiazole derivative (5.0 mmol) and potassium carbonate (2 g) in dry acetone was stirred for 10 minutes under an inert atmosphere. A solution of propargyl bromide (10.0 mmol, 1.17g) was slowly added and the resultant mixture was refluxed at 60°C. After completion, the mixture was filtered through cotton wool and the product was purified using column chromatography.

- **Synthesis of 2-(3-(prop-2-yn-1-yloxy)phenyl)-1H-benzo[*d*]imidazole (7a)**



Stirring time = 24 h. Purified by column chromatography (40% ethyl acetate/hexane) to afford a Dark brown oil (0.902g, 36%). R_f (40% EtOAc/hexane) 0.53. **IR (KBr) (cm^{-1}):** 3326, 2981, 1464, 1255, 858. **^{1}H NMR (500 MHz, DMSO-d_6)** δ 13.01 (s, 1H), 7.73 (s, 1H), 7.59 – 7.47 (m, 1H), 7.44 (t, *J* = 8.1 Hz, 1H), 7.35 – 7.23 (m, 1H), 7.18 (t, *J* = 7.9 Hz, 1H), 7.09 (dd, *J* = 8.3, 2.5 Hz, 1H), 6.95 – 6.85 (m, 1H), 6.79 (d, *J* = 10.6 Hz, 1H), 4.87 – 4.66 (m, 2H), 3.56 – 3.39 (m, 1H). **^{13}C NMR (126 MHz, DMSO-d_6)** δ 157.6, 152.5, 147.5, 143.3, 130.7, 129.5, 120.0, 119.0, 117.4, 113.5, 112.7, 79.8, 78.5, 69.2. **HRMS** m/z calcd for $C_{16}H_{12}N_2O$:248.0950, found $[M+H]^+$ 249.0978.

- **Synthesis of 2-(4-(prop-2-yn-1-yloxy)phenyl)-1*H*-benzo[*d*]imidazole (7c)**



Stirring time = 24 h. Purified by column chromatography (40% ethyl acetate/hexane) to afford a Dark brown solid (0.902g, 36%). R_f (40% EtOAc/hexane) 0.43. MP = 203 – 205 °C (Lit. 200 - 201°C).[289] **^{1}H NMR (500 MHz, DMSO-d_6)** δ 12.81 (s, 1H), 8.18 (d, *J* = 8.9 Hz, 2H), 7.62 (d, *J* = 57.4 Hz, 2H), 7.36 – 7.08 (m, 4H), 5.04 – 4.87 (m, 2H), 3.68 (s, 1H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 158.9, 151.6, 130.9, 130.5, 128.3, 127.8, 123.8, 122.8, 122.5, 121.9, 119.0, 117.7, 115.7, 115.7, 115.5, 115.5, 111.4, 79.5, 79.0, 56.0.

PREPARATION OF PHENYL AZIDE 15a-f

Following the standard azide synthesis approach, several aromatic amines were successively converted into azides from the reaction of sodium nitrite with *in situ* generated diazonium salts.[218]

Analine derivative (0.05 mol) was dissolved in 1:1 HCl/H_2O and the contents were agitated at 0 – 5. Sodium nitrite (0.06 mol) was dissolved in water and added dropwise; sodium azide (0.05 mol) dissolved in water was added dropwise into the solution. Then the reaction was allowed to stir for 5 hours. The reactions yielded various aromatic azide products as yellow and brown oils and solids, with yields ranging from 93% to 98%. The azide products were confirmed by FTIR (typical absorption band at 2099-2100 cm^{-1}) of the crude product and were used directly in the next step without further purification.

THE FOLLOWING METHODS WERE USED FOR THE PREPARATION OF 1,2,3-Triazoles Derivatives (20/21a-h)

- **Method F:**

Trametes versicolor laccase

A mixture of the propargyl unit (1.0 mmol) and phenylazide (1.0 mmol) in acetate buffer pH 4.5 (2 ml) and acetone (2.0 ml) was stirred for 5 minutes. *Trametes versicolor* laccase (10 mg) was added, and the mixture was stirred overnight at room temperature. After the reaction period, the contents of the reaction mixture were extracted with ethyl acetate (30.0 mL) and water (3 × 20.0 mL). The organic layer was separated, dried over $MgSO_4$. The products were concentrated and purified by column chromatography to afford the desired product.

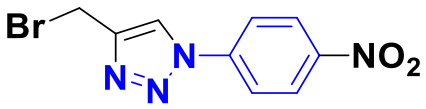
- **Method G.**

The same as **Method F**, except that Acetonitrile (2.0 mL) was used instead of acetone.

- **Method H.**

A mixture of $CuSO_4.5H_2O$ (0.03 mmol), sodium ascorbate (0.12 mmol) and DABCO (0.06 mmol) in water (2 mL) was stirred vigorously at room temperature for 5 min. To it acetic acid (0.06 mmol), alkynes (1 mmol) and azides (1 mmol) were added sequentially, and the resultant mixture was stirred overnight at room temperature. After the reaction period, the contents of the reaction mixture were extracted with ethyl acetate (30.0 mL) and water (3 × 20.0 mL). The organic layer was separated, dried over $MgSO_4$, and the products were concentrated and purified by column chromatography to afford the desired product.[218]

- **Synthesis of 4-(Bromomethyl)-1-(4-nitrophenyl)-1*H*-1,2,3-triazole (20a)**



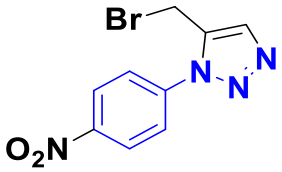
Method F.

Propargyl unit (0.118 g, 1.0 mmol), phenylazide (0.164 g, 1.0 mmol). Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford a dark brown oil (64.2 mg, 24%). R_f (40% EtOAc/hexane) 0.57. **IR (KBr) (cm^{-1})**: 3393, 2916, 1656, 1597, 1210. **^{1}H NMR (400 MHz, Chloroform-*d*)** δ 8.45 (d, *J* = 9.0 Hz, 1H), 8.19 (s, 1H), 8.16 (d, *J* = 4.2 Hz, 1H), 7.99 (d, *J* = 9.0 Hz, 1H), 6.93 (d, *J* = 9.1 Hz, 1H), 4.66 (s, 2H). **^{13}C NMR (101 MHz, Chloroform-*d*)** δ 126.2, 125.6, 120. 9, 120.6, 115.7, 31.0. **HRMS** m/z calcd for $C_9H_8BrN_4O_2$: 282.9825, found $[M+H]^+$ 282.9848.

Method G.

Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford a dark brown oil (56.3 mg, 20%).

- **Synthesis of 5-bromo-1-(4-nitrophenyl)-1H-1,2,3-triazole (21a)**



Method F.

Propargyl unit (0.118 g, 1.0 mmol), phenylazide (0.164 g, 1.0 mmol). Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford a light brown oil (31.1 mg, 11%) 0.34. . **IR (KBr) (cm^{-1})**: 3393, 2916, 1656, 1597, 1210. **^{1}H NMR (500 MHz, Chloroform-*d*)** δ 8.50 (d, *J* = 8.9 Hz, 1H), 7.92 (s, 1H), 7.89 (d, *J* = 8.9 Hz, 1H), 7.02 – 6.94 (m, 1H), 6.90 (dd, *J* = 8.2, 2.5 Hz, 1H), 4.70 (s, 1H), 4.52 (s, 1H). **^{13}C NMR (126 MHz, Chloroform-*d*)** δ 129.6, 125.5, 125.3, 118.7, 114.0, 112.1, 30.7. **HRMS** m/z calcd for $C_9H_8BrN_4O_2$: 281.9825, found $[M+H]^+$ 282.9844.

Method G.

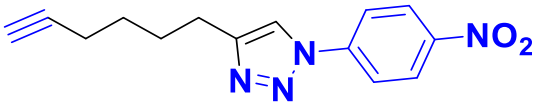
Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford a light brown oil (25.4 mg, 9%).

- **Synthesis of 4-butyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazole (20b)**

Method H.

Propargyl unit (0.0821 g, 1.0 mmol), phenylazide (0.164 g, 1.0 mmol). Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford a brown oil (0.133 g, 54%). R_f (40% EtOAc/hexane) 0.63. **IR (KBr) (cm^{-1})**: 3162, 2917, 1653, 1597, 1236. **^{1}H NMR (300 MHz, Chloroform-*d*)** δ 8.41 (d, *J* = 8.6 Hz, 2H), 7.98 (d, *J* = 8.6 Hz, 2H), 7.87 (s, 1H), 2.83 (t, *J* = 7.6 Hz, 2H), 1.69 (s, 2H), 1.44 (q, *J* = 7.4 Hz, 2H), 0.97 (t, *J* = 7.3 Hz, 3H). **^{13}C NMR (75 MHz, Chloroform-*d*)** δ 146.9, 141.4, 125.5, 120.19, 31.3, 25.2, 22.2, 13.8. **HRMS** m/z calcd for $C_{12}H_{15}N_4O_2$: 247.1190, found $[M+H]^+$ 247.1185.

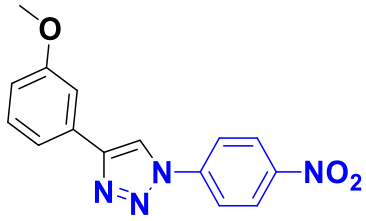
- **Synthesis of 4-(hex-5-yn-1-yl)-1-(4-nitrophenyl)-1*H*-1,2,3-triazole (20c)**



Method H.

Propargyl unit (0.106 g, 1.0 mmol), phenylazide (0.164g, 1.0 mmol). Stirring time = 4 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford an orange brown oil (0.281 g, 62%). R_f (40% EtOAc/hexane) 0.62. **IR (KBr) (cm^{-1})**: 3310, 3108, 2898, 1643, 1562, 1216. **^{1}H NMR (300 MHz, Chloroform-*d*)** δ 8.42 (d, *J* = 9.2 Hz, 2H), 7.97 (d, *J* = 9.2 Hz, 2H), 7.86 (s, 1H), 2.86 (t, *J* = 7.6 Hz, 2H), 2.33 – 2.22 (m, 2H), 1.96 (s, 1H), 1.93 – 1.83 (m, 2H), 1.66 (dt, *J* = 13.8, 6.7 Hz, 2H). **^{13}C NMR (75 MHz, Chloroform-*d*)** δ 125.5, 120.2, 84.0, 68.6, 30.3, 28.1, 27.8, 25.0, 18.1. **HRMS** m/z calcd for $C_{14}H_{15}N_4O_2$: 271.1190, found $[M+H]^+$ 271.1186.

- **Synthesis of 4-(3-Methoxyphenyl)-1-(4-nitrophenyl)-1*H*-1,2,3-triazole (20e)**



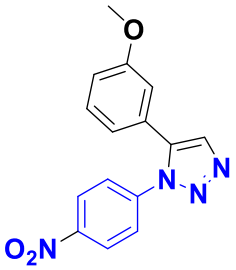
Method F.

Propargyl unit (0.118 g, 1.0 mmol), phenylazide (0.164 g, 1.0 mmol). Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford a yellow oil (41.5 mg, 14%). R_f (40% EtOAc/hexane**)** 0.64**. IR (KBr) (cm^{-1})**: 3124, 2918, 1613, 1581, 1227. **^{1}H NMR (500 MHz, Chloroform-*d*)** δ 8.46 (d, *J* = 9.0 Hz, 2H), 8.29 (s, 1H), 8.05 (d, *J* = 9.0 Hz, 2H), 7.53 (s, 1H), 7.46 (d, *J* = 7.7 Hz, 1H), 7.40 (t, *J* = 7.9 Hz, 1H), 6.96 (dd, *J* = 8.0, 2.6 Hz, 1H), 3.91 (s, 3H). **^{13}C NMR (126 MHz, Chloroform-*d*)** δ 160.1, 149.1, 130.1, 125.6, 120.4, 118.3, 117.3, 114.9, 111.1, 55.4. **HRMS** m/z calcd for $C_{15}H_{13}N_4O_3$: 297.0982, found $[M+H]^+$ 297.0907.

Method H.

Propargyl unit (0.118 g, 1.0 mmol), phenylazide (0.164 g, 1.0 mmol). Stirring time = 4 h. Purified by column chromatography to afford a yellow oil (79.9 mg, 27%).

- **Synthesis of 5-(3-methoxyphenyl)-1-(4-nitrophenyl)-1H-1,2,3-triazole (21e)**

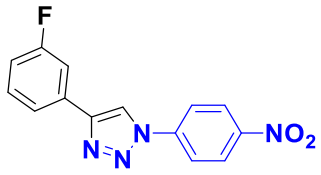


Method F.

Propargyl unit (0.118 g, 1.0 mmol), phenylazide (0.164 g, 1.0 mmol). Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford an orange brown oil (17.8 mg, 6%). **IR (KBr) (cm^{-1})**: 3083, 2975, 1681, 1582, 1227. R_f (40% EtOAc/hexane**)** 0.42. **^{1}H NMR (400 MHz, Chloroform-*d*)** δ 8.31 (d, *J* = 9.0 Hz, 2H), 7.89

(s, 1H), 7.61 (d, *J* = 9.0 Hz, 2H), 7.31 (t, *J* = 7.9 Hz, 1H), 6.97 (d, *J* = 7.9 Hz, 1H), 6.86 – 6.71 (m, 2H), 3.78 (s, 3H). **^{13}C NMR (101 MHz, Chloroform-*d*)** δ 159.9, 147.5, 141.2, 137.8, 134.2, 130.4, 127.2, 125.2, 124.8, 121.0, 115.1, 114.6, 55.4. **HRMS** m/z calcd for $C_{15}H_{13}N_4O_3$: 297.0982, found $[M+H]^+$ 297.0983.

- **Synthesis of 4-(3-Fluorophenyl)-1-(4-nitrophenyl)-1*H*-1,2,3-triazole (20f)**



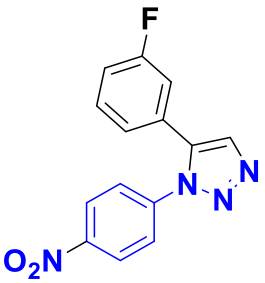
Method F.

Propargyl unit (0.120 g, 1.0 mmol), phenylazide (0.16 4g, 1.0 mmol). Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford a brown oil (73.9 mg, 26%). R_f (40% EtOAc/hexane) 0.68. **IR (KBr) (cm^{-1})**: 3099, 2962, 1617, 1587, 1237. **^{1}H NMR (400 MHz, Chloroform-*d*)** δ 8.47 (d, *J* = 9.0 Hz, 2H), 8.32 (s, 1H), 8.05 (d, *J* = 9.0 Hz, 2H), 7.73 – 7.65 (m, 1H), 7.52 – 7.44 (m, 2H), 7.11 (td, *J* = 8.4, 2.5 Hz, 1H). **^{13}C NMR (101 MHz, Chloroform-*d*) δ** 160.3 (d, J_{C-F} = 276.5 Hz), 140.7, 131.3 (d, J_{C-F} = 10.9 Hz), 130.50 (d, J_{C-F} = 8.4 Hz), 128.7, 127.8, 125.4, 124.5, 124.2, 123.6 (d, J_{C-F} = 3.0 Hz), 121.35 (d, J_{C-F} = 3.1 Hz), 120.2, 117.3, 115.6 (d, J_{C-F} = 21.3 Hz), 112.7 (d, J_{C-F} = 23.2 Hz). **HRMS** m/z calcd for $C_{14}H_{10}FN_4O_2$: 285.0782, found $[M+H]^+$ 285.0778.

Method H.

Propargyl unit (0.120 g, 1.0 mmol), phenylazide (0.164 g, 1.0 mmol). Stirring time = 4 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford a brown oil (0.111g, 39%).

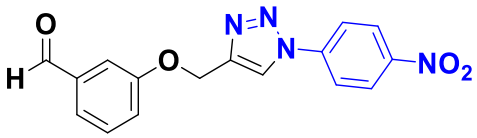
- **Synthesis of 5-(3-methoxyphenyl)-1-(4-nitrophenyl)-1H-1,2,3-triazole (21f)**



Method F.

Propargyl unit (0.120 g, 1.0 mmol), phenylazide (0.164 g, 1.0 mmol). Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford a brown oil (17.8 mg, 6%). R_f (40% EtOAc/hexane**)** 0.52**. IR (KBr) (cm^{-1})**: 3090, 2920, 1611, 1597, 1237. **^{1}H NMR (400 MHz, Chloroform-*d*)** δ 8.34 (d, *J* = 8.9 Hz, 2H), 7.92 (s, 1H), 7.60 (d, *J* = 8.9 Hz, 2H), 7.41 (td, *J* = 8.0, 5.7 Hz, 1H), 7.22 – 7.14 (m, 1H), 7.05 – 6.93 (m, 2H). **^{13}C NMR (101 MHz, Chloroform-*d*) δ** 160.6 (d, $J_{C\text{-}F}$ = 249.3 Hz), 145.6, 138.8, 134.7, 132.2, 129.0 (d, $J_{C\text{-}F}$ = 8.7 Hz), 125.8 (d, $J_{C\text{-}F}$ = 8.3 Hz), 124.1, 123.5 123.2, 122.9, 122.4 (d, $J_{C\text{-}F}$ = 3.3 Hz), 119.47 (d, $J_{C\text{-}F}$ = 3.0 Hz), 118.3, 115.54, 115.02 (d, $J_{C\text{-}F}$ = 21.0 Hz), 113.73 (d, $J_{C\text{-}F}$ = 23.3 Hz). **HRMS** m/z calcd for $C_{14}H_{10}FN_4O_2$: 285.0782, found $[M+H]^+$ 285.0801.

- **Synthesis of 3-((1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy) benzaldehyde (20g)**



Method F.

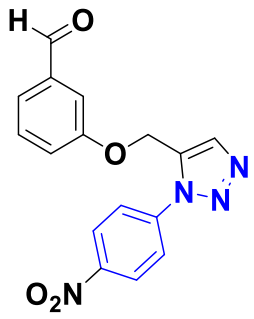
propargyl unit (0.161 g, 1.0 mmol), phenylazide (0.164 g, 1.0 mmol). Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford an orange solid (74.5 mg, 23%). R_f (40% EtOAc/hexane) 0.48**. IR (KBr) (cm^{-1})**: **)**: 3098, 2926, 1673, 1595, 1234. **^{1}H NMR (400 MHz, DMSO-d_6)** δ 10.01 (s, 1H), 9.20 (s, 1H), 8.47 (d, *J* = 9.1 Hz, 2H), 8.26 (d, *J* = 9.1 Hz, 2H), 7.61 (s, 1H), 7.57-7.55 (m, 2H), 7.46-7.42 (m, 1H), 5.39 (s, 2H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 193.3, 158.9, 147.2, 144.7, 141.2, 138.1,

130.9, 126.0, 123.8, 123.3, 122.0, 121.2, 114.7, 61.7. **HRMS** m/z calcd for $C_{16}H_{13}N_4O_4$: 325.0931, found $[M+H]^+$ 325.0914.

Method H.

Propargyl unit (0.161 g, 1.0 mmol), phenylazide (0.164 g, 1.0 mmol). Stirring time = 4 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford an orange solid (0.119 g, 37%).

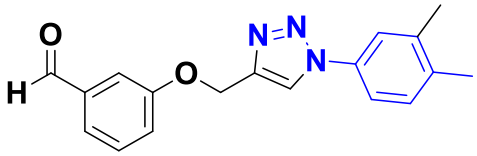
- **Synthesis of 3-((1-(4-nitrophenyl)-1H-1,2,3-triazol-5-yl)methoxy) benzaldehyde (21g)**



Method F.

Propargyl unit (0.161 g, 1.0 mmol), phenylazide (0.164 g, 1.0 mmol). Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford an orange oil (61.5 mg, 19%). R_f (40% EtOAc/hexane**)** 0.41**. IR (KBr) (cm^{-1})**: 3090, 2924, 1692, 1596, 1234. **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 9.97 (s, 1H), 8.45 (d, *J* = 9.0 Hz, 2H), 8.16 (s, 1H), 8.02 (d, *J* = 9.0 Hz, 2H), 7.62 – 7.47 (m, 2H), 7.43 (s, 1H), 7.31 (m, 1H), 5.49 (s, 2H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 204.6, 169.6, 159.6, 152.6, 149.6, 147.4, 145.8, 142.5, 137.5, 137.1, 135.5, 133.7, 126.2, 70.56. **HRMS** m/z calcd for $C_{16}H_{13}N_4O_4$: 325.0931, found $[M+H]^+$ 325.0914.

- **Synthesis of 3-((1-(3,4-Dimethylphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy) benzaldehyde (20h)**

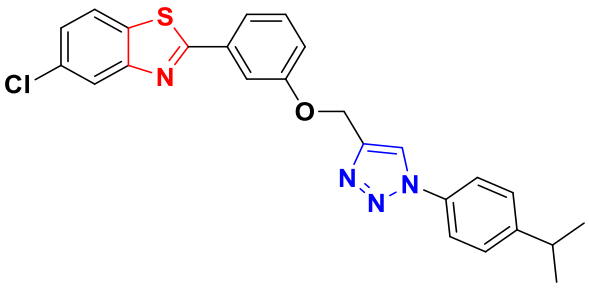


Method H.

Propargyl unit (0.161 g, 1.0 mmol), phenylazide (0.164 g, 1.0 mmol). Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford a colorless oil (74.5 mg, 23%). R_f (40% EtOAc/hexane) 0.43. **^{1}H NMR (300 MHz, Chloroform-*d*)** δ 9.95 (s, 1H), 7.85 (s, 1H), 7.58 – 7.43 (m, 3H), 7.33 – 7.27 (m, 1H), 7.25 – 7.06 (m, 3H), 5.34 (s, 2H), 2.36 (s, 3H), 2.20 (s, 3H). **^{13}C NMR (75 MHz, Chloroform-*d*)** δ 192.3, 159.1, 140.4, 138.1, 134.2, 133.6, 132.4, 130.5, 127.7, 126.0, 125.0, 124.0, 122.2, 114.0, 62.4, 21.4, 18.07. **HRMS** m/z calcd for $C_{18}H_{18}N_3O_2$: 308.1394, found $[M+H]^+$ 308.1377.

PREPARATION OF NOVEL BENZIMIDAZOLE AND BENZOTHIAZOLE FUSED TRIAZOLE DERIVATIVES (23/24)

- **Synthesis of 5-Chloro-2-(3-((1-(4-isopropylphenyl)-1*H*-1,2,3-triazol-4-yl) methoxy)phenyl) benzo[*d*]thiazole (23b)**



Method F.

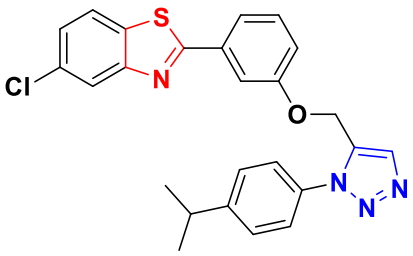
Propargyl unit (0.150 g, 0.5 mmol), phenylazide (0.082 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford a yellow oil (78.2 mg, 34%). R_f (40% EtOAc/hexane) 0.21. **IR (KBr) (cm^{-1}):** 3088, 2926, 1612, 1495, 1440, 1421. **^{1}H NMR (500 MHz, Chloroform-*d*)** δ 8.10 – 8.02 (m, 2H), 7.82 (d, *J* = 8.5 Hz, 1H), 7.80 (t, *J* = 2.1 Hz, 1H), 7.69 (t, *J* = 1.2 Hz, 1H), 7.66 (dd, *J* = 8.8, 2.2 Hz, 2H),

7.44 (t, *J* = 8.0 Hz, 1H), 7.41 – 7.33 (m, 3H), 7.19 (dd, *J* = 8.3, 2.6 Hz, 1H), 5.41 (s, 2H), 2.99 (hept, *J* = 6.9 Hz, 1H), 1.34 – 1.18 (m, 6H). **^{13}C NMR (126 MHz, Chloroform-*d*)** δ 158.6, 154.87, 150.0, 144.2, 134.8, 134.6, 133.3, 132.3, 130.3, 127.7, 125.7, 123.0, 122.3, 121.0, 120.7, 120.6, 118.0, 113.4, 62.2, 33.8, 23.8. **HRMS** m/z calcd for $C_{25}H_{22}ClN_4OS$: 461.1197, found $[M+H]^+$ 461.1196.

Method H.

Propargyl unit (0.150 g, 0.5 mmol), phenylazide (0.082 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford a yellow oil (74.5 mg, 23%)

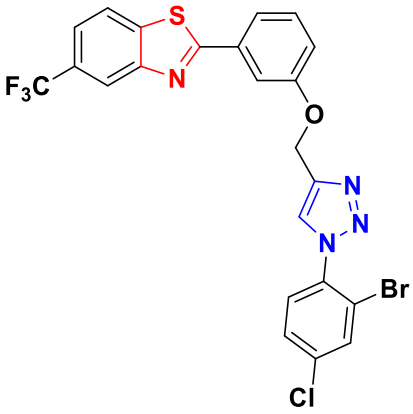
- **Synthesis of 5-chloro-2-(3-((1-(4-isopropylphenyl)-1*H*-1,2,3-triazol-5-yl)methoxy)phenyl) benzo[*d*]thiazole (24b)**



Method F.

Propargyl unit (0.150 g, 0.5 mmol), phenylazide (0.082 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford a light brown oil (16.2 mg, 7%). R_f (40% EtOAc/hexane) 0.19**. IR (KBr) (cm^{-1}):** 3085, 2923, 1606, 1492, 1440, 1422. **^{1}H NMR (300 MHz, Chloroform-*d*)** δ 8.10 (d, *J* = 2.9 Hz, 2H), 7.86 (s, 1H),7.84 – 7.79 (m, 1H), 7.80 – 7.63 (m, 2H), 7.60 – 7.32 (m, 4H), 7.22 (ddd, *J* = 8.3, 2.6, 1.0 Hz, 1H), 7.02 (s, 1H), 5.40 (s, 2H), 3.40 – 2.74 (m, 1H), 1.34 (dd, *J* = 8.0, 6.9 Hz, 6H). **^{13}C NMR (75 MHz, Chloroform-*d*)** δ 159.0, 155.2, 150.3, 130.6, 128.0, 127.7, 126.1, 125.8, 123.4, 122.6, 121.0, 118.4, 113.8, 62.6, 34.2, 30.6, 24.23. **HRMS** m/z calcd for $C_{25}H_{22}ClN_4OS$: 461.1197, found $[M+H]^+$ 461.1165.

- **Synthesis of 2-(3-((1-Phenyl-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)benzo[*d*] thiazole (23c)**



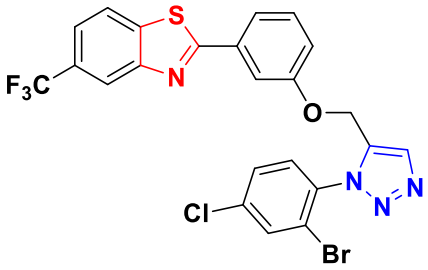
Method F.

Propargyl unit (0.167 g, 0.5 mmol), phenylazide (0.115 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford a cream solid (45.1 mg, 16%). R_f (40% EtOAc/hexane**)** 0.69**.** MP = 154 – 156 °C. **IR (KBr) (cm^{-1}):** 3075, 2932, 1597, 1486, 1445, 1422,1112. **^{1}H NMR (400 MHz, DMSO-*d*6)** δ 8.78 (s, 1H), 8.57 – 8.42 (m, 2H), 8.17 (s, 1H,), 7.85 (q, *J* = 1.5 Hz, 2H), 7.78 (m, 3H), 7.62 (t, *J* = 8.0 Hz, 1H), 7.43 (dd, *J* = 8.3, 2.7, 1H), 5.47 (s, 2H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 159.0, 153.5, 143.1, 136.1, 135.6, 134.1, 133.4, 131.3, 130.3, 129.4, 128.08 (q, $J_{C\text{-}F}$ = 31.9 Hz), 127.4, 124.4, 124.7, 122.0 (q, $J_{C\text{-}F}$ = 3.6 Hz), 120.9, 120.4, 120.1 (q, $J_{C\text{-}F}$ = 4.4 Hz), 119.1, 113.8, 61.7. **HRMS** m/z calcd for $C_{23}H_{14}BrClF_3N_4OS$: 564.9707, found $[M+H]^+$ 564.9701.

Method H.

Propargyl unit (0.167 g, 0.5 mmol), phenylazide (0.115 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography to afford a cream solid (0.189 g, 67%).

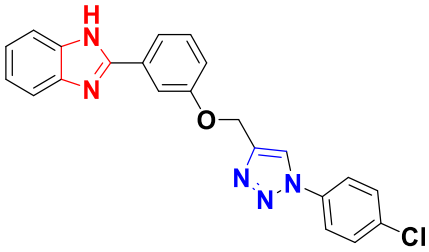
- **Synthesis of 2-(3-((1-(2-bromo-4-chlorophenyl)-1*H*-1,2,3-triazol-5-yl)methoxy)phenyl)-5-(trifluoromethyl)benzo[*d*]thiazole (24c)**



Method F.

Propargyl unit (0.167 g, 0.5 mmol), phenylazide (0.115 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford a brown solid (39.6 mg, 14%). R_f (40% EtOAc/hexane) 0.48. MP = 203 – 205 °C. **IR (KBr) (cm^{-1}):** 3073, 2932, 1595, 1489, 1441, 1422, 1114. **^{1}H NMR (400 MHz, Chloroform-*d*)** δ 8.43 (d, *J* = 8.6 Hz, 2H), 8.13 (s, 1H), 8.10 (d, *J* = 2.1 Hz, 1H), 7.80 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.77 – 7.64 (m, 3H), 7.54 – 7.44 (m, 2H), 7.14 (dd, *J* = 8.2, 2.6 Hz, 1H), 5.30 (s, 2H). **^{13}C NMR (101 MHz, Chloroform-*d*)** δ 158.2, 143.9, 141.4, 139.9, 139.2, 139.0, 138.8, 138.1, 136.0, 135.8, 134.1, 129.1, 128.4 (q, J_{C-F} = 34.5 Hz), 127.2, 126.8 (q, J_{C-F} = 3.6 Hz), 126.1, 124.9 (q, J_{C-F} = 2.3 Hz), 124.8, 123.7, 118.3, 63.7. **HRMS** m/z calcd for $C_{23}H_{14}BrClF_3N_4OS$: 564.9707, found $[M+H]^+$ 564.9698.

- **Synthesis of 2-(3-((3-(4-Chlorophenyl)-3*H*-pyrazol-5-yl)methoxy)phenyl)-1*H*-benzo[*d*]imidazole (23f)**



Method F.

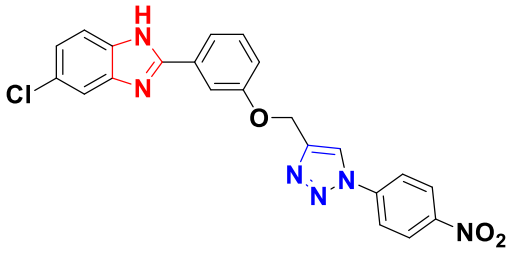
Propargyl unit (0.124 g, 0.5 mmol), phenylazide (0.0767 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford a brown solid (18.1 mg, 9%). R_f (40% EtOAc/hexane) 0.15. MP = 217 – 220 °C. **IR (KBr) (cm^{-1}):** 3386, 2932, 1602, 1449, 1232, 1023. **^{1}H NMR (400 MHz, DMSO-d_6)** δ 13.00 (s, 1H),

9.05 (s, 1H), 8.04 – 7.98 (m, 2H), 7.96 – 7.92 (m, 1H), 7.85 (d, *J* = 7.8 Hz, 1H), 7.77 – 7.65 (m, 3H), 7.54 (t, *J* = 8.0 Hz, 1H), 7.25 (dd, *J* = 7.9, 2.9 Hz, 4H), 5.40 (s, 2H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 158.8, 151.4, 144.4, 135.7, 133.5, 131.9, 130.7, 130.3, 123.4, 122.3, 119.7, 119.3, 116.9, 112.9, 111.8, 61.6. **HRMS** m/z calcd for $C_{22}H_{17}ClN_5O$: 402.1116; found $[M+H]^+$ 402.1113.

Method H.

Propargyl unit (0.124 g, 0.5 mmol), phenylazide (0.0767 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography to afford a brown solid (46.2 mg, 23%).

- **Synthesis of 5-Chloro-2-(3-((1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[*d*]imidazole (23g)**



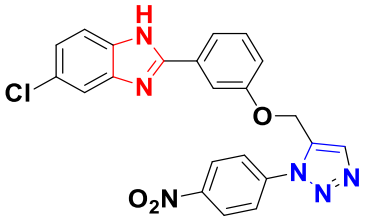
Method F.

Crude propargyl unit (0.141 g, 0.5 mmol), phenylazide (0.082 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography to afford a dark brown solid (11.1 mg, 5%). R_f (40% EtOAc/hexane) 0.23. MP = 78 °C. **IR (KBr) (cm^{-1}):** 3392, 2973, 1654, 1455, 1232, 1023. **^{1}H NMR (500 MHz, DMSO-*d*$_6$)** δ 13.17 (s, 1H), 9.26 (s, 1H), 8.52 (d, *J* = 9.2 Hz, 2H), 8.32 (d, *J* = 9.2 Hz, 2H), 7.95 (t, *J* = 2.1 Hz, 1H), 7.85 (d, *J* = 8.0 Hz, 1H), 7.79-7.68 (m, 2H), 7.57 (t, *J* = 8.0 Hz, 1H), 7.30 (dt, *J* = 8.6, 2.1 Hz, 2H), 5.45 (s, 2H). **^{13}C NMR (126 MHz, DMSO-*d*$_6$)** δ 158.8, 147.2, 144.9, 141.2, 131.5, 130.8, 126.0, 123.7, 121.2, 119.9, 117.3, 113.1, 61.6. **HRMS** m/z calcd for $C_{22}H_{16}ClN_6O_3$: 447.0967; found $[M+H]^+$ 447.0975.

Method H.

Crude propargyl unit (0.141 g, 0.5 mmol), phenylazide (0.082 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford a dark brown oil (69.2 mg, 31%).

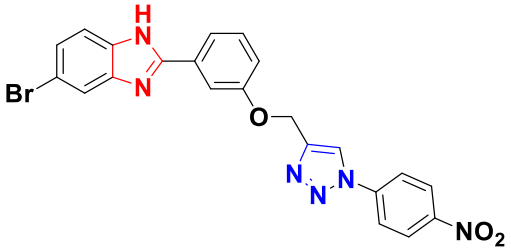
- **Synthesis of 5-Chloro-2-(3-((1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[*d*]imidazole (24g)**



Method F.

Crude propargyl unit (0.141 g, 0.5 mmol), phenylazide (0.082 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography to afford a dark brown oil (6.8 mg, 3%). R_f (40% EtOAc/hexane) 0.13. **IR (KBr) (cm^{-1}):** 3403, 2967, 1655, 1466, 1260, 1023. **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 9.11 (s, 1H), 8.40 (d, *J* = 9.2 Hz, 2H), 8.19 (d, *J* = 9.2 Hz, 2H), 7.87 (d, *J* = 9.2 Hz, 1H), 7.54 (d, *J* = 16.3 Hz, 1H), 7.39 (s, 1H), 7.33 – 7.24 (m, 1H), 7.11 – 6.93 (m, 2H), 6.80 (d, *J* = 16.4 Hz, 1H), 6.58 – 6.49 (m, 1H), 5.27 (s, 2H). **^{13}C NMR (126 MHz, DMSO-*d*$_6$)** δ 141.2, 130.5, 128.1, 126.8, 123.8, 121.2, 114.2, 112.8, 61.5. **HRMS** m/z calcd for $C_{22}H_{16}ClN_6O_3$: 447.0967; found $[M+H]^+$ 447.0967.

- **Synthesis of 5- Bromo-2-(3-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[*d*]imidazole (23h)**



Method F.

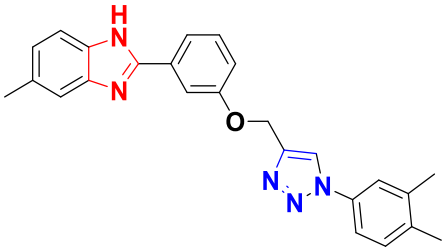
Propargyl unit (0.163 g, 0.5 mmol), phenylazide (0.082 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography to afford an orange-brown solid (17.3 mg, 7%). R_f (40% EtOAc/hexane) 0.20. MP = 115 – 118 °C. **IR (KBr) (cm^{-1}):** 3201, 2936, 1633, 1459, 1207, 1033. **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 12.23 (s, 1H), 8.31 (s, 1H), 7.57 (d, *J* = 9.2 Hz, 2H), 7.36 (d, *J* = 9.2 Hz, 2H), 6.99 (s, 1H), 6.90 (d, *J* = 7.9 Hz, 2H), 6.62 (t, *J* = 8.0 Hz, 2H), 6.46 (d, *J* = 8.6 Hz, 1H), 6.35 (dd, *J* = 8.1, 2.2 Hz, 1H), 4.50 (s, 2H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 158.8, 147.2, 144.9, 141.2, 131.4, 130.8, 126.0, 123.7, 121.1, 119.9,

117.3, 113.1, 61.65. **HRMS** m/z calcd for $C_{22}H_{16}BrN_6O_3$:490.0462; found $[M+H]^+$ 491.0475.

Method H.

Propargyl unit (0.163 g, 0.5 mmol), phenylazide (0.082 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography to afford an orange-brown solid (51.3 mg, 21%).

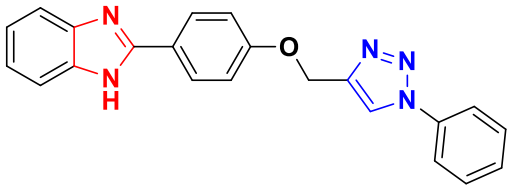
- **Synthesis of 5-Bromo-2-(3-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[*d*]imidazole (23J)**



Method F.

Propargyl unit (0.163 g, 0.5 mmol), phenylazide (0.082 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography to afford a peach solid (26.8 mg, 13%). R_f (40% EtOAc/hexane) 0.23. MP = 224 – 226 °C. **IR (KBr) (cm^{-1}):** 3178, 2964, 1664, 1447, 1247, 1033. **^{1}H NMR (400 MHz, DMSO-d_6)** δ 12.74 (s, 1H), 8.60 (s, 1H), 7.87 (s, 1H), 7.78 (d, *J* = 7.7 Hz, 1H), 7.49 (t, *J* = 8.0 Hz, 2H), 7.34 (d, *J* = 8.0 Hz, 2H), 7.30 (s, 1H), 7.27 – 7.14 (m, 2H), 7.04 (d, *J* = 8.1 Hz, 1H), 5.35 (s, 2H), 2.44 (s, 3H), 2.38 (s, 3H), 2.12 (s, 3H**). ^{13}C NMR (101 MHz, DMSO-d_6)** δ 158.8, 143.1, 139.9, 134.3, 133.1, 132.2, 132.1, 130.5, 127.8, 126.7, 126.2, 119.5, 116.7, 112.9, 61.7, 21.8, 21.0, 17.7. **HRMS** m/z calcd for $C_{25}H_{24}N_5O$: 410.1975; found $[M+H]^+$ 410.1982.

- **Synthesis of 2-(4-((1-Phenyl-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-*1H*-indole (23k)**



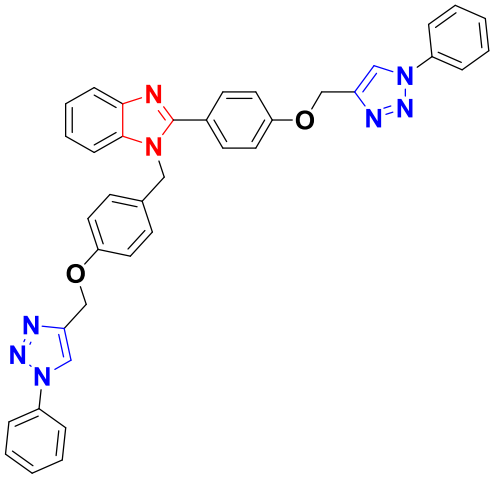
Method F.

Propargyl unit (0.124 g, 0.5 mmol), phenylazide (0.059 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography to afford a brown solid (78.8 mg, 43%). R_f (40% EtOAc/hexane) 0.19. MP = 232 – 234 °C. **IR (KBr) (cm^{-1}):** 3156, 2974, 1580, 1450, 1216, 1033. **^{1}H NMR (500 MHz, DMSO-d_6)** δ 12.76 (s, 1H), 8.99 (s, 1H), 8.14 (d, *J* = 8.8 Hz, 2H), 7.92 (d, *J* = 7.3 Hz, 2H), 7.69 – 7.56 (m, 3H), 7.56 – 7.43 (m, 2H), 7.26 (d, *J* = 8.9 Hz, 2H), 7.24 – 7.09 (m, 2H), 5.34 (s, 2H). **^{13}C NMR (126 MHz, DMSO-d_6)** δ 159.7, 151.7, 144.1, 137.0, 136.8, 130.4, 129.2, 128.5, 123.6, 123.5, 120.6, 119.5, 115.6, 114.2. **HRMS** m/z calcd for $C_{22}H_{18}N_5O$: 368.1506; found $[M+H]^+$ 368.1523.

Method H.

Propargyl unit (0.124 g, 0.5 mmol), phenylazide (0.059 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography to afford a brown solid (130.3 mg, 71%).

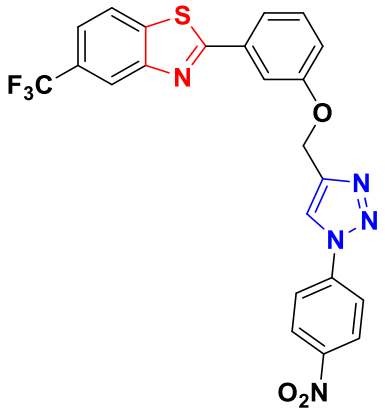
- **Synthesis of 1-(4-((1-Phenyl-1*H*-1,2,3-triazol-4-yl)methoxy)benzyl)-2-(4-((1-phenyl-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-1*H*-benzo[*d*]imidazole (23l)**



Method F.

Crude propargyl unit (0.196 g, 0.5 mmol), phenylazide (0.118 g, 1.0 mmol). Stirring time = 24 h. Purified by column chromatography to afford a colorless oil (12.7 mg, 4%). R_f (40% EtOAc/hexane) 0.17. **IR (KBr) (cm^{-1}):** 3336, 2963, 1609, 1458, 1237, 1033. **^{1}H NMR (500 MHz, DMSO-d_6)** δ 9.05 (s, 1H), 8.96 (s, 1H), 7.96 (ddt, *J* = 15.7, 6.4, 1.3 Hz, 4H), 7.83 – 7.72 (m, 3H), 7.67 (q, *J* = 8.1 Hz, 4H), 7.61 – 7.50 (m, 2H), 7.36 – 7.25 (m, 3H), 7.24 – 7.17 (m, 3H), 7.11 – 6.98 (m, 3H), 5.56 (s, 2H), 5.39 (s, 2H), 5.23 (s, 2H). **^{13}C NMR (126 MHz, DMSO-d_6)** δ 188.6, 151.2, 141.9, 141.6, 135.7, 133.6, 131.3, 130.3, 124.8, 124.6, 124.19, 123.8, 123.6, 122.4, 122.3, 121.9, 115.7, 115.3, 69.0, 63.5, 56.2. **HRMS** m/z calcd for $C_{38}H_{31}N_8O_2$: 631.2564; found $[M+H]^+$ 631.2567.

- **Synthesis of 2-(3-((1-(4-Nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-(trifluoromethyl) benzo[*d*]thiazole (23n)**



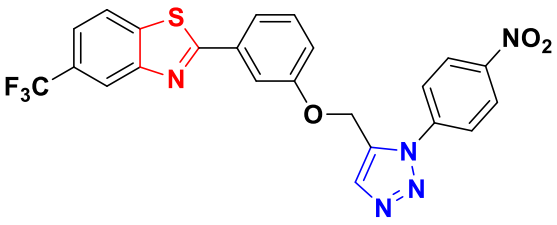
Method F.

Propargyl unit (0.167 g, 0.5 mmol), phenylazide (0.082 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography to afford a cream solid (69.7 mg, 28%). R_f (40% EtOAc/hexane) 0.53. MP = 220 – 222 °C. **IR (KBr) (cm^{-1}):** 3095, 2932, 1597, 1486, 1445, 1422, 1112. **^{1}H NMR (400 MHz, Chloroform-*d*)** δ 8.43 (d, *J* = 9.1 Hz, 2H), 8.34 (s, 1H), 8.22 (s, 1H), 8.02 (t, *J* = 8.4 Hz, 3H), 7.84 (s, 1H), 7.71 (d, *J* = 7.6 Hz, 1H), 7.64 (d, *J* = 8.4 Hz, 1H), 7.47 (t, *J* = 8.0 Hz, 1H), 7.20 (dd, *J* = 8.1, 2.2 Hz, 1H), 5.45 (s, 2H). **^{13}C NMR (101 MHz, Chloroform-*d*)** δ 158.5, 153.6, 145.5, 134.5, 130.4, 125.5, 122.2, 121.6 (q, J_{C-F} = 3.2 Hz), 121.1, 120.4 (q, J_{C-F} = 3.8 Hz), 120.7, 120.5, 120.5, 118.2, 113.4, 62.06. **HRMS** m/z calcd for $C_{23}H_{15}F_3N_5O_3S$: 498.0842; found $[M+H]^+$ 498.0844.

Method H.

Propargyl unit (0.167 g, 0.5 mmol), phenylazide (0.082 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford a cream solid (0.153 g, 62%).

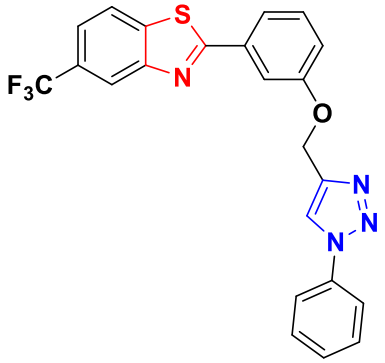
- **Synthesis of 2-(3-((1-(4-nitrophenyl)-1H-1,2,3-triazol-5-yl)methoxy)phenyl)-5-(trifluoromethyl)benzo[d]thiazole (24n)**



Method F.

Propargyl unit (0.167 g, 0.5 mmol), phenylazide (0.082 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford a colorless oil (52.3 mg, 21%). R_f (40% EtOAc/hexane) 0.33. **IR (KBr) (cm^{-1}):** 3095, 2924, 1597, 1486, 1445, 1422, 1114. **^{1}H NMR (400 MHz, Chloroform-*d*)** δ 8.43 (d, *J* = 9.0 Hz, 2H), 8.33 (s, 1H), 8.04 (s, 1H), 8.02 (d, *J* = 3.8 Hz, 1H), 7.94 (d, *J* = 9.0 Hz, 2H), 7.79 – 7.73 (m, 1H), 7.72 (d, *J* = 7.7 Hz, 1H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.48 (t, *J* = 8.0 Hz, 1H), 7.09 (dd, *J* = 8.3, 2.5 Hz, 1H), 5.26 (s, 2H). **^{13}C NMR (101 MHz, Chloroform-*d*)** δ 169.0, 157.6, 153.5, 148.1, 140.8, 138.4, 136.2, 132.5, 130.7, 125.2, 125.0, 122.3, 122.1, 121.8 (q, J_{C-F} = 3.4 Hz), 120.4 (q, J_{C-F} = 4.1 Hz), 118.6, 112.7, 58.2. **HRMS** m/z calcd for $C_{23}H_{15}F_3N_5O_3S$: 498.0842; found $[M+H]^+$ 498.0845.

- **Synthesis of 2-(3-((1-phenyl-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-(trifluoromethyl) benzo[*d*]thiazole (23o)**



Method F.

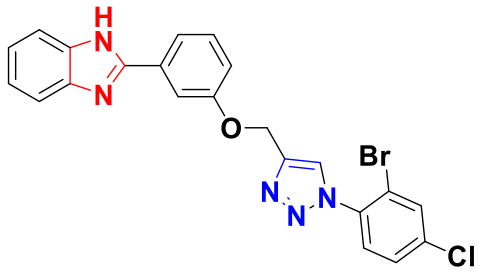
Propargyl unit (0.167 g, 0.5 mmol), phenylazide (0.0590 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography (ethyl acetate in hexane 2:3) to afford a light brown solid (5.3 mg, 2.4%). R_f (40% EtOAc/hexane) 0.61. MP = 108 – 182 °C. **IR (KBr) (cm^{-1}):** 3095, 2932, 1597, 1486, 1445, 1422, 1112. **IR (KBr) (cm^{-1}):** 3074, 2922, 1596,

1474, 1440, 1421, 1120. **^{1}H NMR (400 MHz, Chloroform-*d*)** δ 8.34 (s, 1H), 8.11 (s, 1H), 8.02 (d, *J* = 8.4 Hz, 1H), 7.86 – 7.81 (m, 1H), 7.80 – 7.73 (m, 2H), 7.71 (d, *J* = 7.7, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.54 (t, *J* = 7.7 Hz, 2H), 7.45 (t, *J* = 8.0 Hz, 2H), 7.21 (dd, *J* = 7.9, 2.9 Hz, 1H), 5.43 (s, 2H). **^{13}C NMR (101 MHz, Chloroform-*d*)** δ 158.7, 153.6, 144.4, 136.9, 134.4, 130.4, 129.8, 128.9, 122.2, 121.61 (q, J_{C-F} = 3.3 Hz), 121.0, 120.9, 120.6, 120.4 (q, J_{C-F} = 4.2 Hz), 118.28, 113.5, 62.2. **HRMS** m/z calcd for $C_{23}H_{16}F_3N_4OS$: 453.0991; found $[M+H]^+$ 453.1001.

Method H.

Propargyl unit (0.167 g, 0.5 mmol), phenylazide (0.0590 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography to afford a light brown solid (24.9 mg, 11%).

- **Synthesis of 2-(3-((1-(2-Bromo-4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-1*H*-benzo[*d*]imidazole (23r)**



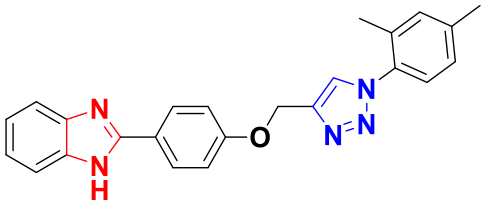
Method F.

Propargyl unit (0.124 g, 0.5 mmol) phenylazide (0.115 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography to afford a purple solid (14.4 mg, 6%). R_f (40% EtOAc/hexane) 0.57. MP = 242 – 246 °C. **IR (KBr) (cm^{-1}):** 3184, 2929, 1591, 1447, 1232, 1023. **^{1}H NMR (400 MHz, DMSO-d_6)** δ 12.93 (s, 1H), 8.77 (s, 1H), 8.16 (s, 1H), 7.94 (s, 1H), 7.86 (d, *J* = 7.8 Hz, 1H), 7.81 – 7.67 (m, 3H), 7.63 – 7.50 (m, 2H), 7.32 – 7.17 (m, 3H), 5.43 (s, 2H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 158.8, 151.4, 144.1, 143.2, 136.1, 135.6, 135.4, 133.4, 132.0, 130.6, 130.3, 129.4, 127.3, 123.0, 122.1, 120.4, 119.7, 119.3, 117.0, 113.1, 111.7, 61.6. **HRMS** m/z calcd for $C_{22}H_{16}BrClN_5O$: 480.0221; found $[M+H]^+$ 480.0221.

Method H.

Propargyl unit (0.124 g, 0.5 mmol), phenylazide (0.115 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography to afford a purple solid (0.132 g, 55%).

- **Synthesis of 2-(4-((1-(2,4-Dimethylphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-1*H*-benzo[*d*]imidazole (23s)**



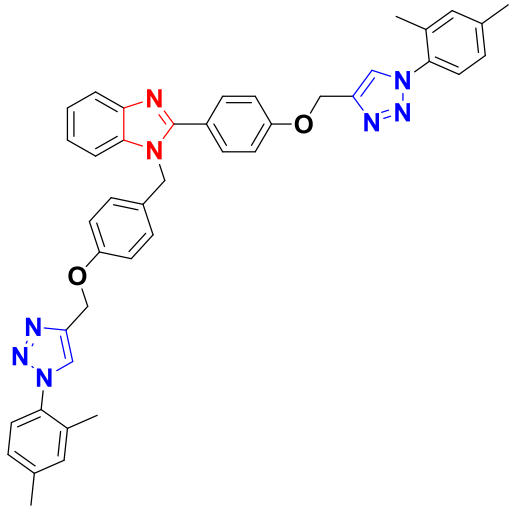
Method F.

Crude propargyl unit (0.124 g, 0.5 mmol), phenylazide (0.082 g, 0.5 mmol). Stirring time = 24 h. Purified by column chromatography to afford a yellow oil (43.3 mg, 22%). R_f (40% EtOAc/hexane) 0.22. **IR (KBr) (cm^{-1}):** 3102, 2958, 1611, 1438, 1245, 1025. **^{1}H NMR (400 MHz, DMSO-d_6)** δ 12.80 (s, 1H), 8.65 (s, 1H), 8.19 (d, *J* = 8.8 Hz, 2H), 7.80 – 7.43 (m, 2H), 7.38 (d, *J* = 8.0 Hz, 1H), 7.35 – 7.29 (m, 3H), 7.24 (m, 3H), 5.38 (s, 2H), 2.42 (s, 3H), 2.16 (s, 3H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 159.8, 151.7, 142.9, 140.0, 134.2, 133.1, 132.2, 128.4, 127.8, 126.8, 126.3, 115.67, 61.67, 21.09, 17.76. **HRMS** m/z calcd for $C_{24}H_{22}N_5O$: 396.1819; found $[M+H]^+$ 396.1836.

Method H.

Crude propargyl unit (0.124 g, 0.5 mmol), phenylazide (0.082 g, 0.5 mmol). Stirring time = 4 h. Purified by column chromatography to afford a yellow oil (94.8 mg, 48%).

- **Synthesis of 1-(4-((1-(2,4-Dimethylphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzyl)-2-(4-((1-(2,4-dimethylphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-1*H*-benzo[*d*] imidazole (23t)**



Method F.

Crude propargyl unit (0.196 g, 0.5 mmol), phenylazide (0.164 g, 1.0 mmol). Stirring time = 24 h. Purified by column chromatography to afford a pale yellow oil (18.3 mg, 5.3%). R_f (40% EtOAc/hexane) 0.18. **IR (KBr) (cm^{-1}):** 3201, 2961, 1611, 1412, 1259, 1024. **^{1}H NMR (400 MHz, DMSO-d_6)** δ 8.53 (s, 1H), 8.44 (s, 1H), 7.67 (s, 3H), 7.45 (s, 1H), 7.28 – 7.05 (m, 10H), 6.97 – 6.76 (m, 4H), 5.46 (s, 2H), 5.25 (s, 2H), 5.09 (s, 2H), 2.30 (d, *J* = 4.0 Hz, 6H), 2.03 (s, 3H), 1.99 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.7, 140.3, 140.2, 134.0, 133.1, 132.2, 127.9, 126.7, 126.2, 115.5, 61.2, 21.0, 17.6. **HRMS** m/z calcd for $C_{42}H_{39}N_8O_2$: 687.3190; found $[M+H]^+$ 687.3205

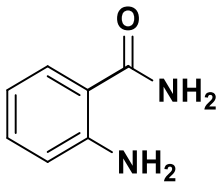
Method H.

Crude propargyl unit (0.124 g, 0.5 mmol), phenylazide (0.164 g, 0.5 mmol). Stirring time = 4 h. Purified by column chromatography to afford a pale yellow oil (44.7 mg, 13%).

GENERAL PROCEDURE FOR THE PREPARATION OF 2-AMINOBENZALDEHYDE DERIVES 26a-b

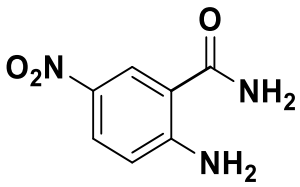
A wet whole cell paste of NHs (*Rhodococuss rhodochrous* ATCC BAA-870) (50 mL) was added to a solution of 2-aminobenzonitrile derivatives (10.0 mmol) in 18 mL of Tris buffer (7.6 pH) and 10% acetone (v/v). The reaction mixture was stirred overnight at room temperature. The contents of the reaction mixture were extracted with ethyl acetate (30.0 mL) and washed with water (3 × 20.0 mL). The organic layer was separated, dried over $MgSO_4$ and the products were recrystallized from cold ethanol or purified by column chromatography (60% ethyl acetate/hexane) to afford desired product **26a-b.**

- **2-aminobenzamide (26a)**



Stirring time = 24 h. Purified by column chromatography to afford a purple solid (1.27 g, 95%). R_f (40% EtOAc/hexane) 0.64. MP = 110 – 122 °C (lit. 109 – 110 °C).[290] **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 7.73 (s, 1H), 7.53 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.13 (ddd, *J* = 8.4, 7.0, 1.6 Hz, 1H), 7.07 (s, 1H), 6.68 (dd, *J* = 8.3, 1.2 Hz, 1H), 6.57 (s, 2H), 6.48 (ddd, *J* = 8.1, 7.0, 1.2 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ** 171.7, 150.6, 132.3, 129.2, 116.8, 114.8, 114.1.

- **2-amino-5-nitrobenzamide (26b)**

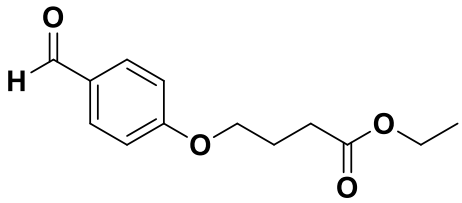


Stirring time = 24 h. Purified by recrystallisation to afford a yellow solid (0.833 g, 46%). R_f (60% EtOAc/hexane) 0.40. MP = 130 – 133 °C (lit. 128 – 134 °C).[291] **^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ** 8.56 (d, *J* = 2.7 Hz, 1H), 8.23 (s, 1H), 8.02 (dd, *J* = 9.2, 2.6 Hz, 1H), 7.97 – 7.85 (s, 2H), 7.42 (s, 1H), 6.80 (d, *J* = 9.3 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ** 170.1, 156.1, 135.2, 127.9, 126.8, 116.4, 112.4.

GENERAL PROCEDURE FOR THE SYNTHESIS OF ETHER BENZALDEHYDES 27(d-f).

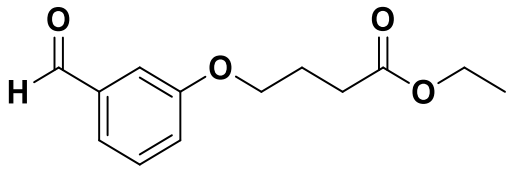
Hydroxy benzaldehyde derivative (1 equivalent) was dissolved in anhydrous acetone and potassium carbonate (2 equivalents) was added and the contents were stirred at 50°C for 5 minutes under nitrogen gas. Then corresponding Ethyl 4-bromobutyrate (1 equivalent) was added to the stirring solution. Finally, potassium iodide (1 equivalent) was added. The reaction mixture was refluxed for 24 h. After completion of reaction, the resultant mixture was concentrated and ethyl acetate (60.0 mL) and washed with water (3 × 20.0 mL). The organic layer was separated and treated with brine and dried over $MgSO_4$. The crude mixture was purified by silica chromatography using hexane: ethyl acetate (9:1) to afford the desired ether hydroxy benzaldehyde derivatives **27(d-f)**.

- **Ethyl 4-(4-formylphenoxy)butanoate (27d)**



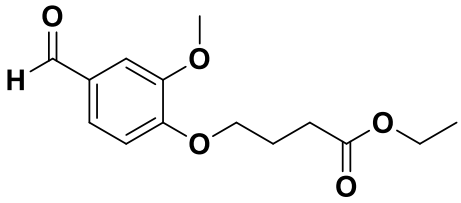
The crude product was purified by column chromatography eluding with 10% ethyl acetate in hexane, to obtain a pale-yellow oil containing a small amount of ethyl 4-bromobutyrate. R_f (60% EtOAc/hexane) 0.83. (lit.).[292] The product mixture was used in the subsequent reaction. **^{1}H NMR (400 MHz, CDCl3) δ** 9.88 (s, 1H), 7.83 (d, J = 8.8 Hz, 2H), 6.99 (d, J = 8.7 Hz, 2H), 4.18 – 4.07 (m, 4H), 2.52 (t, 7.2 Hz, 2H), 2.19 – 2.11 (m, 2H), 1.30 – 1.23 (m, 3H). **^{13}C NMR (101 MHz, CDCl3) δ** 190.7, 172.9, 163.8, 131.9, 129.94, 114.73, 67.12, 60.55, 30.59, 24.40, 14.21.

- **Ethyl 4-(3-formylphenoxy)butanoate (27e)**



The crude product was purified by silica chromatography eluding with 10% ethyl acetate in hexane, to obtain a colourless oil (2.27 g, 96%). R_f (60% EtOAc/hexane) 0.83. (BP. lit. 175 – 185 °C).[293] **^{1}H NMR (400 MHz, DMSO-d6) δ** 9.91 (s, 1H), 7.49 – 7.42 (m, 2H), 7.35 (s, 1H), 7.22 – 7.19 (m, 1H), 4.02 – 3.97 (m, 4H), 2.41 (t, J = 7.3 Hz, 2H), 1.93 (p, J = 6.8 Hz, 2H), 1.11 (t, J = 7.1 Hz, 3H). **^{13}C NMR (101 MHz, DMSO-d6) δ** 193.4, 172.9, 159.4, 138.0, 130.8, 130.1, 122.8, 114.0, 67.3, 60.3, 30.5, 24.5, 14.5.

- **Ethyl 4-(4-formyl-2-methoxyphenoxy)butanoate (26f)**

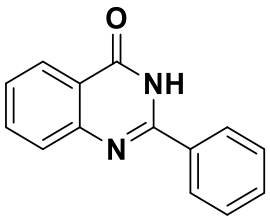


Purified by silica chromatography eluding with 10% ethyl acetate in hexane, to obtain a colourless oil (2.57 g, 97%). R_f (60% EtOAc/hexane) 0.84. (MP. lit. 75 – 76 °C).[294] **^{1}H NMR (400 MHz, DMSO-d6) δ** 9.83 (s, 1H), 7.53 (dd, J = 8.2, 1.9 Hz, 1H), 7.39 (d, J = 1.9 Hz, 1H), 7.16 (d, J = 8.3 Hz, 1H), 4.12 – 4.03 (m, 4H), 3.84 (s, 3H), 2.47 (t, J = 7.3 Hz, 2H), 2.01 (p, J = 6.8 Hz, 2H), 1.18 (t, J = 7.1 Hz, 3H). **^{13}C NMR (101 MHz, DMSO-d6) δ** 191.7, 172.8, 153.8, 149.7, 130.1, 126.4, 112.5, 110.0, 67.9, 60.3, 55.9, 30.4, 24.5, 14.5.

GENERAL PROCEDURE FOR THE PREPARATION OF 2-SUBSTITUTED QUINAZOLINONE DERIVATIVES 29

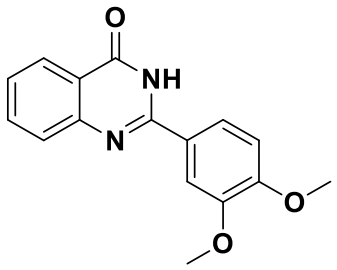
A mixture of the 2-aminobenzamide derivative **26** (10 mmol) and benzaldehydes 2**7** (10 mmol) in a sealed microwave tube (neat). The mixture was then irradiated under microwave conditions (150 W, 130 °C) for 5 minutes while maintaining adequate stirring. After complete conversion of the starting materials (TLC) to aminal intermediate **28**, the contents were transferred to 250 mL round-bottomed flask equipped with a magnetic stirrer bar, by washing with DMSO (5 ml) to ensure quantitative transfer of all the solids. Additional DMSO (10 mL), laccase Novoprime B.268 (0.50 g), acetate buffer solution (0.1 M, pH 4.0, 5 mL) were added to the mixture. The mixture was then stirred at 70°C with the flask open to air for the time given in **Table 16**. The crude product was precipitated out of solution by addition of water into the solution. Trituration of the crude product with ethanol afforded the quinazolinone product **29**.

- **2-phenylquinazolin-4(3*H*)-one (29a)**



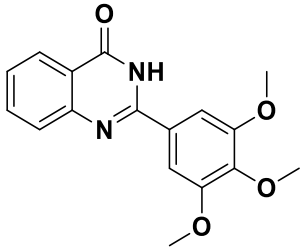
Trituration of the crude product with ethanol to obtain a white solid (2.57 g, 95%). R_f (60% EtOAc/hexane) 0.84. MP = 240 – 242 °C (lit. 241 – 242 °C).[295] **^{1}H NMR (400 MHz, DMSO-d_6)** δ 8.20 (s, 1H), 8.17 (d, *J* = 8.8 Hz, 2H), 7.92 (d, *J* = 8.0 Hz, 2H), 7.70 – 7.64 (m, 1H), 7.61 (t, *J* = 7.5 Hz, 3H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 161.9, 154.6, 145.8, 135.5, 132.8, 130.8, 129.1, 129.0, 127.8, 126.6, 125.5, 120.9.

- **2-(3,4-dimethoxyphenyl)quinazolin-4(3*H*)-one (29b)**



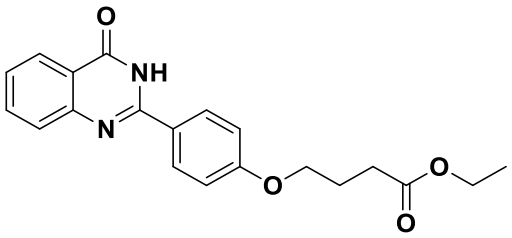
Trituration of the crude product with ethanol to obtain a white solid (2.78 g, 99%). R_f (60% EtOAc/hexane) 0.32. MP = 230 – 232 °C (lit. 222 – 224 °C).[296] **^{1}H NMR (400 MHz, DMSO-d_6)** δ 12.42 (s, 1H), 8.14 (d, *J* = 7.6 Hz, 1H), 7.88 (d, *J* = 8.3 Hz, 1H), 7.82 (m, 2H), 7.72 (d, *J* = 7.8 Hz, 1H), 7.49 (t, *J* = 6.9 Hz, 1H), 7.12 (d, *J* = 8.1 Hz, 1H), 3.89 (s, 3H), 3.86 (s, 3H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 162.8, 152.3, 152.0, 149.2, 149.0, 134.9, 127.6, 126.5, 126.2, 125.2, 121.6, 121.1, 111.8, 111.1, 56.1.

- **2-(3,4,5-trimethoxyphenyl)quinazolin-4(*3H*)-one (29c)**



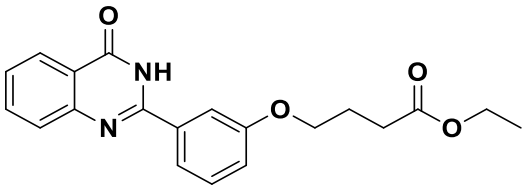
Trituration of the crude product with ethanol to obtain a white solid (2.78 g, 99%). R_f (60% EtOAc/hexane) 0.74. MP = 251 – 255 °C (lit. 258 – 260 °C).[297] **^{1}H NMR (400 MHz, DMSO-d_6)** δ 12.51 (s, 1H), 8.15 (d, *J* = 7.8 Hz, 1H), 7.83 (t, *J* = 7.5 Hz, 1H), 7.75 (d, *J* = 8.0 Hz, 1H), 7.57 (s, 2H), 7.51 (t, *J* = 7.4 Hz, 1H), 3.91 (s, 6H), 3.76 (s, 3H). **^{13}C NMR (101 MHz, DMSO-d_6) δ** 162.7, 153.3, 152.1, 149.1, 140.7, 135.0, 128.1, 127.9, 126.9, 126.3, 121.2, 105.6, 60.6, 56.5.

- **Ethyl 4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanoate (29d)**



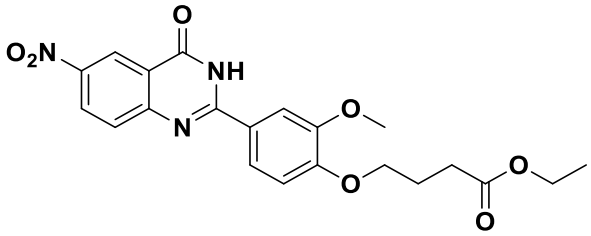
Trituration of the crude product with ethanol to obtain a cream-white solid (3.37 g, 96%). R_f (60% EtOAc/hexane) 0.73. MP = 180 – 184 °C. **IR (KBr) (cm^{-1})**: 3179, 2964, 1730, 1447, 1339. **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 12.37 (s, 1H), 8.18 (d, *J* = 8.8 Hz, 2H), 8.13 (d, *J* = 7.9 Hz, 1H), 7.81 (t, *J* = 8.3 Hz, 1H), 7.70 (d, *J* = 8.1 Hz, 1H), 7.48 (t, *J* = 7.5 Hz, 1H), 7.07 (d, *J* = 8.8 Hz, 2H), 4.08 (q, *J* = 7.3 Hz, 4H), 2.52 – 2.43 (m, 2H), 2.01 (p, *J* = 6.9 Hz, 2H), 1.19 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 172.9, 162.8, 161.5, 152.37, 149.3, 134.9, 129.9, 126.5, 126.2, 125.2, 121.1, 114.8, 67.2, 60.3, 30.5, 24.5, 14.5. **HRMS** m/z calcd for $C_{20}H_{20}N_2O_4$: 352.1423, found $[M+H]^+$ 353.1478.

- **Ethyl 4-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanoate (29e)**



Trituration of the crude product with ethanol to obtain a cream-white solid (3.17 g, 90%). R_f (60% EtOAc/hexane) 0.75. MP = 154 – 160 °C. **IR (KBr) (cm^{-1})**: 3165, 2927, 1735, 1452, 1328. **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 12.54 (s, 1H), 8.16 (dd, *J* = 7.9, 1.2 Hz, 1H), 7.87 – 7.72 (m, 4H), 7.57 – 7.50 (m, 1H), 7.45 (t, *J* = 8.0 Hz, 1H), 7.14 (dd, *J* = 8.2, 1.8 Hz, 1H), 4.15 – 4.04 (m, 4H), 2.48 (m, 2H), 2.02 (p, *J* = 6.7 Hz, 2H), 1.19 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 173.0, 162.7, 159.0, 149.1, 135.1, 134.4, 130.2, 127.9, 127.1, 126.3, 121.4, 120.6, 118.5, 113.5, 67.2, 60.3, 30.6, 24.6, 14.5. **HRMS** m/z calcd for $C_{20}H_{20}N_2O_4$: 352.1423, found $[M+H]^+$ 353.1479.

- **Ethyl 4-(2-methoxy-4-(6-nitro-4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy) butanoate (29f)**



Trituration of the crude product with ethanol to obtain a yellow solid (4.21 g, 99%). R_f (60% EtOAc/hexane) 0.65. MP = 225 – 227 °C. **IR (KBr) (cm^{-1})**: 3115, 2905, 1665, 1472, 1332. **^{1}H NMR (400 MHz, DMSO) δ** 12.81 (s, 1H), 8.75 (d, J = 2.8 Hz, 1H), 8.47 (dd, J = 9.0, 2.8 Hz, 1H), 7.86 (dd, J = 8.5, 2.2 Hz, 1H), 7.82 (s, 1H), 7.80 (d, J = 2.0 Hz, 1H), 7.08 (d, J = 8.6 Hz, 1H),3.99-4.03 (m, 4H), 3.83 (s, 2H), 2.42 (t, *J* = 7.2 Hz, 2H) 1.95 (p, J = 6.9 Hz, 2H), 1.13 (t, J = 7.1 Hz, 3H). **^{13}C NMR (101 MHz, DMSO) δ** 172.9, 155.7, 151.9, 149.1, 144.6, 129.2, 128.8, 122.5, 122.4, 121.0, 112.8, 111.61, 67.8, 60.3, 56.2, 30.5, 24.6, 14.5. **HRMS m/z** calcd for $C_{21}H_{21}N_3O_7$: 427.1380, found $[M+H]^+$ 427.1430.

GENERAL PROCEDURE FOR THE PREPARATION OF 2-SUBSTITUTED QUINAZOLINONE ACIDS 30

The following methods were used in the synthesis of quinazolinone acids **30**.

- **Method I.**

In a 100mL round-bottom flask was added the quinazolinone ester **29** (5 mmol) in a phosphate buffer (0.2M, pH 7.4) and 70% DMSO (v/v) (total volume of 10 mL). Then 3Å molecular sieve (0.10 g) and immobilized CAL-B (0.10 g) were finally added to the mixture. The reaction mixture was heated to 30°C for 3 days. The product was precipitated out of solution by addition of water into the solution and 3 M HCl (2 x 2 mL) was added to bring the pH of the solution to 3. Trituration of the crude product with ethanol afforded the quinazolinone acid **30**.

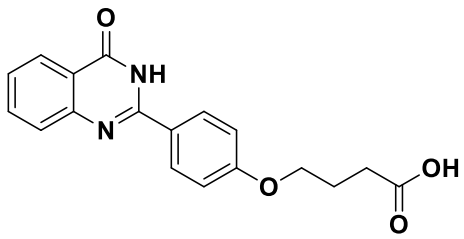
- **Method J.**

The same as **Method I**, except that DMF was used instead of DMSO.

- **Method K.**

The same as **Method I**, except that Acetone was used instead of DMSO.

- **4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanoic acid (30d)**



Method I.

Trituration of the crude product with ethanol to obtain a cream white solid (1.38 g, 86%). R_f (60% EtOAc/hexane) 0.29. MP = 220 – 224 °C. **IR (KBr) (cm^{-1})**: 3175, 2919, 1699, 1473, 1349. **^{1}H NMR (300 MHz, DMSO-d_6) δ** 12.40 (s, 1H), 12.19 (s, 1H), 8.18 (d,

J = 8.9 Hz, 2H), 8.13 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.81 (ddd, *J* = 8.5, 7.0, 1.6 Hz, 1H), 7.70 (dd, *J* = 8.3, 1.1 Hz, 1H), 7.48 (ddd, *J* = 8.1, 7.0, 1.3 Hz, 1H), 7.08 (d, *J* = 8.9 Hz, 2H), 4.09 (t, *J* = 6.5 Hz, 2H), 2.41 (t, *J* = 7.3 Hz, 2H), 1.98 (p, *J* = 6.9 Hz, 2H). **^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ** 174.0, 162.2, 161.0, 151.8, 148.8, 134.5, 129.4, 127.2, 126.0, 125. 124. 120.6, 114.3, 66.8, 30.0, 24.1. **HRMS** m/z calcd for $C_{18}H_{16}N_2O_4$: 324.1110, found $[M+H]^+$ 325.1173.

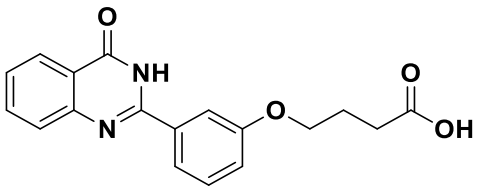
Method J.

Stirring time = 24 h. Trituration of the crude product with ethanol to obtain a cream white solid (1.33 g, 82 %).

Method K.

Stirring time = 5 days. Purified by column chromatography (80% ethyl acetate/hexane) to afford a cream white solid (0.91 g, 56 %).

- **4-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanoic acid (30e)**



Method I.

Trituration of the crude product with ethanol to obtain a cream white solid (1.06 g, 65%). R_f (60% EtOAc/hexane) 0.31. MP = 218 – 222 °C. **IR (KBr) (cm^{-1})**: 3125, 2889, 1700, 1462, 1322. **^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ** 12.57 (s, 1H), 8.15 (d, *J* = 7.8 Hz, 1H), 7.84 (t, *J* = 7.4 Hz, 1H), 7.82 – 7.70 (m, 3H), 7.53 (t, *J* = 7.4 Hz, 1H), 7.45 (t, *J* = 8.0 Hz, 1H), 7.15 (dd, *J* = 8.2, 2.5 Hz, 1H), 4.10 (t, *J* = 6.4 Hz, 2H), 2.43 (t, *J* = 7.3 Hz, 2H), 1.99 (p, *J* = 6.6 Hz, 2H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ** 174.5, 162.7, 159.0, 152.5, 148.9, 135.0, 134.3, 130.2, 127.8, 127.1, 126.3, 121.4, 120.6, 118.5, 113.5, 67.3, 30.6, 24.7. **HRMS m/z** calcd for $C_{18}H_{16}N_2O_4$: 324.1110, found $[M+H]^+$ 325.1172.

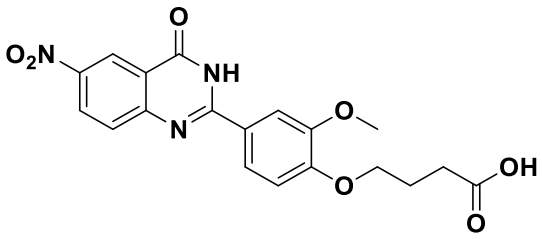
Method J.

Stirring time = 72 h. Trituration of the crude product with ethanol to obtain a cream white solid (1.24 g, 76 %).

Method K.

Stirring time = 5 days. Purified by column chromatography (80% ethyl acetate/hexane) to afford a cream white solid (0.77 g, 48 %).

- **4-(2-methoxy-4-(6-nitro-4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy) butanoic acid (30f)**



Method I.

Trituration of the crude product with ethanol to obtain yellow solid (1.39 g, 70%). R_f (60% EtOAc/hexane) 0.51. MP = 240 – 244 °C. **IR (KBr) (cm^{-1})**: 3162, 2991, 1712, 1558, 1325. **^{1}H NMR (400 MHz, DMSO-d_6) δ** 12.59 (s, 1H), 8.76 (d, *J* = 2.7 Hz, 1H), 8.48 (dd, *J* = 9.0, 2.8 Hz, 1H), 7.88 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.82 (s, 1H), 7.80 (d, *J* = 6.4 Hz, 1H), 7.10 (d, *J* = 8.6 Hz, 1H), 4.07 (t, *J* = 6.4 Hz, 2H), 2.41 (t, *J* = 7.3 Hz, 2H), 1.98 (p, *J* = 7.0 Hz, 2H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 174.5, 162.3, 155.6, 153.6, 151.9, 149.0, 144.5, 129.1, 128.7, 124.4, 122.5, 122.4, 120.9, 112.7, 111.5, 67.8, 56.1, 30.4, 24.6. **HRMS** m/z calcd for $C_{19}H_{17}N_3O_7$: 399.1066, found $[M+H]^+$ 400.1116.

Method J.

Stirring time = 24 h. Trituration of the crude product with ethanol to obtain a cream white solid (1.03 g, 52 %).

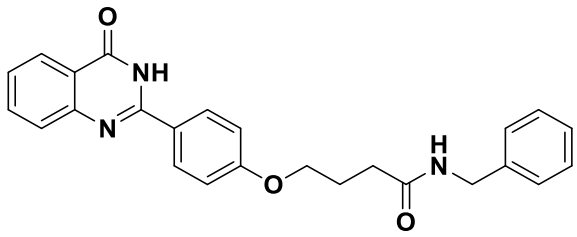
Method K.

Stirring time = 5 days. Purified by column chromatography (80% ethyl acetate/hexane) to afford a cream white solid (1.01 g, 51 %).

GENERAL PROCEDURE FOR THE PREPARATION OF 2-SUBSTITUTED QUINAZOLINONE AMIDES 32

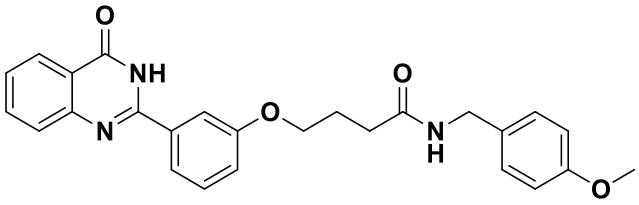
In a 100mL round-bottom flask was successively introduced the amine **31** (0.35 mmol, 1 eq), quinazolinone acid **30** (0.35 mmol, 1 eq) in DMF (5 mL). *N*-methylmorpholine (0.35 mmol, 1 eq) and HBTU (0.35 mmol, 1 eq) are finally introduced. The reaction mixture is stirred at room temperature for 2h. The crude product was precipitated out of solution by addition of water into the mixture. Trituration of the crude product with acetone afforded the quinazolinone amide **32**.

- ### *N*-benzyl-4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanamide (32a)



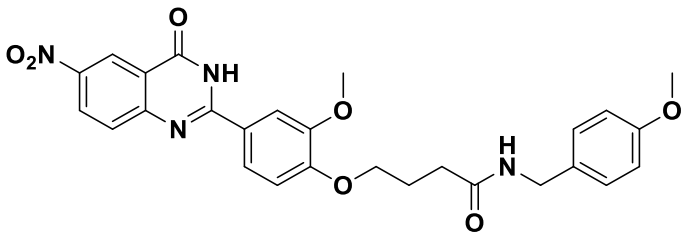
Trituration of the crude product with ethanol to obtain a white solid (137.4 mg, 95%). R_f (60% EtOAc/hexane) 0.28. MP = 244 – 247 °C. **IR (KBr) (cm^{-1})**: 3300, 3175, 2921, 1656, 1447, 1325. **^{1}H NMR (400 MHz, DMSO-d_6) δ** 12.25 (s, 1H), 8.45 (t, *J* = 6.0 Hz, 1H), 8.19 (d, *J* = 8.5 Hz, 2H), 8.14 (d, *J* = 8.0 Hz, 1H), 7.82 (t, *J* = 7.7 Hz, 1H), 7.71 (d, *J* = 8.2 Hz, 1H), 7.49 (t, *J* = 7.6 Hz, 1H), 7.30 (d, *J* = 7.1 Hz, 2H), 7.25 – 7.21 (m, 3H), 7.07 (d, *J* = 8.5 Hz, 2H), 4.29 (d, *J* = 6.0 Hz, 2H), 4.08 (t, *J* = 6.3 Hz, 2H), 2.36 (t, *J* = 7.4 Hz, 2H), 2.02 (q, *J* = 6.8 Hz, 2H). **^{13}C NMR (101 MHz, DMSO-d_6) δ** 171.9, 162.8, 161.6, 152.3, 149.3, 140.0, 135.0, 129.9, 128.7, 127.6, 127.1, 126.5, 126.2, 125.1, 121.1, 114.8, 67.6, 42.5, 32.0, 25.2. **HRMS** m/z calcd for $C_{25}H_{23}N_3O_3$: 413.1739; found $[M+H]^+$ 414.1839.

- ***N*-(4-methoxybenzyl)-4-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy) butanamide (32b)**



Trituration of the crude product with ethanol to obtain a white solid (148.9 mg, 95%). R_f (60% EtOAc/hexane) 0.56. MP = 225 – 229 °C. **IR (KBr) (cm^{-1})**: 3289, 3208, 2959, 1681, 1448, 1323. **^{1}H NMR (400 MHz, DMSO-d_6) δ** 12.52 (s, 1H), 8.36 (t, *J* = 5.9 Hz, 1H), 8.16 (d, *J* = 7.9 Hz, 1H), 7.88 – 7.71 (m, 4H), 7.53 (t, *J* = 7.5 Hz, 1H), 7.45 (t, *J* = 8.0 Hz, 1H), 7.16 (d, *J* = 8.5 Hz, 2H), 7.12 (s, 1H), 6.82 (d, *J* = 8.2 Hz, 2H), 4.21 (d, *J* = 5.8 Hz, 2H), 4.09 (t, *J* = 6.3 Hz, 2H), 3.67 (s, 3H), 2.35 (t, *J* = 7.3 Hz, 2H), 2.02 (p, *J* = 6.7 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-d_6) δ** 171.87, 162.7, 159.1, 158.5, 152.5, 149.0, 135.0, 134.4, 132.0, 130.2, 128.9, 127.9, 127.0, 126.3, 121.4, 120.5, 118.5, 114.0, 113.5, 67.6, 55.4, 41.9, 32.1, 25.3. **HRMS** m/z calcd for $C_{26}H_{25}N_3O_4$: 443.1845; found $[M+H]^+$ 444.1845.

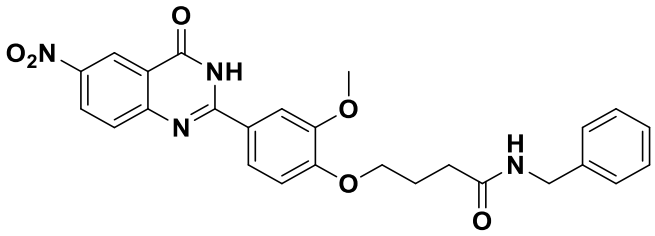
- **4-(2-methoxy-4-(6-nitro-4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)-*N*-(4-methoxybenzyl)butanamide (32c)**



Trituration of the crude product with ethanol to obtain a yellow solid (177.9 mg, 98%). R_f (60% EtOAc/hexane) 0.58. MP = 252 – 256 °C. **IR (KBr) (cm^{-1})**: 3182, 3120, 2918, 1664, 1423, 1300. **^{1}H NMR (400 MHz, DMSO-d_6) δ** 12.89 (s, 1H), 8.82 (d, *J* = 2.8 Hz, 1H), 8.54 (dd, *J* = 9.0, 2.8 Hz, 1H), 8.36 (t, *J* = 5.9 Hz, 1H), 7.93 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.88 (s, 1H), 7.86 (s, 1H), 7.17 (d, *J* = 8.6 Hz, 2H), 7.13 (d, *J* = 8.7 Hz, 1H), 6.86 (d, *J* = 8.7 Hz, 2H), 4.20 (d, *J* = 5.9 Hz, 2H), 4.08 (t, *J* = 6.4 Hz, 2H), 3.90 (s, 3H), 3.71 (s, 3H), 2.33 (t, *J* = 7.4 Hz, 2H), 2.00 (p, *J* = 6.8 Hz, 2H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 171.7, 162.3, 158.5, 155.7, 152.1, 149.1, 144.6, 132.0, 129.2, 128.9, 124.3, 122.5, 121.0, 114.1, 112.7,

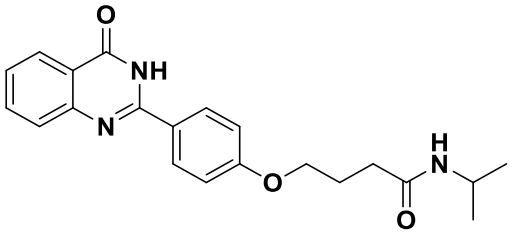
111.5, 68.2, 56.2, 55.4, 32.0, 25.2. **HRMS** m/z calcd for $C_{27}H_{26}N_4O_7$: 518.1802; found $[M+H]^+$ 519.1892.

- ***N*-benzyl-4-(2-methoxy-4-(6-nitro-4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy) butanamide (32d)**



Trituration of the crude product with ethanol to obtain a yellow solid (177.9 mg, 98%). R_f (60% EtOAc/hexane) 0.58. MP = 262 – 266 °C. **IR (KBr) (cm^{-1})**: 3295, 3180, 2955, 1632, 1440, 1339. **^{1}H NMR (400 MHz, DMSO-d_6)** δ 12.85 (s, 1H), 8.79 (d, *J* = 2.7 Hz, 1H), 8.51 (dd, *J* = 9.0, 2.8 Hz, 1H), 8.44 (t, *J* = 6.0 Hz, 1H), 7.91 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.86 (s, 1H), 7.85 – 7.83 (m, 1H), 7.34 – 7.29 (m, 2H), 7.25 (dt, *J* = 8.3, 2.2 Hz, 3H), 7.12 (d, *J* = 8.7 Hz, 1H), 4.29 (d, *J* = 5.9 Hz, 2H), 4.08 (t, *J* = 6.4 Hz, 2H), 3.89 (s, 3H), 2.36 (t, *J* = 7.4 Hz, 2H), 2.02 (p, *J* = 6.9 Hz, 2H). **^{13}C NMR (101 MHz, DMSO-d6) δ** 171.9, 162.7, 162.2, 155.6, 152.1, 149.1, 144.6, 140.0, 129.2, 128.8, 128.7, 127.6, 127.1, 124.3, 122.5, 120.9, 112.7, 111.5, 68.2, 56.1, 42.5, 32.02, 25.25. **HRMS** m/z calcd for $C_{26}H_{24}N_4O_6$: 488.1696; found $[M+H]^+$ 489.1793.

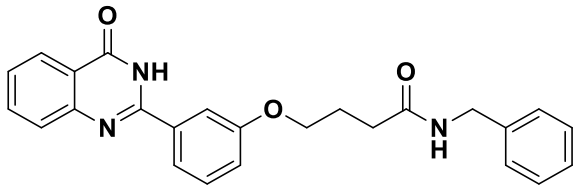
- ***N*-isopropyl-4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanamide (32f)**



Trituration of the crude product with ethanol to obtain a white solid (116.4 mg, 91%). R_f (60% EtOAc/hexane) 0.18. MP = 272 – 274 °C. **IR (KBr) (cm^{-1})**: 3302, 3176, 2971, 1634, 1447, 1328. **^{1}H NMR (400 MHz, DMSO-d_6) δ** 12.42 (s, 1H), 8.18 (d, *J* = 8.9 Hz, 2H), 8.13 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.84 – 7.76 (m, 2H), 7.71 (d, *J* = 7.9 Hz, 1H), 7.49 (ddd, *J* = 8.1, 7.0, 1.2 Hz, 1H), 7.08 (d, *J* = 9.0 Hz, 2H), 4.06 (t, *J* = 6.4 Hz, 2H), 3.84 (dq, *J* = 13.2, 6.6 Hz, 1H), 2.23 (t, *J* = 7.4 Hz, 2H), 1.95 (p, *J* = 6.8 Hz, 2H), 1.04 (d, *J* = 6.6 Hz, 6H). **^{13}C**

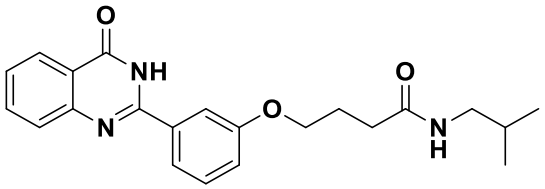
NMR (101 MHz, DMSO-d6) δ 170.9, 162.8, 161.6, 152.5, 149.3, 135.0, 129.9, 127.7, 126.5, 126.2, 125.1, 121.1, 114.8, 67.7, 32.1, 25.2, 22.9. **HRMS** m/z calcd for $C_{21}H_{23}N_3O_3$: 365.1739; found $[M+H]^+$ 366.1837.

- ***N*-benzyl-4-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanamide (32g)**



Trituration of the crude product with ethanol to obtain a white solid (112,6 mg, 78 %). R_f (60% EtOAc/hexane) 0.46. MP = 217– 219 °C. **IR (KBr) (cm^{-1})**: 3325, 3160, 2962, 1662, 1420, 1343.**^{1}H NMR (400 MHz, DMSO-d_6) δ** 11.42 (s, 1H), 8.44 (t, *J* = 5.8 Hz, 1H), 8.16 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.85 (ddd, *J* = 8.4, 7.2, 1.5 Hz, 1H), 7.82 – 7.73 (m, 3H), 7.54 (ddd, J = 8.1, 7.1, 1.1 Hz, 1H), 7.46 (t, *J* = 8.0 Hz, 1H), 7.29 – 7.17 (m, 5H), 7.14 (dd, *J* = 8.2, 1.9 Hz, 1H), 4.29 (d, *J* = 5.9 Hz, 2H), 4.10 (t, *J* = 6.3 Hz, 2H), 2.38 (t, *J* = 7.3 Hz, 2H), 2.03 (p, *J* = 6.8 Hz, 2H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 172.0, 162.7, 159.1, 152.5, 140.0, 135.0, 134.4, 130.2, 128.7, 127.9, 127.6, 127.1, 127.1, 126.3, 121.4, 120.5, 118.5, 113.5, 67.6, 42.5, 32.1, 25.3. **HRMS** m/z calcd for $C_{25}H_{23}N_3O_3$: 413.1739; found $[M+H]^+$ 414.1837.

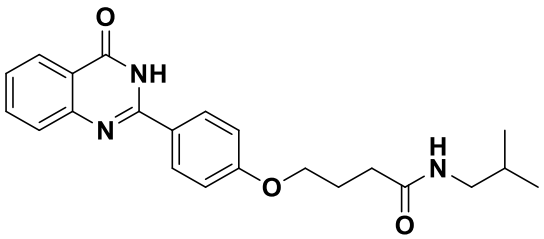
- ***N*-isobutyl-4-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanamide (32h)**



Trituration of the crude product with ethanol to obtain a white solid (87.6 mg, 66 %). R_f (60% EtOAc/hexane) 0.33. MP = 225 – 226 °C. **IR (KBr) (cm^{-1})**: 3280, 3117, 2971, 1654, 1427, 1329. **^{1}H NMR (400 MHz, DMSO-d_6) δ** 12.54 (s, 1H), 8.16 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.90 (t, *J* = 5.8 Hz, 1H), 7.85 (ddd, *J* = 8.5, 7.1, 1.6 Hz, 1H), 7.81 – 7.72 (m, 3H), 7.53 (ddd, *J* = 8.1, 7.1, 1.2 Hz, 1H), 7.45 (t, *J* = 8.0 Hz, 1H), 7.14 (dd, *J* = 8.3, 1.7 Hz, 1H), 4.08 (t, *J* = 6.3 Hz, 2H), 2.88 (t, *J* = 6.3 Hz, 2H), 2.30 (t, *J* = 7.4 Hz, 2H), 1.98 (p, *J* = 6.5 Hz, 2H), 1.72 – 1.59

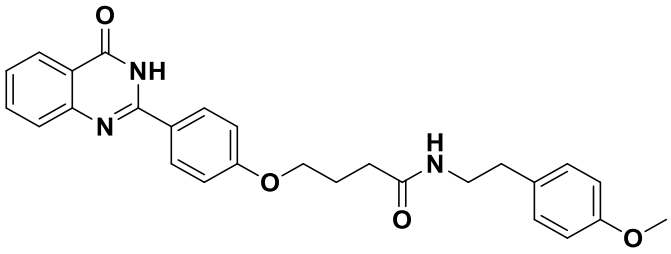
(m, *J* = 6.7 Hz, 1H), 0.82 (d, *J* = 6.7 Hz, 6H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ** 171.9, 159.1, 135.0, 134.4, 130.2, 127.0, 126.3, 121.4, 120.5, 118.5, 113.5, 67.7, 46.5, 32.1, 28.5, 25.4, 20.5. **HRMS** m/z calcd for $C_{22}H_{25}N_3O_3$: 379.1896; found $[M+H]^+$ 380.1990.

- ***N-i*sobutyl-4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanamide (32j)**



Trituration of the crude product with ethanol to obtain a white solid (112,9 mg, 85 %). R_f (60% EtOAc/hexane) 0.32. MP = 247 – 250 °C. **IR (KBr) (cm^{-1})**: 3304, 3175, 2965, 1669, 1447, 1325. **^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ** 12.42 (s, 1H), 8.18 (d, *J* = 8.9 Hz, 2H), 8.13 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.89 (t, *J* = 5.9 Hz, 1H), 7.82 (ddd, *J* = 8.5, 7.1, 1.6 Hz, 1H), 7.70 (d, *J* = 7.9 Hz, 1H), 7.49 (ddd, *J* = 8.1, 7.1, 1.2 Hz, 1H), 7.08 (d, *J* = 8.9 Hz, 2H), 4.06 (t, *J* = 6.4 Hz, 2H), 2.88 (t, *J* = 6.3 Hz, 2H), 2.28 (t, *J* = 7.4 Hz, 2H), 1.97 (p, *J* = 6.7 Hz, 2H), 1.73 – 1.60 (m, *J* = 6.6 Hz, 1H), 0.83 (d, *J* = 6.7 Hz, 6H). **^{13}C NMR (101 MHz, DMSO-d6) δ** 171.8, 162.7, 152.3, 135.0, 129.9, 127.7, 126.5, 126.2, 125.1, 121.1, 114.8, 67.7, 46.4, 32.0, 28.5, 25.3, 20.5. **HRMS** m/z calcd for $C_{22}H_{25}N_3O_3$: 379.1896; found $[M+H]^+$ 380.1995.

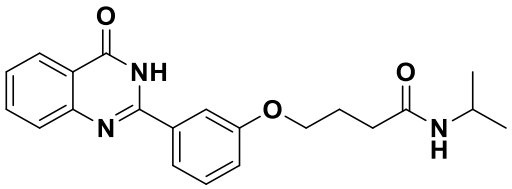
- ***N*-(4-methoxyphenethyl)-4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy) butanamide (32l)**



Trituration of the crude product with ethanol to obtain a white solid (118,2 mg, 74 %). R_f (60% EtOAc/hexane) 0.22. MP = 246 – 249 °C. **IR (KBr) (cm^{-1})**: 3305, 3175, 2931, 1639, 1447, 1326. **^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ** 12.43 (s, 1H), 8.19 (d, *J* = 8.8 Hz, 2H), 8.14 (d, *J* = 7.7 Hz, 1H), 7.96 (t, *J* = 5.5 Hz, 1H), 7.82 (t, *J* = 7.2 Hz, 1H), 7.71 (d, *J* = 8.1 Hz, 1H), 7.49 (t, *J* = 7.4 Hz, 1H), 7.09 (dd, *J* = 13.8, 8.5 Hz, 4H), 6.82 (d, *J* = 8.5 Hz, 2H),

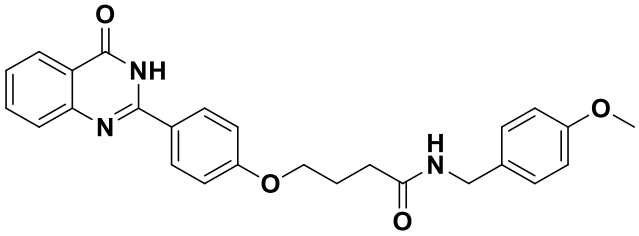
4.03 (t, *J* = 6.3 Hz, 2H), 3.69 (s, 3H), 3.24 (q, *J* = 6.8 Hz, 2H), 2.64 (t, *J* = 7.3 Hz, 2H), 2.25 (t, *J* = 7.3 Hz, 2H), 1.96 (p, *J* = 6.5 Hz, 2H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ** 171.8, 162.7, 161.6, 158.0, 152.3, 149.3, 135.0, 131.8, 130.0, 129.9, 127.7, 126.5, 126.2, 125.1, 121.1. 114.8, 114.1, 67.6, 55.3, 34.7, 32.0, 25.2. **HRMS** m/z calcd for $C_{27}H_{27}N_3O_4$: 457.2002; found $[M+H]^+$ 458.2096.

- ***N*-isopropyl-4-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanamide (32n)**



Trituration of the crude product with ethanol to obtain a white solid (78.6 mg, 60 %). R_f (60% EtOAc/hexane) 0.22. MP = 245 – 247 °C. **IR (KBr) (cm^{-1})**: 3307, 3143, 2964, 1638, 1448, 1344. **^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ** 12.55 (s, 1H), 8.16 (d, *J* = 7.8 Hz, 1H), 7.85 (ddd, *J* = 8.5, 7.1, 1.6 Hz, 1H), 7.81 – 7.70 (m, 4H), 7.53 (t, *J* = 7.2 Hz, 1H), 7.45 (t, *J* = 8.0 Hz, 1H), 7.14 (dd, *J* = 8.1, 2.5 Hz, 1H), 4.08 (t, *J* = 6.3 Hz, 2H), 3.92 – 3.78 (m, *J* = 6.8 Hz, 1H), 2.24 (t, *J* = 7.4 Hz, 2H), 1.98 (q, *J* = 7.1, 6.4 Hz, 2H), 1.04 (d, *J* = 6.5 Hz, 6H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ** 170.9, 162.6, 159.1, 152.4, 149.1, 135.0, 134.4, 130.2, 127.9, 127.1, 126.3, 121.4, 120.5, 118.5, 113.5, 67.7, 32.2, 25.3, 22.9. **HRMS** m/z calcd for $C_{21}H_{23}N_3O_3$: 365.1739; found $[M+H]^+$ 366.1841.

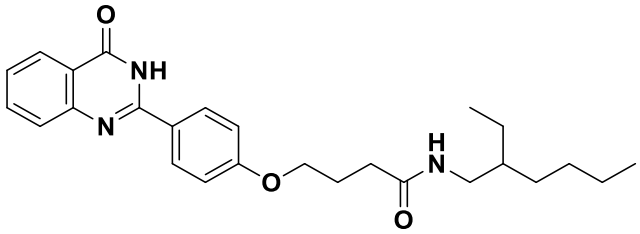
- ***N*-(4-methoxybenzyl)-4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy) butanamide (32o)**



Trituration of the crude product with ethanol to obtain a white solid (142.8 mg, 92 %). R_f (60% EtOAc/hexane) 0.27. MP = 228 – 233 °C. **IR (KBr) (cm^{-1})**: 3379, 3185, 2967, 1666, 1472, 1345. **^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ** 12.43 (s, 1H), 8.36 (t, *J* = 5.9 Hz, 1H), 8.19 (d, *J* = 9.0 Hz, 2H), 8.14 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.82 (ddd, *J* = 8.5, 7.1, 1.6 Hz, 1H), 7.71 (d, *J* = 7.7 Hz, 1H), 7.49 (ddd, *J* = 8.1, 7.1, 1.2 Hz, 1H), 7.16 (d, *J* = 8.6 Hz, 2H), 7.06 (d,

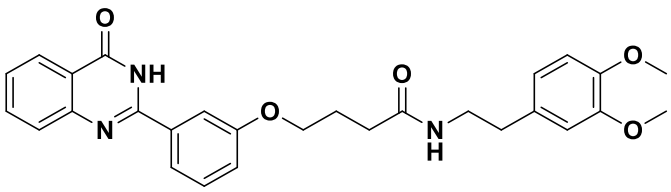
J = 9.0 Hz, 2H), 6.85 (d, *J* = 8.7 Hz, 2H), 4.21 (d, *J* = 5.9 Hz, 2H), 4.07 (t, *J* = 6.4 Hz, 2H), 3.70 (s, 3H), 2.33 (t, *J* = 7.3 Hz, 2H), 2.00 (p, *J* = 6.7 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ** 171.8, 162.7, 161.6, 158.5, 152.3, 149.3, 135.0, 132.0, 129.9, 128.9, 127.7, 126.5, 126.2, 125.1, 121.1, 114.8, 114.1, 67.6, 55.4, 41.9, 32.0, 25.2. **HRMS** m/z calcd for $C_{26}H_{25}N_3O_4$: 443.1845; found $[M+H]^+$ 444.1954.

- ***N*-(2-ethylhexyl)-4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy) butanamide (32p)**



Trituration of the crude product with ethanol to obtain a white solid (138.6 mg, 91 %). R_f (60% EtOAc/hexane) 0.36. MP = 180 – 182 °C. **IR (KBr) (cm^{-1})**: 3305, 3173, 2957, 1652, 1447, 1345. **^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ** 12.43 (s, 1H), 8.19 (d, *J* = 8.9 Hz, 2H), 8.13 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.86 – 7.77 (m, 2H), 7.70 (d, *J* = 7.2 Hz, 1H), 7.49 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 1H), 7.07 (d, *J* = 8.9 Hz, 2H), 4.06 (t, *J* = 6.4 Hz, 2H), 2.99 (t, *J* = 5.5 Hz, 2H), 2.28 (t, *J* = 7.3 Hz, 2H), 1.97 (p, *J* = 6.8 Hz, 2H), 1.38 – 1.26 (m, 1H), 1.31 – 1.16 (m, 8H), 0.87 – 0.76 (m, 6H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ** 171.9, 161.6, 152.3, 140.0, 135.0, 129.9, 128.7, 127.6, 127.1, 126.5, 126.2, 125.1, 121.1, 114.8, 67.6, 42.5, 32.0, 25.2. **HRMS** m/z calcd for $C_{26}H_{33}N_3O_3$: 435.2522; found $[M+H]^+$ 436.2614.

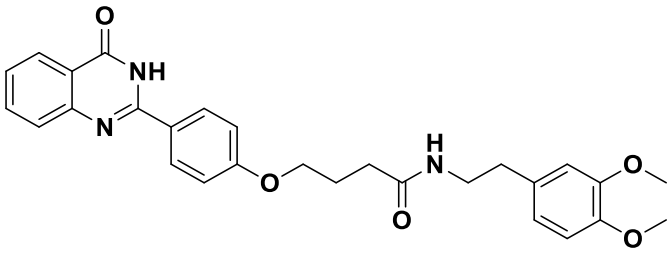
- ***N*-(3,4-dimethoxyphenethyl)-4-(3-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy) butanamide (32q)**



Trituration of the crude product with ethanol to obtain a white solid (98.7 mg, 58 %). R_f (60% EtOAc/hexane) 0.34. MP = 202 – 208 °C. **IR (KBr) (cm^{-1})**: 3249, 3149, 2981, 1623, 1457, 1333. **^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ** 12.60 (s, 1H), 8.22 (d, *J* = 7.7 Hz, 1H), 8.04 (t, *J* = 5.6 Hz, 1H), 7.91 (t, *J* = 7.4 Hz, 1H), 7.85 (d, *J* = 7.8 Hz, 1H), 7.81 (d, *J* = 6.8 Hz, 2H),

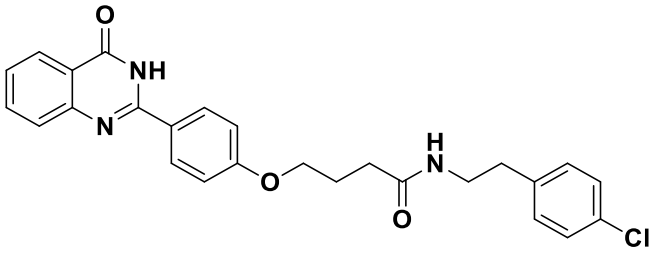
7.59 (t, *J* = 7.4 Hz, 1H), 7.52 (t, *J* = 7.9 Hz, 1H), 7.20 (d, *J* = 7.9 Hz, 1H), 6.86 (d, *J* = 8.0 Hz, 2H), 6.74 (d, *J* = 7.9 Hz, 1H), 4.12 (t, *J* = 5.9 Hz, 2H), 3.78 (s, 3H), 3.73 (s, 3H), 3.35 – 3.29 (m, 2H), 2.70 (t, *J* = 7.0 Hz, 2H), 2.33 (t, *J* = 7.1 Hz, 2H), 2.03 (p, *J* = 7.0 Hz, 2H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ** 171.9, 159.1, 149.0, 147.6, 135.0, 134.4, 132.4, 130.2, 127.1, 126.3, 121.4, 120.8, 120.5, 118.5, 113.5, 112.9, 112.2, 67.6, 55.9, 55.7, 35.2, 32.1, 25.3. **HRMS** m/z calcd for $C_{28}H_{29}N_3O_5$: 487.2107; found $[M+H]^+$ 488.2143.

- ***N*-(3,4-dimethoxyphenethyl)-4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy) butanamide (32r)**



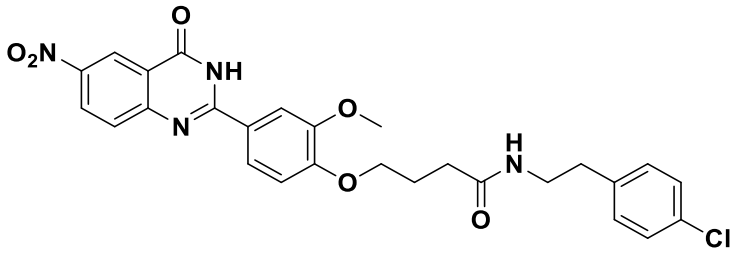
Trituration of the crude product with ethanol to obtain a white solid (151.6 mg, 89 %). R_f (60% EtOAc/hexane) 0.36. MP = 225 – 230 °C. **IR (KBr) (cm^{-1})**: 3330, 3075, 2962, 1640, 1441, 1322. **^{1}H NMR (400 MHz, DMSO-d6) δ** 12.34 (s, 1H), 8.11 (d, J = 8.9 Hz, 2H), 8.07 (dd, J = 7.9, 1.6 Hz, 1H), 7.90 (t, J = 5.6 Hz, 1H), 7.76 (ddd, J = 8.2, 7.0, 1.4 Hz, 1H), 7.64 (d, J = 7.9 Hz, 1H), 7.43 (ddd, J = 8.1, 7.1, 1.2 Hz, 1H), 7.00 (d, J = 8.9 Hz, 2H), 6.76 – 6.72 (m, 2H), 6.62 (dd, J = 8.1, 2.0 Hz, 1H), 3.97 (t, J = 6.3 Hz, 2H), 3.66 (s, 3H), 3.62 (s, 3H), 3.20 (q, J = 6.8 Hz, 2H), 2.57 (t, J = 7.3 Hz, 2H), 2.19 (t, J = 7.3 Hz, 2H), 1.89 (p, J = 6.8 Hz, 2H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ** 171.9, 162.7, 161.6, 152.3, 149.3, 148.9, 147.6, 135.0, 132.3, 129.9, 127.7, 126.6, 126.2, 125.1, 120.9, 114.8, 112.9, 112.2, 67.6, 55.9, 55.8, 35.1, 32.0, 25.2. **HRMS** m/z calcd for $C_{28}H_{29}N_3O_5$: 487.2107; found $[M+H]^+$ 488.2154.

- ***N*-(4-chlorophenethyl)-4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy) butanamide (32s)**



Trituration of the crude product with ethanol to obtain a white solid (150.1 mg, 93 %). R_f (60% EtOAc/hexane) 0.26. MP = 273 – 277 °C. **IR (KBr) (cm^{-1})**: 3329, 3087, 2962, 1644, 1455, 1344. **^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ** 12.35 (s, 1H), 8.12 (d, *J* = 8.9 Hz, 2H), 8.07 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.90 (t, *J* = 5.6 Hz, 1H), 7.75 (ddd, *J* = 8.5, 7.1, 1.6 Hz, 1H), 7.66 – 7.62 (m, 1H), 7.42 (ddd, *J* = 8.1, 7.0, 1.2 Hz, 1H), 7.25 (d, *J* = 8.4 Hz, 2H), 7.15 (d, *J* = 8.4 Hz, 2H), 7.00 (d, *J* = 8.9 Hz, 2H), 3.96 (t, *J* = 6.4 Hz, 2H), 3.21 (q, *J* = 6.8 Hz, 2H), 2.63 (t, *J* = 7.1 Hz, 2H), 2.17 (t, *J* = 7.3 Hz, 2H), 1.87 (p, *J* = 6.6 Hz, 2H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ** 171.8, 162.7, 161.6, 152.3, 149.4, 139.0, 135.0, 131.1, 131.0, 129.9, 128.6, 127.7, 126.5, 126.2, 125.1, 121.1, 114.8, 67.6, 34.8, 32.0, 25.2. **HRMS** m/z calcd for $C_{26}H_{24}ClN_3O_3$: 461.1506; found $[M+H]^+$ 462.1548.

- ***N*-(4-chlorophenethyl)-4-(2-methoxy-4-(6-nitro-4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanamide (32t)**



Trituration of the crude product with ethanol to obtain a white solid (144.4 mg, 77 %). R_f (60% EtOAc/hexane) 0.26. MP = 274 – 278 °C. **IR (KBr) (cm^{-1})**: 3379, 3175, 2967, 1666, 1412, 1345. **^{1}H NMR (400 MHz, DMSO) δ** 12.80 (s, 1H), 8.75 (s, 1H), 8.46 (d, J = 9.1 Hz, 1H), 7.94 – 7.83 (m, 2H), 7.80 (d, J = 8.6 Hz, 2H), 7.25 (d, J = 8.0 Hz, 2H), 7.15 (d, J = 8.0 Hz, 2H), 7.05 (d, J = 8.6 Hz, 1H), 3.96 (t, J = 6.5 Hz, 2H), 3.83 (s, 3H), 3.20 (t, J = 6.6 Hz, 3H), 2.64 (t, J = 7.3 Hz, 2H), 2.17 (t, J = 7.4 Hz, 2H), 1.88 (p, J = 8.0, 6.9 Hz, 2H). **^{13}C NMR (101 MHz, DMSO) δ** 171.8, 149.0, 144.5, 139.0, 131.0, 128.6, 122.6, 122.5, 112.7, 111.6, 68.2, 56.1, 34.8, 32.04, 25.2. **HRMS** m/z calcd for $C_{27}H_{25}ClN_4O_6$: 536.1463; found $[M+H]^+$ 537.1487.

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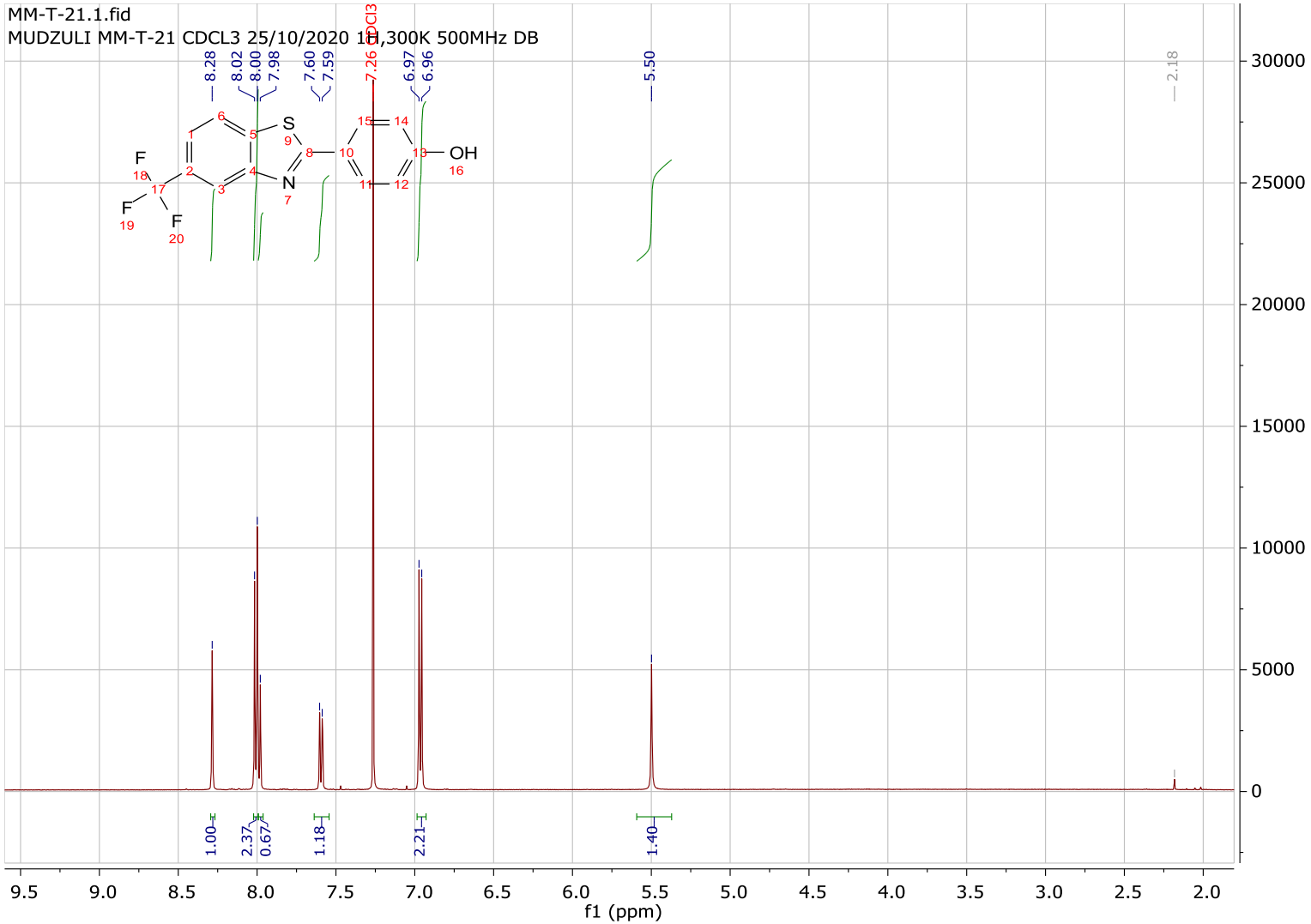
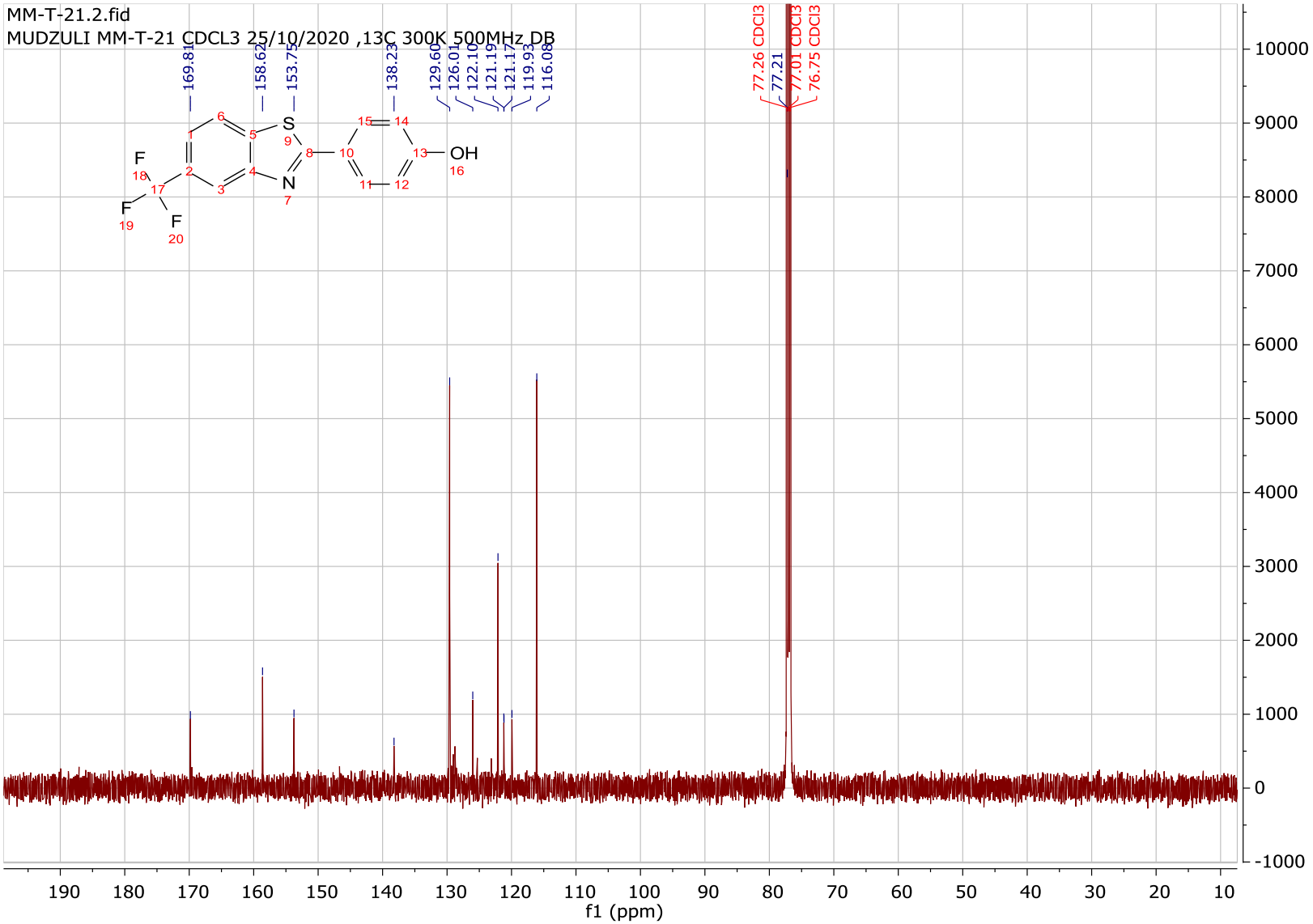
Appendix: NMR Spectra

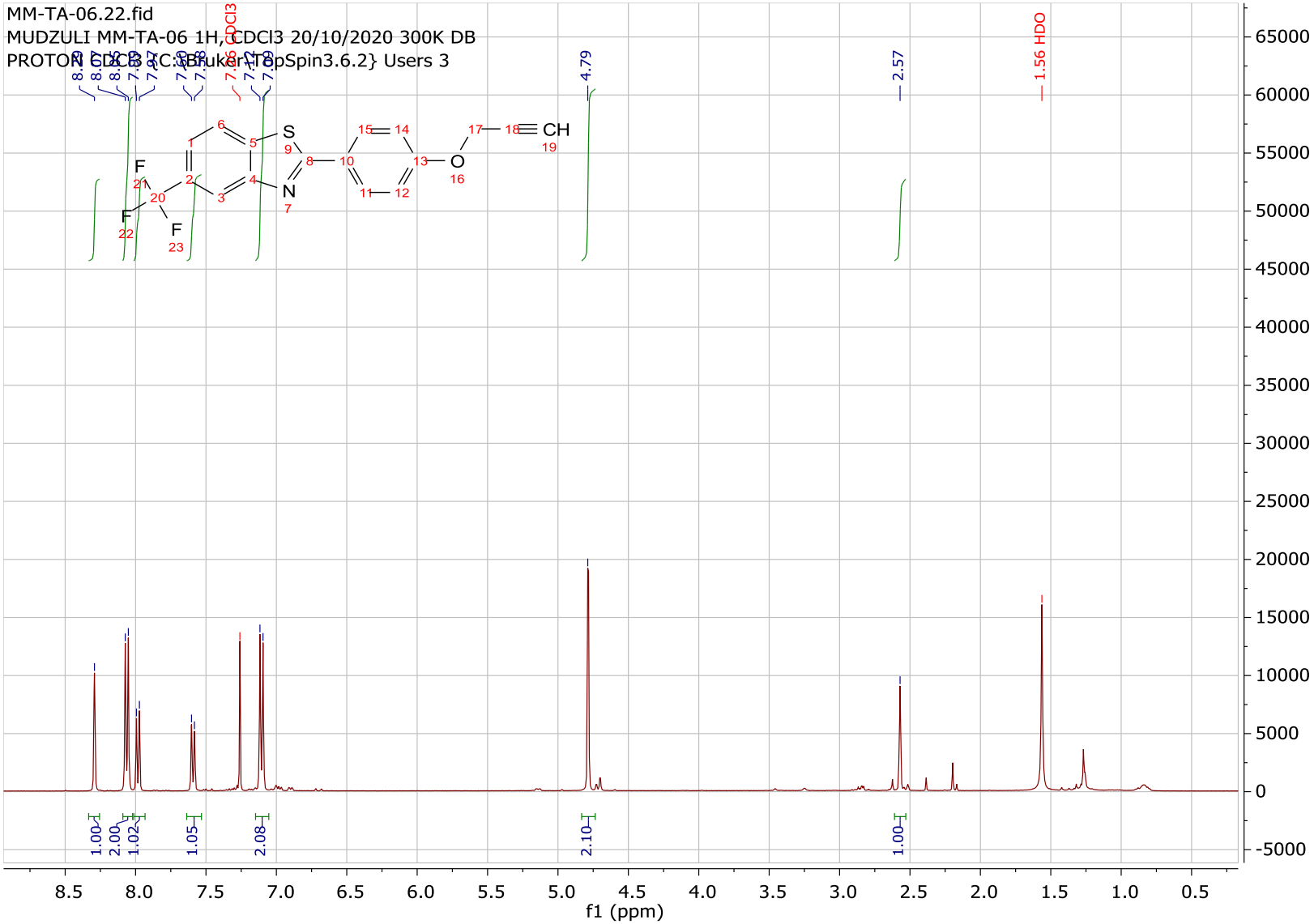
^{1}H NMR of 4-(5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)phenol (3f)



^{13}C NMR of 4-(5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)phenol (3f)



^{1}H NMR of 2-(4-(prop-2-yn-1-yloxy)phenyl)-5-(trifluoromethyl)benzo[*d*]thiazole (6f)



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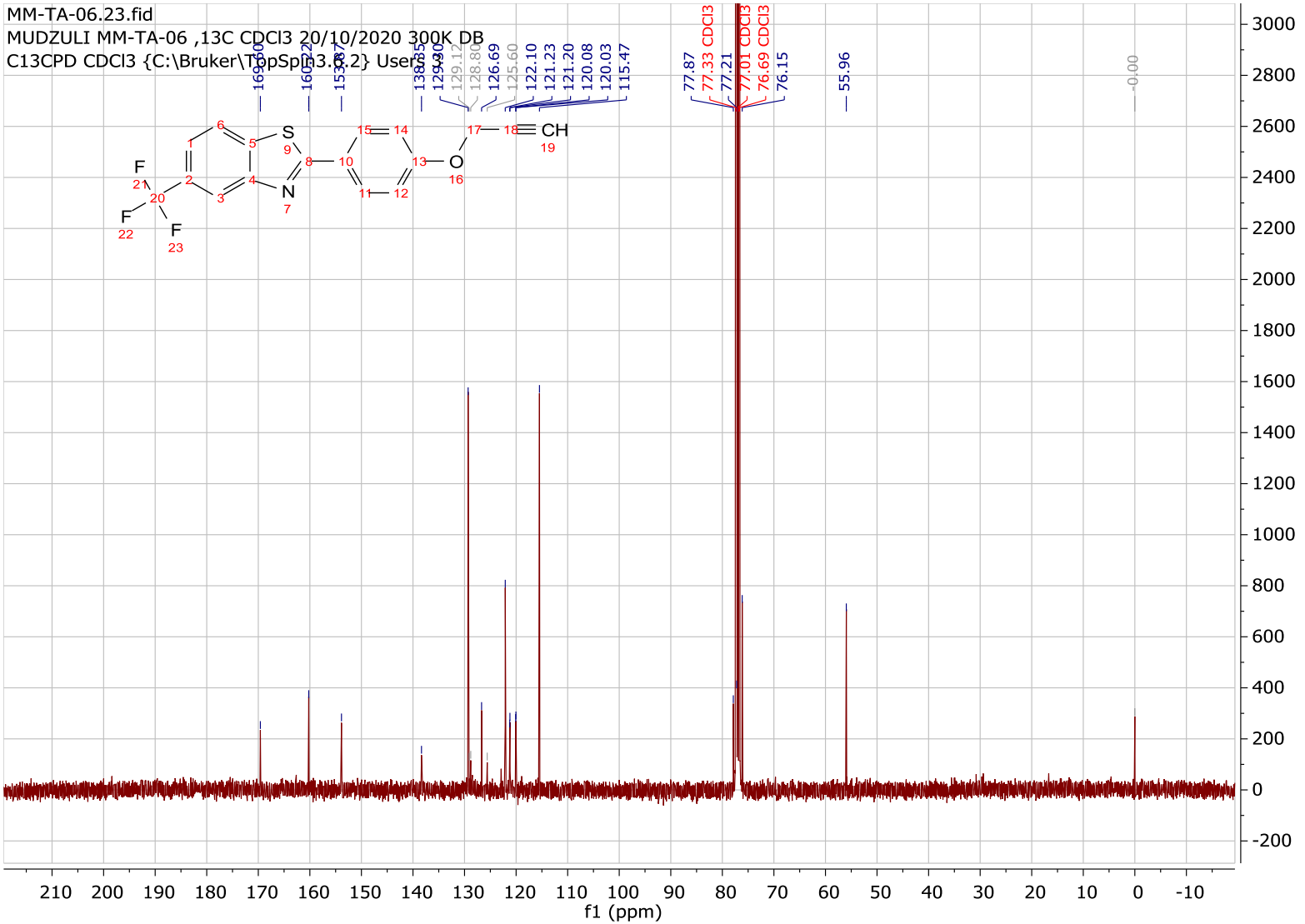

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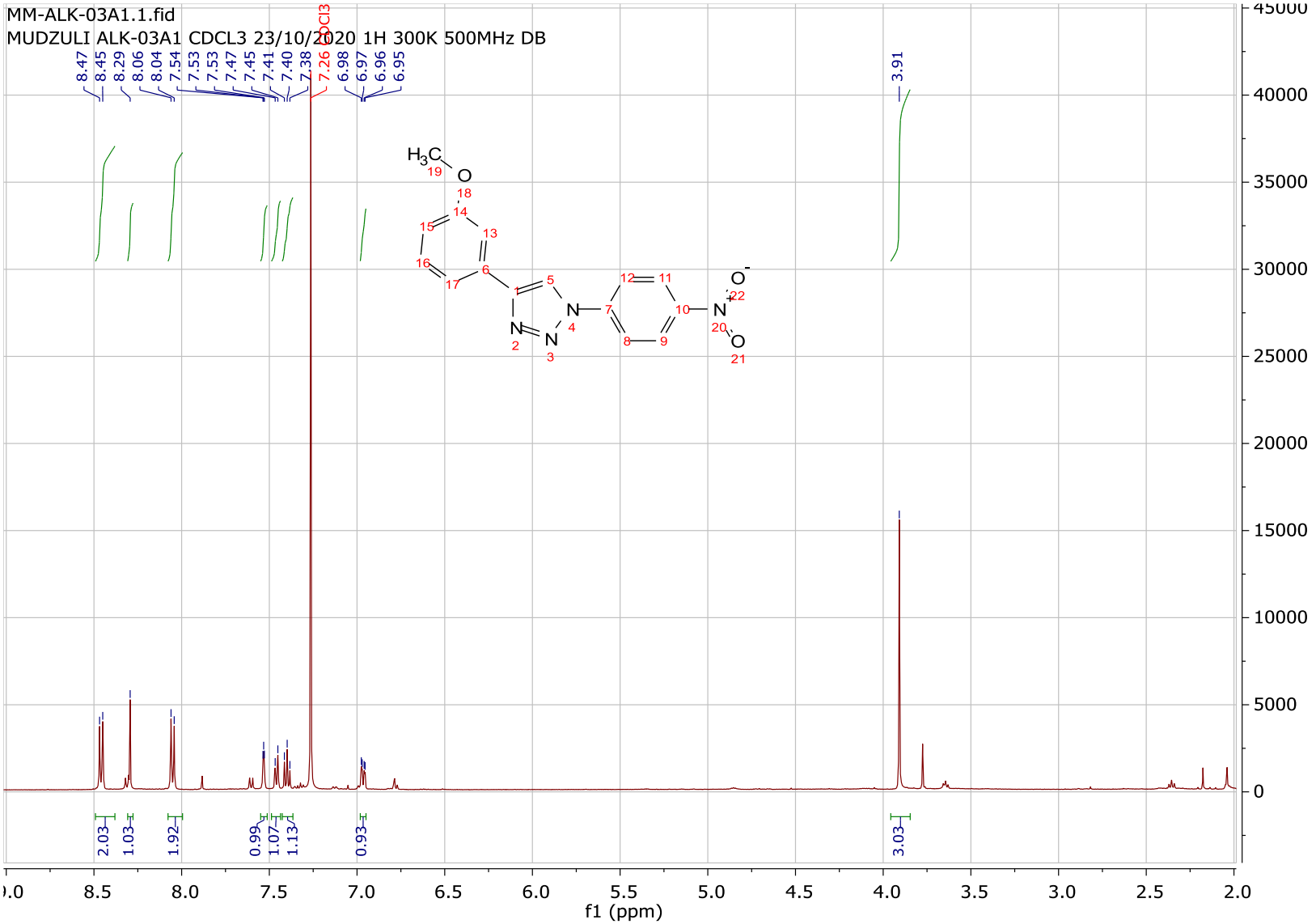

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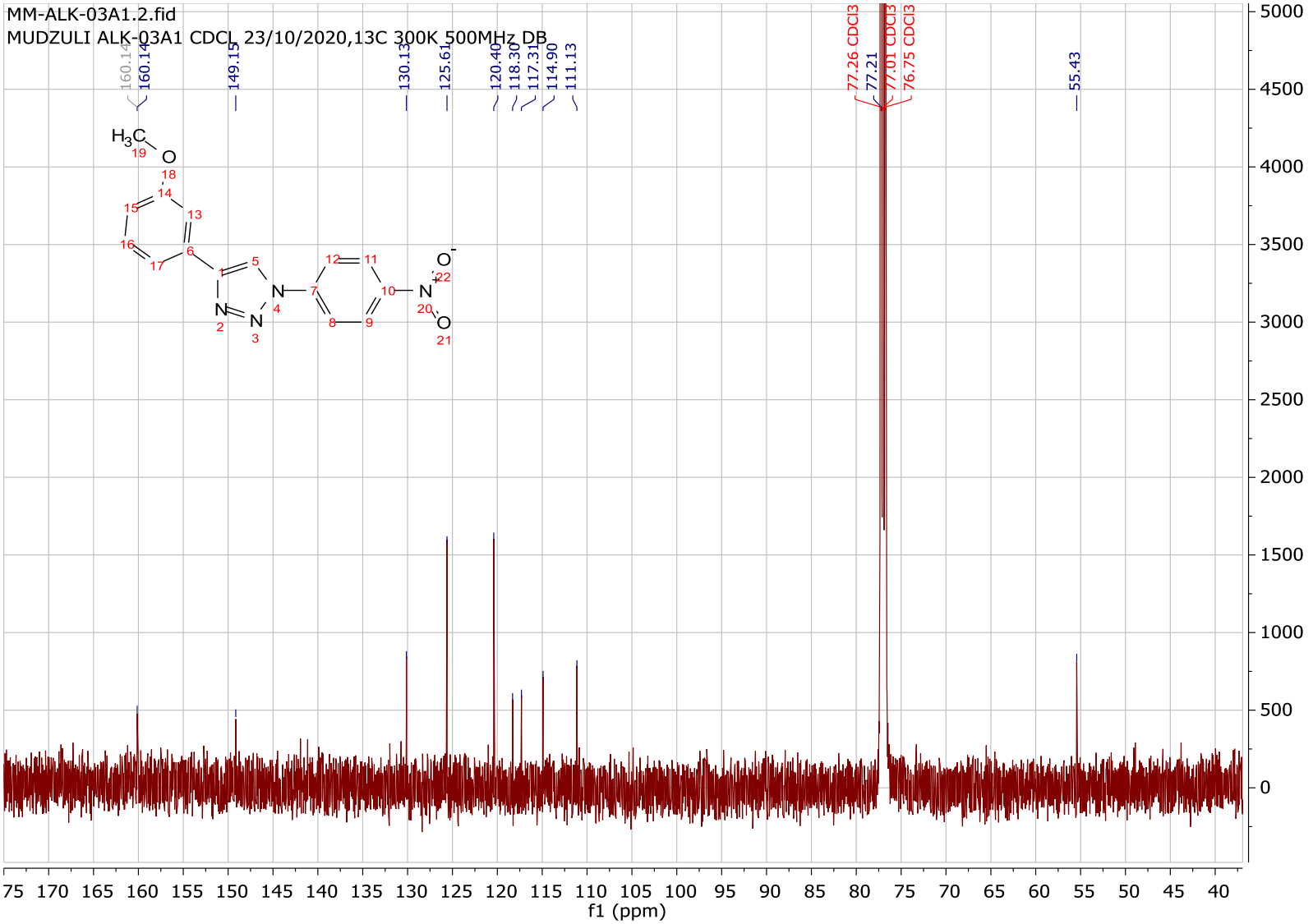
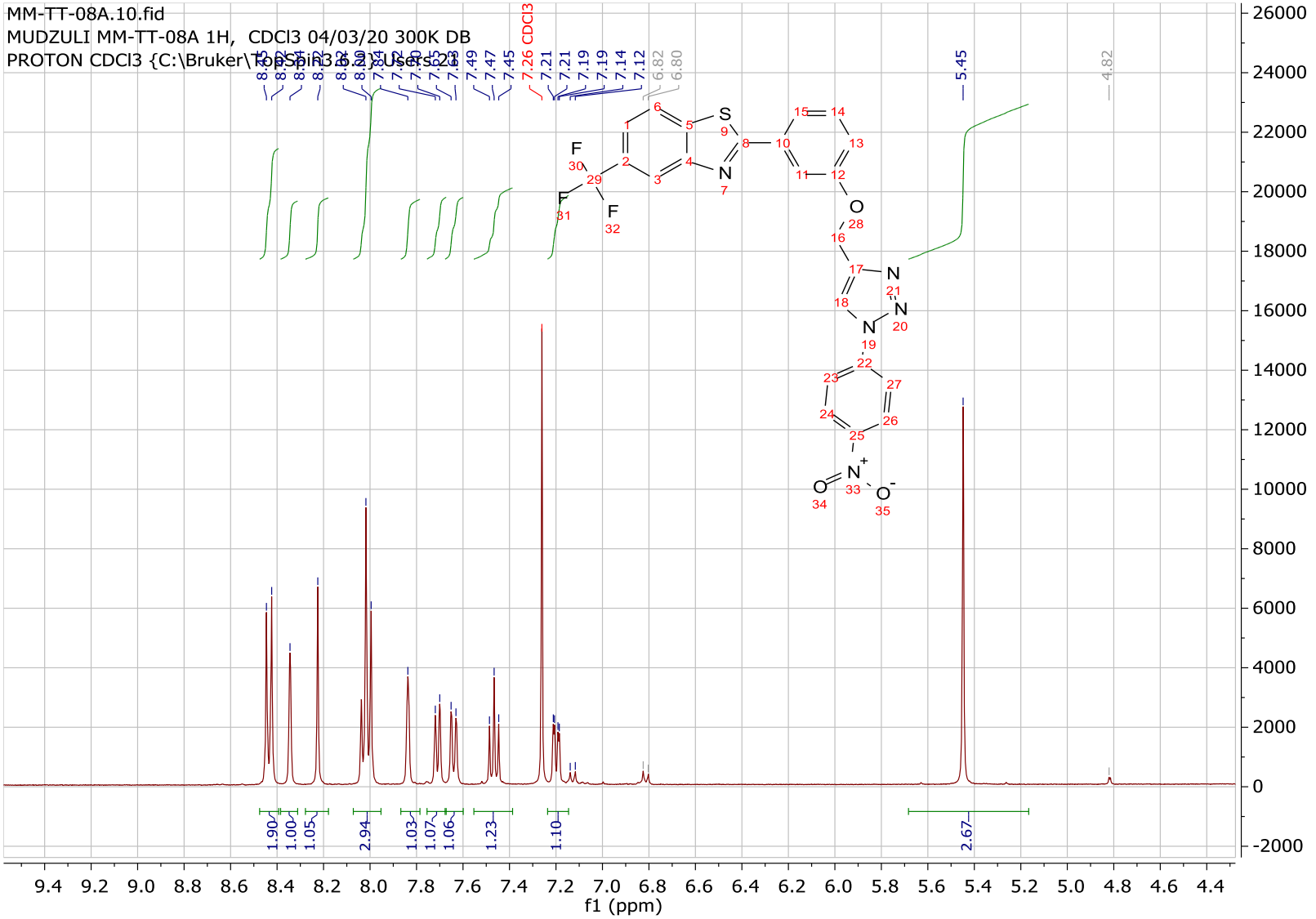
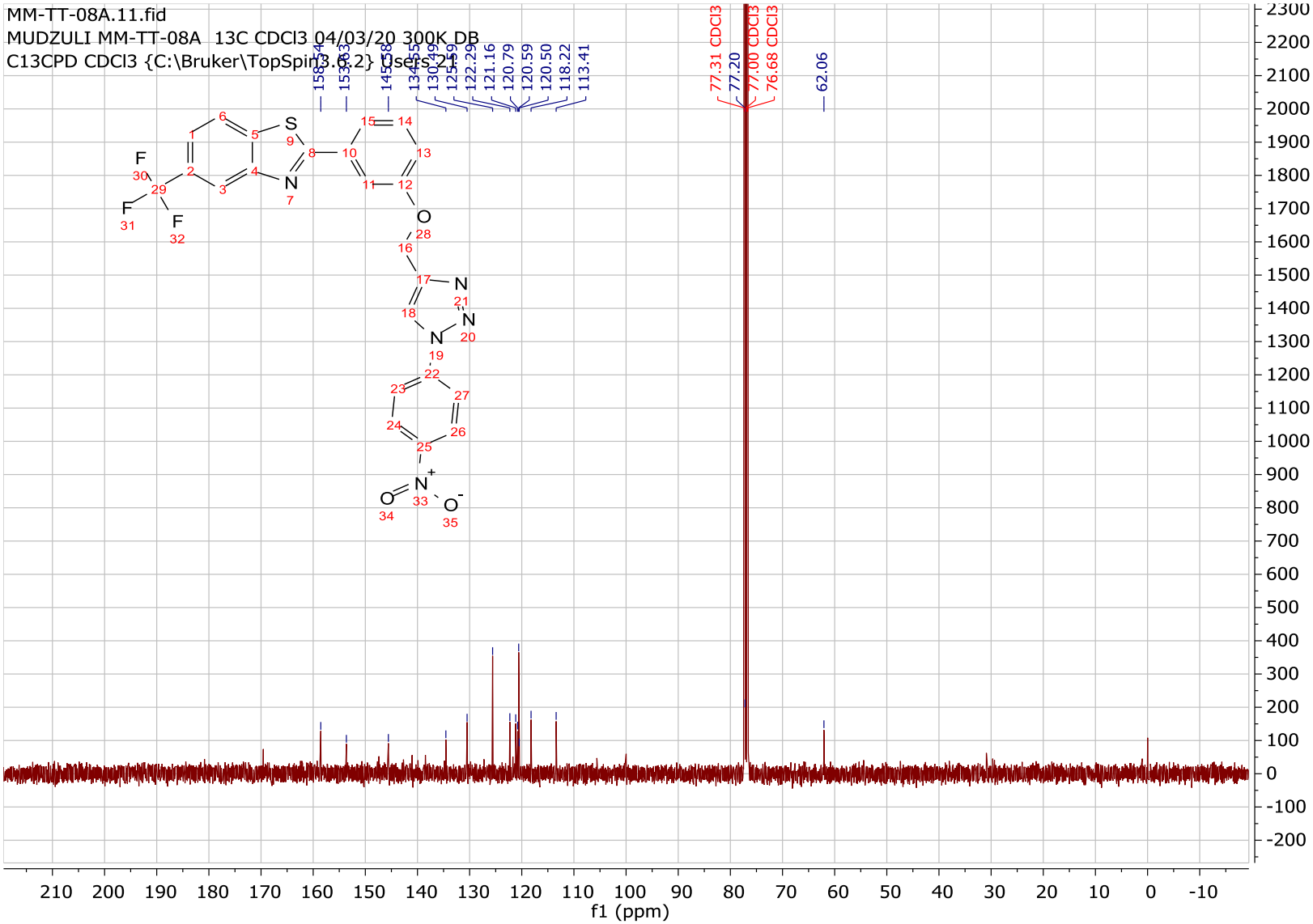


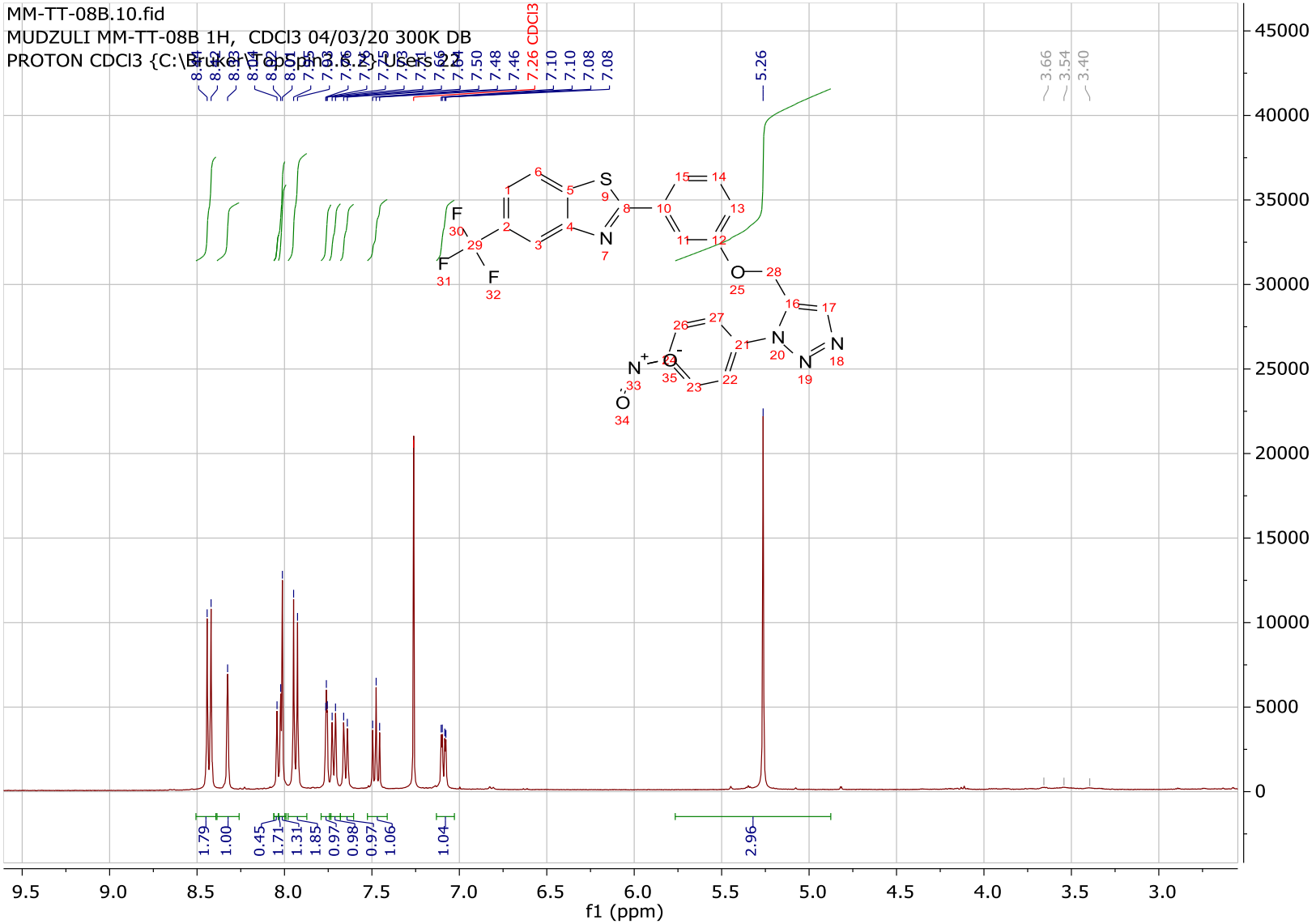
^{1}H NMR of 2-(3-((1-(4-Nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-(trifluoromethyl) benzo[*d*]thiazole (23n)



^{13}C NMR of 2-(3-((1-(4-Nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-5-(trifluoromethyl) benzo[*d*]thiazole (23n)



^{1}H NMR of 2-(3-((1-(4-nitrophenyl)-1H-1,2,3-triazol-5-yl)methoxy)phenyl)-5-(trifluoromethyl) benzo[d]thiazole (24n)



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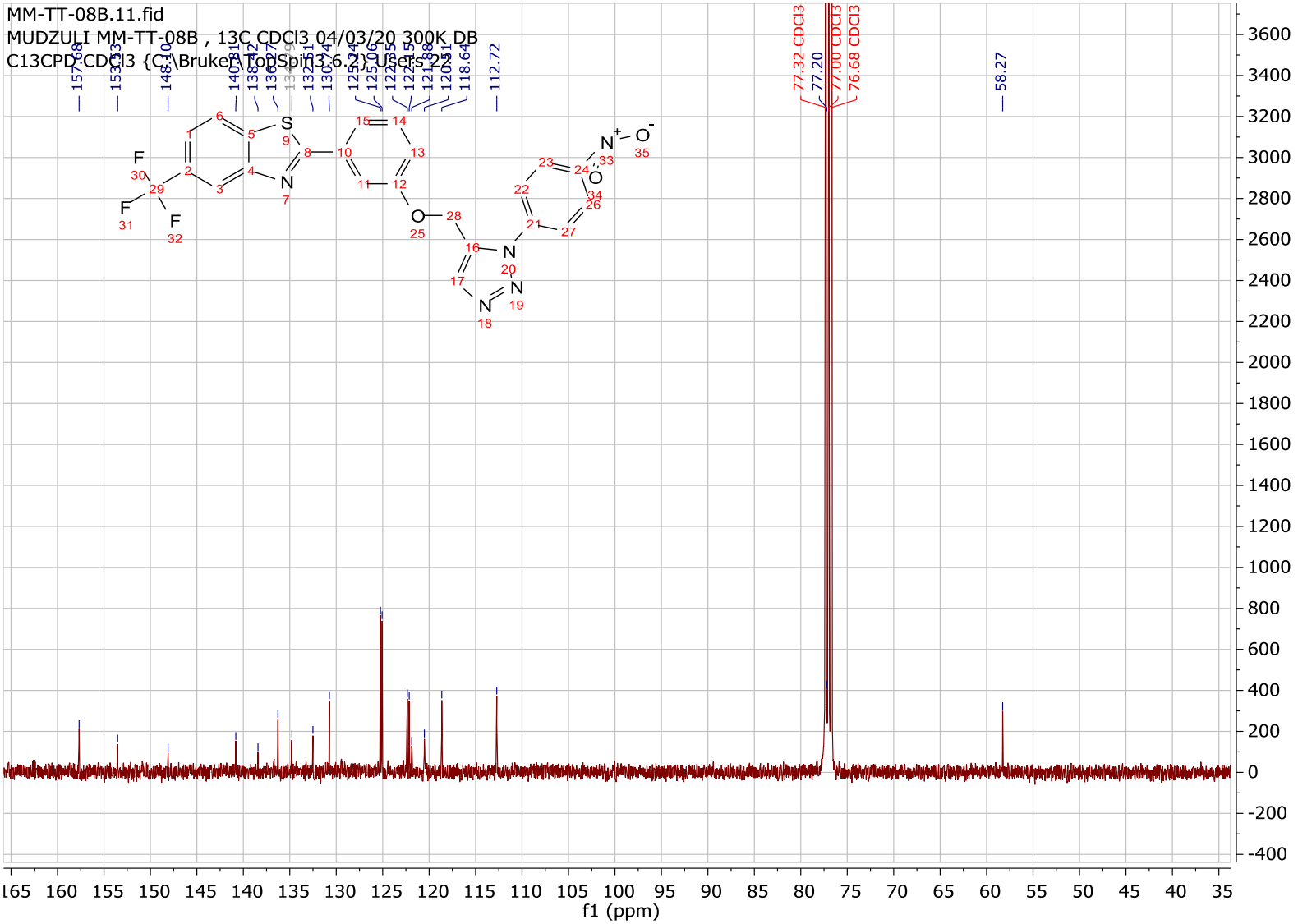

^{1}H NMR of 2-(4-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[*d*]imidazole (23k)

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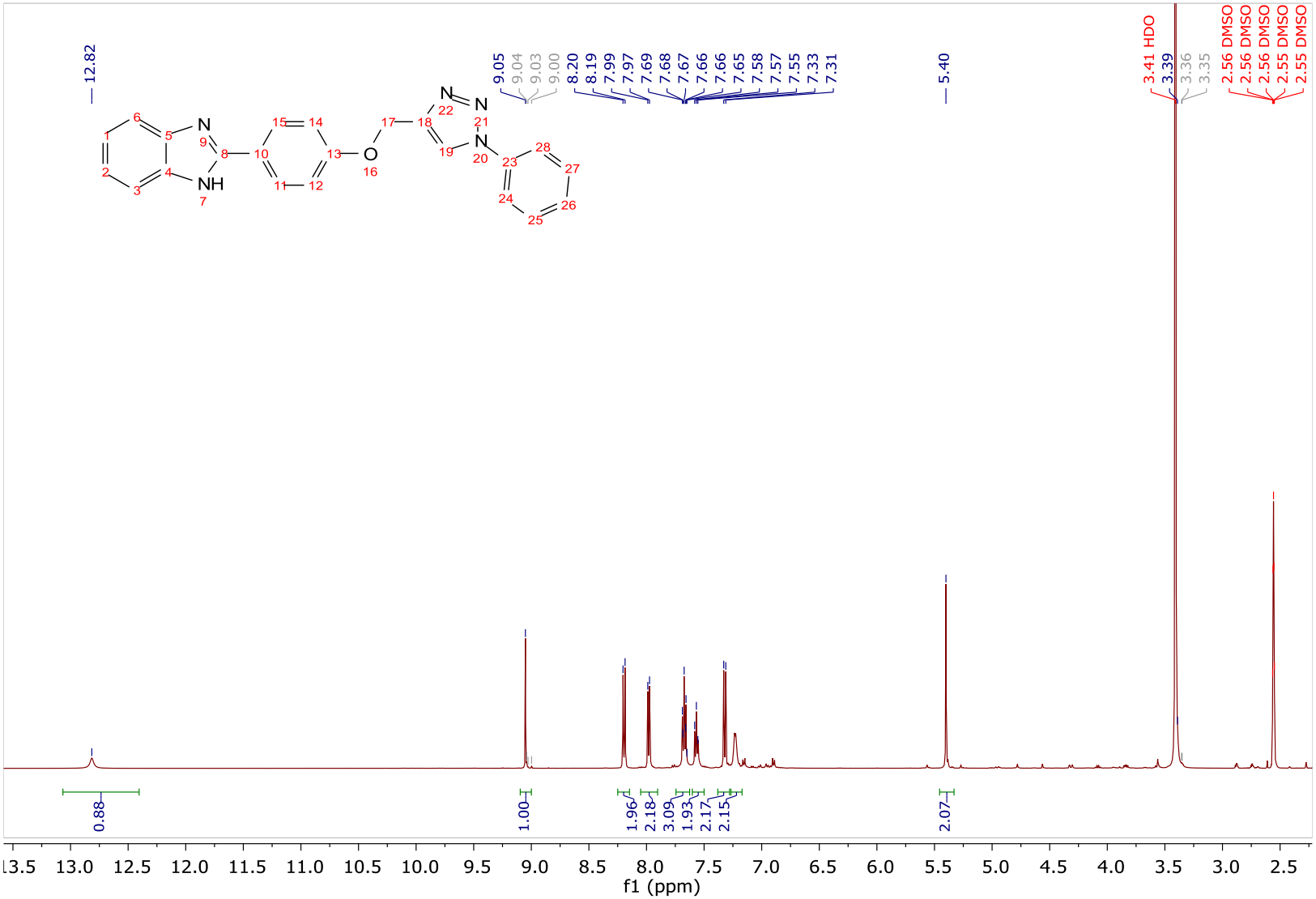

^{13}C NMR of 2-(4-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[*d*]imidazole (23k)

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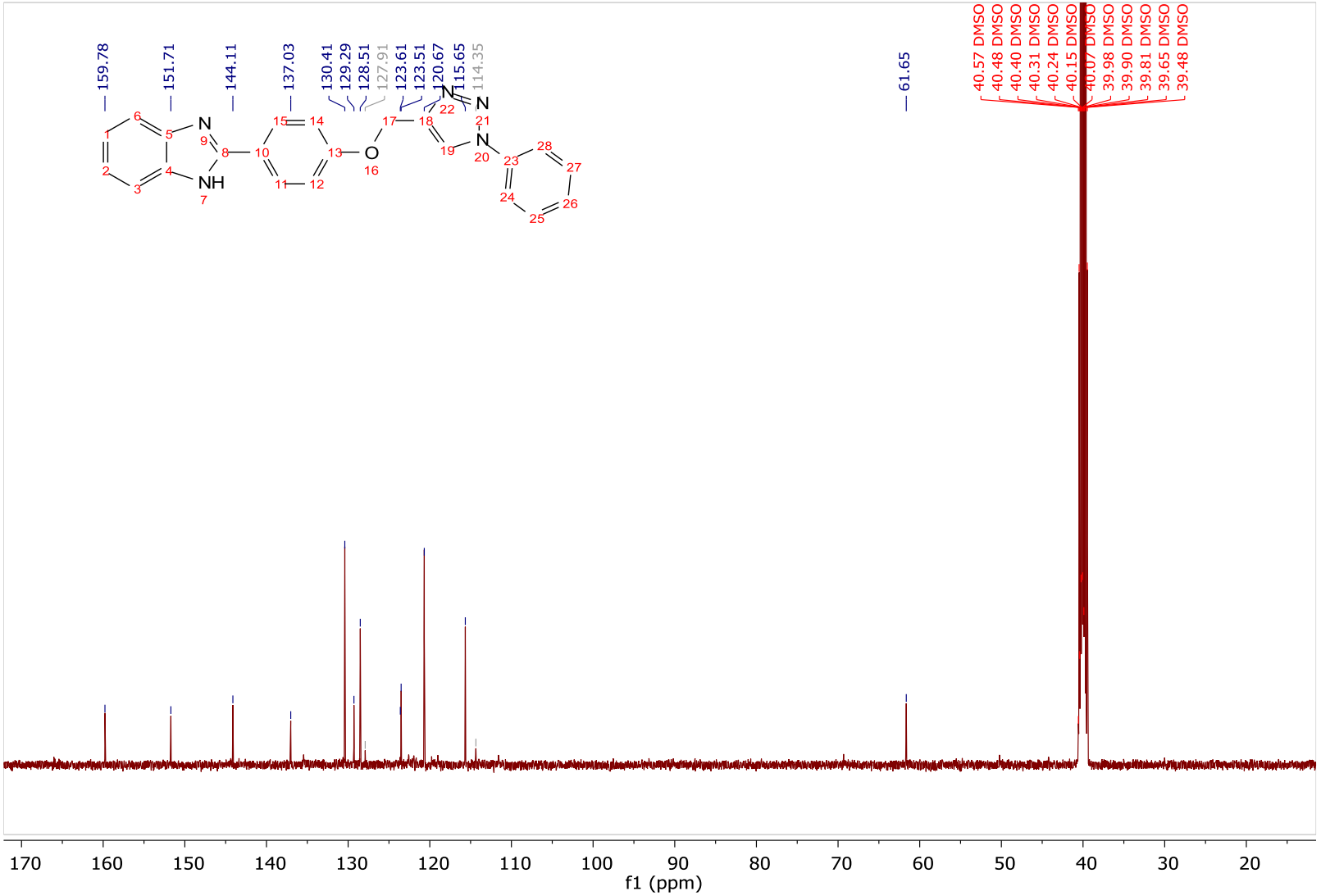
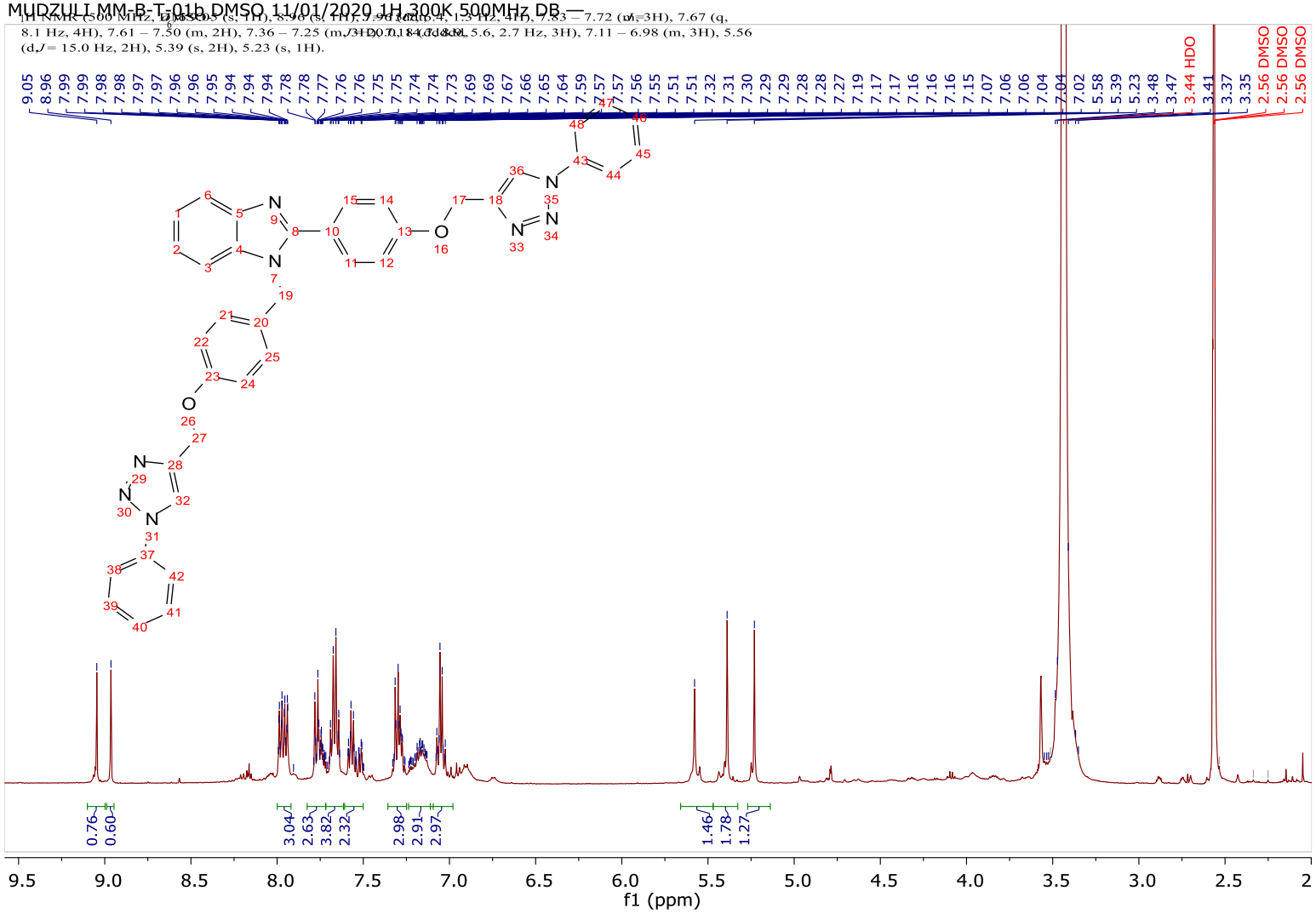


^{1}H NMR of 1-(4-((1-Phenyl-1*H*-1,2,3-triazol-4-yl)methoxy)benzyl)-2-(4-((1-phenyl-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-1*H*-benzo[*d*]imidazole (23l)



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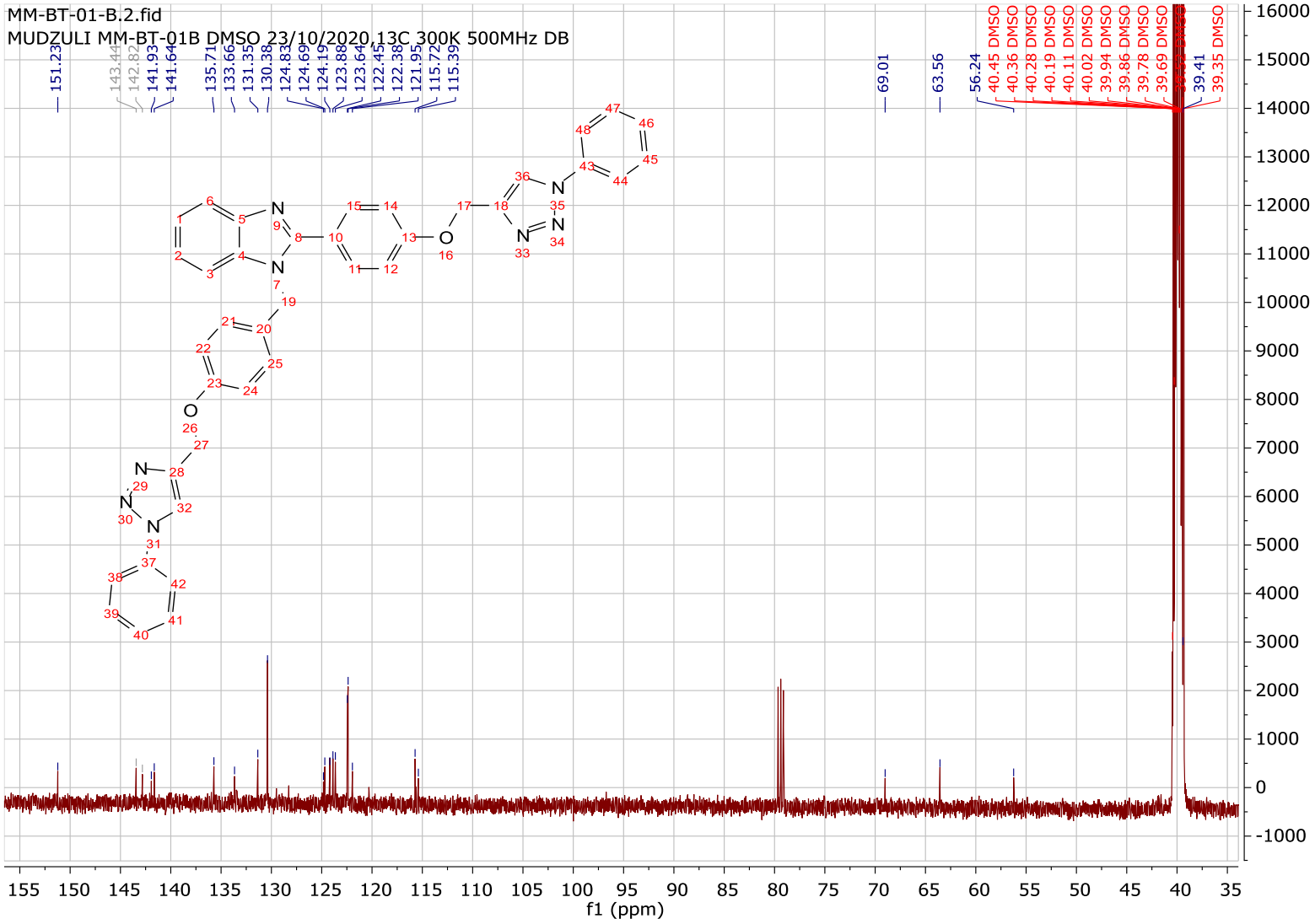
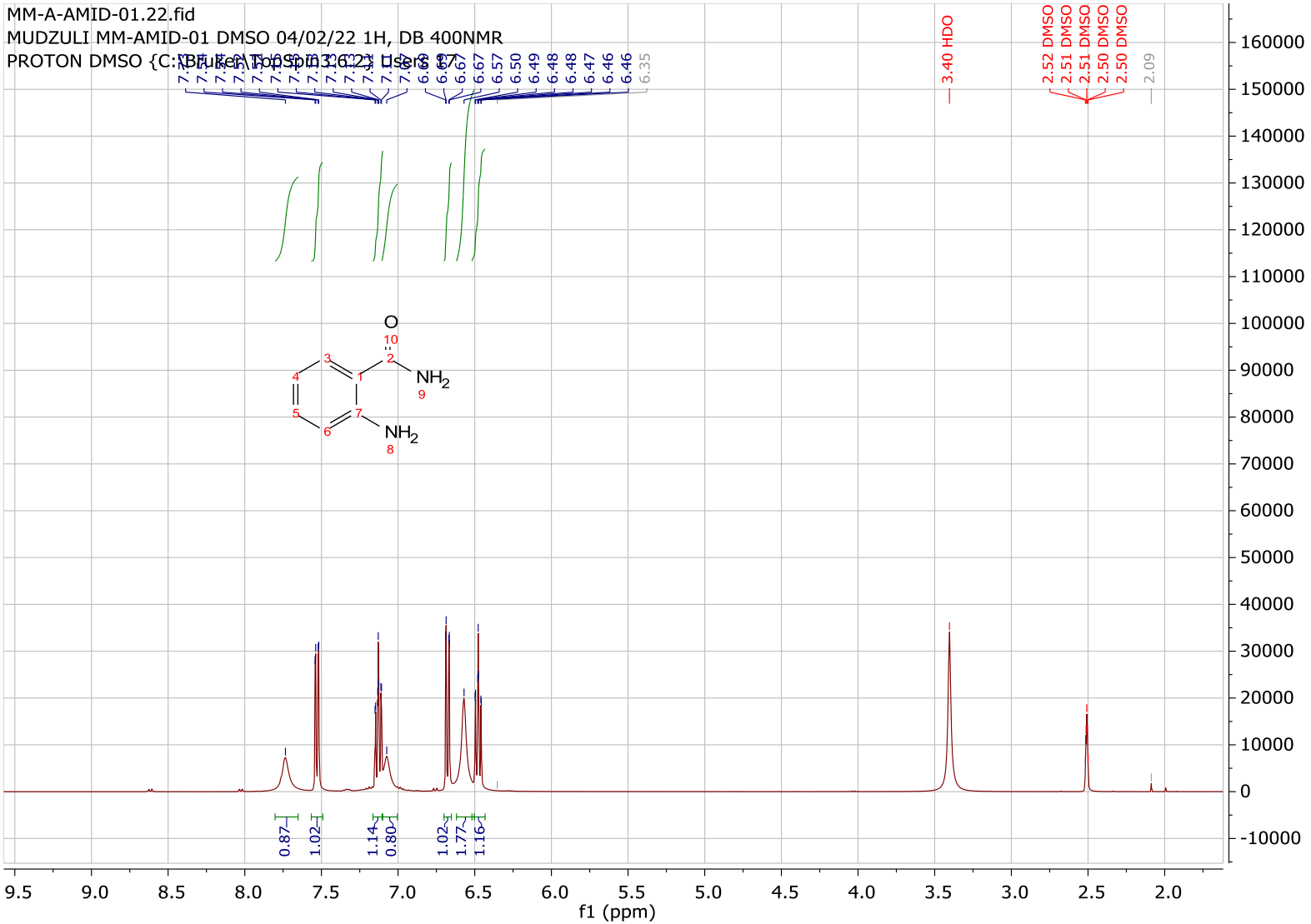


^{1}H NMR of 2-aminobenzamide (26a)



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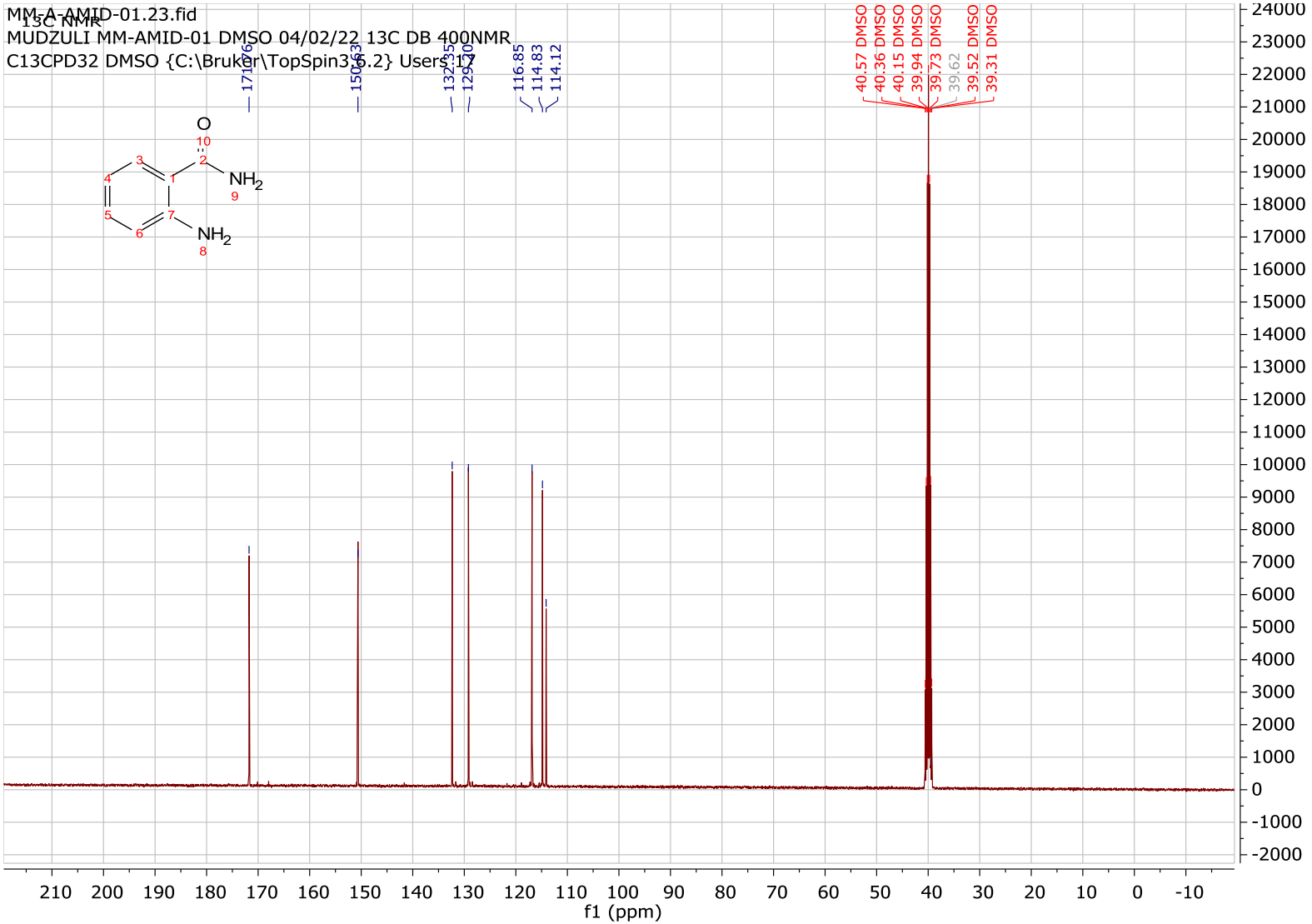
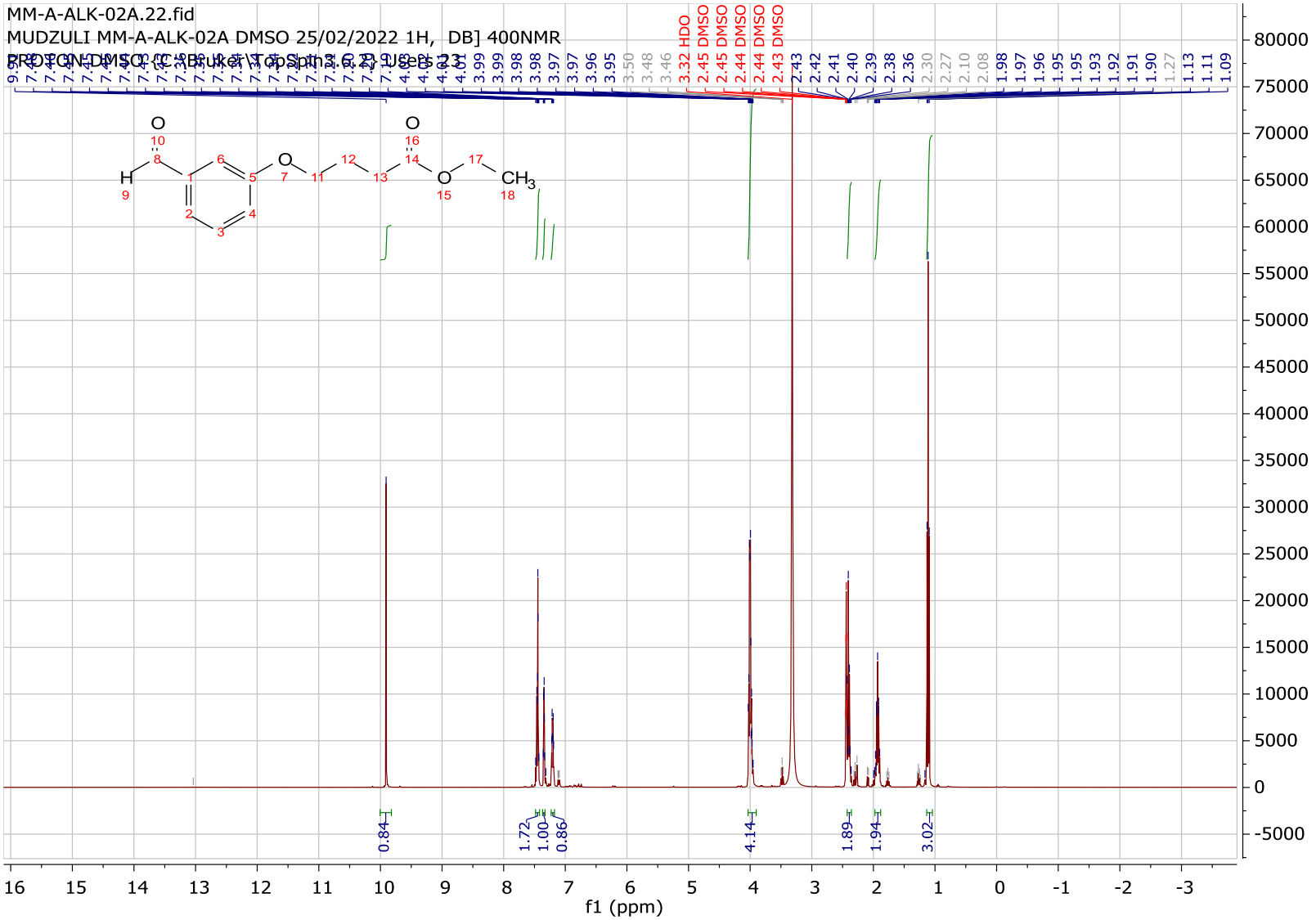
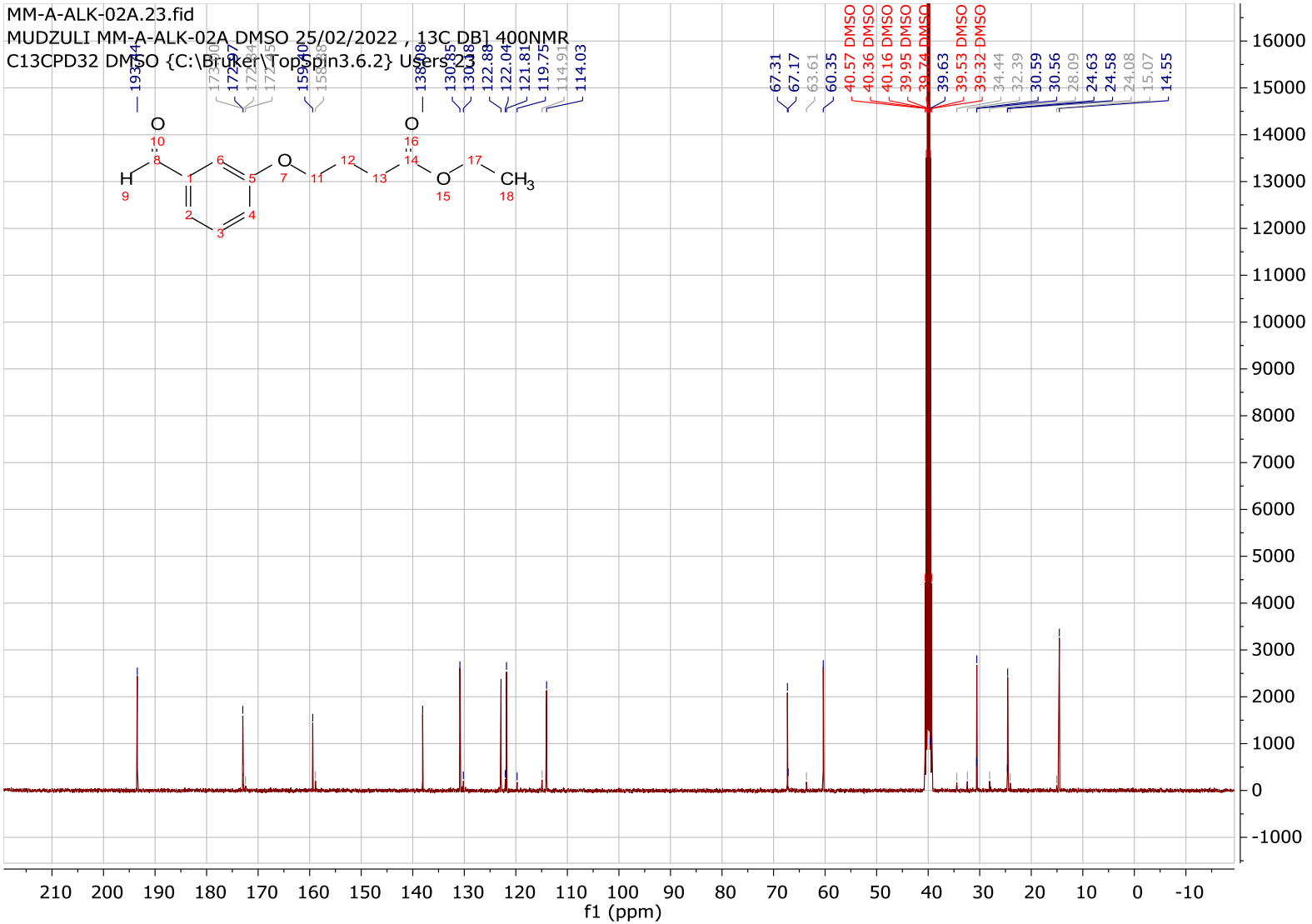


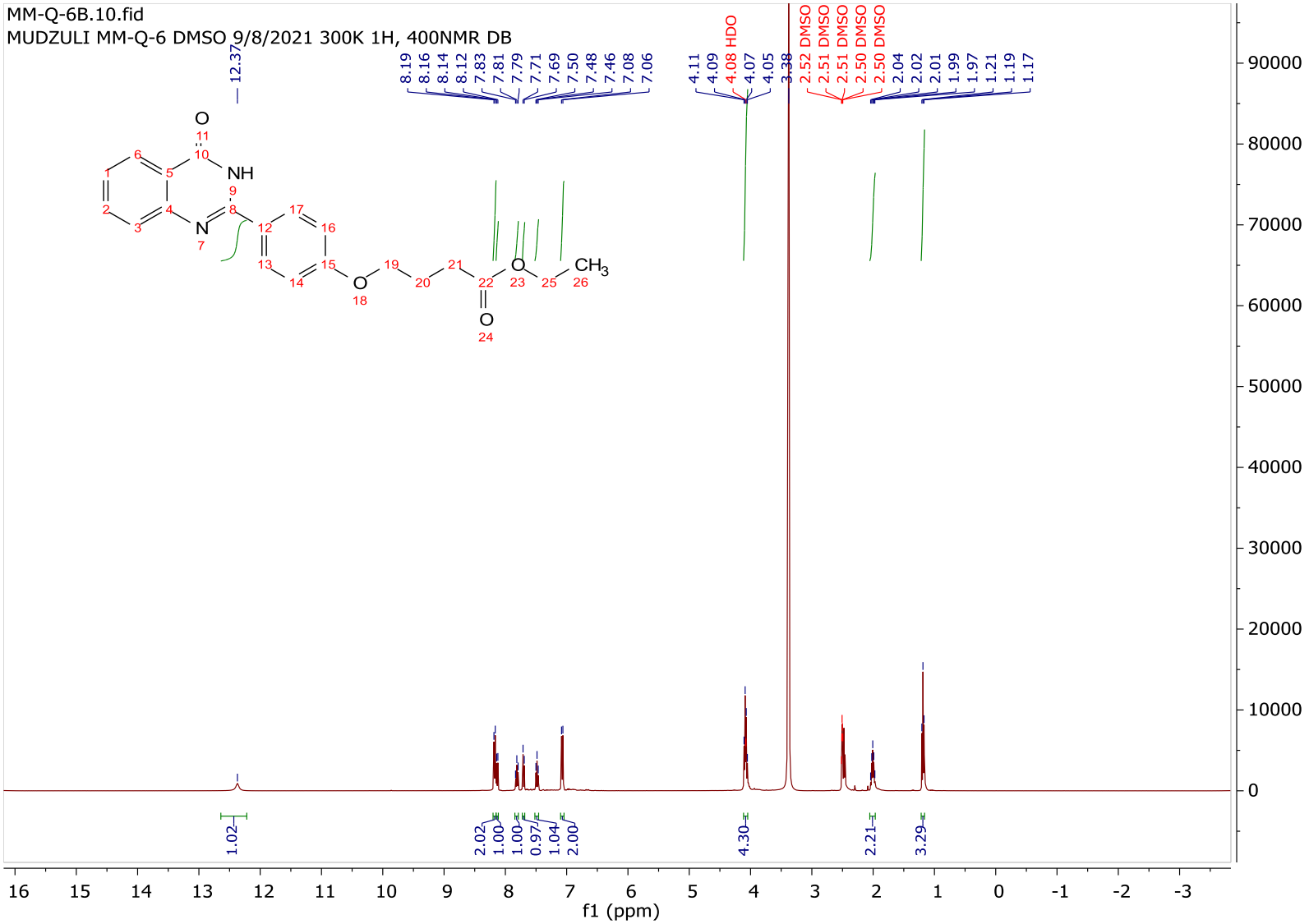
^{1}H NMR of Ethyl 4-(3-formylphenoxy)butanoate (27d)



^{13}C NMR of Ethyl 4-(3-formylphenoxy)butanoate (27d)



^{1}H NMR of Ethyl 4-(4-(4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanoate (29d)



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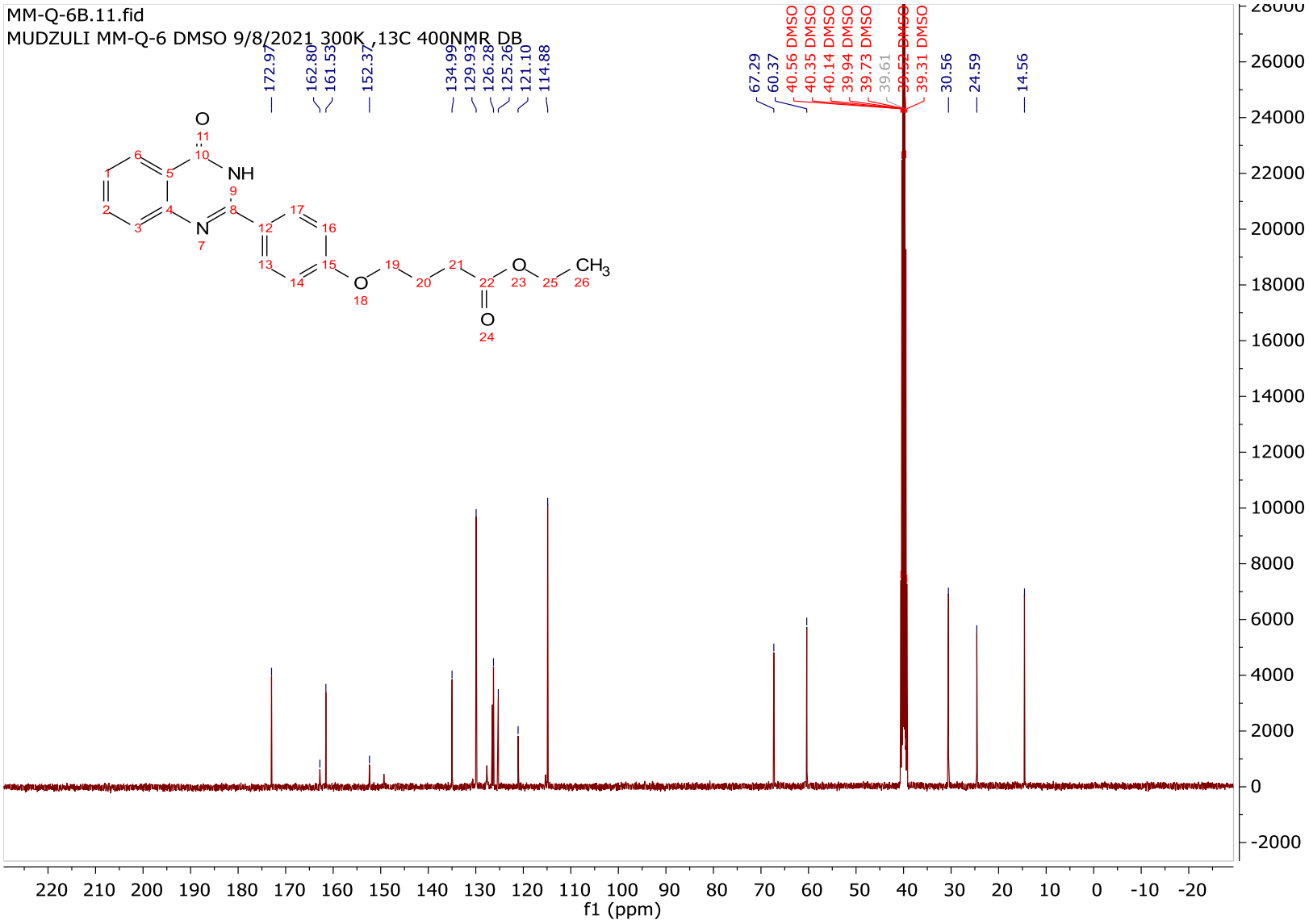

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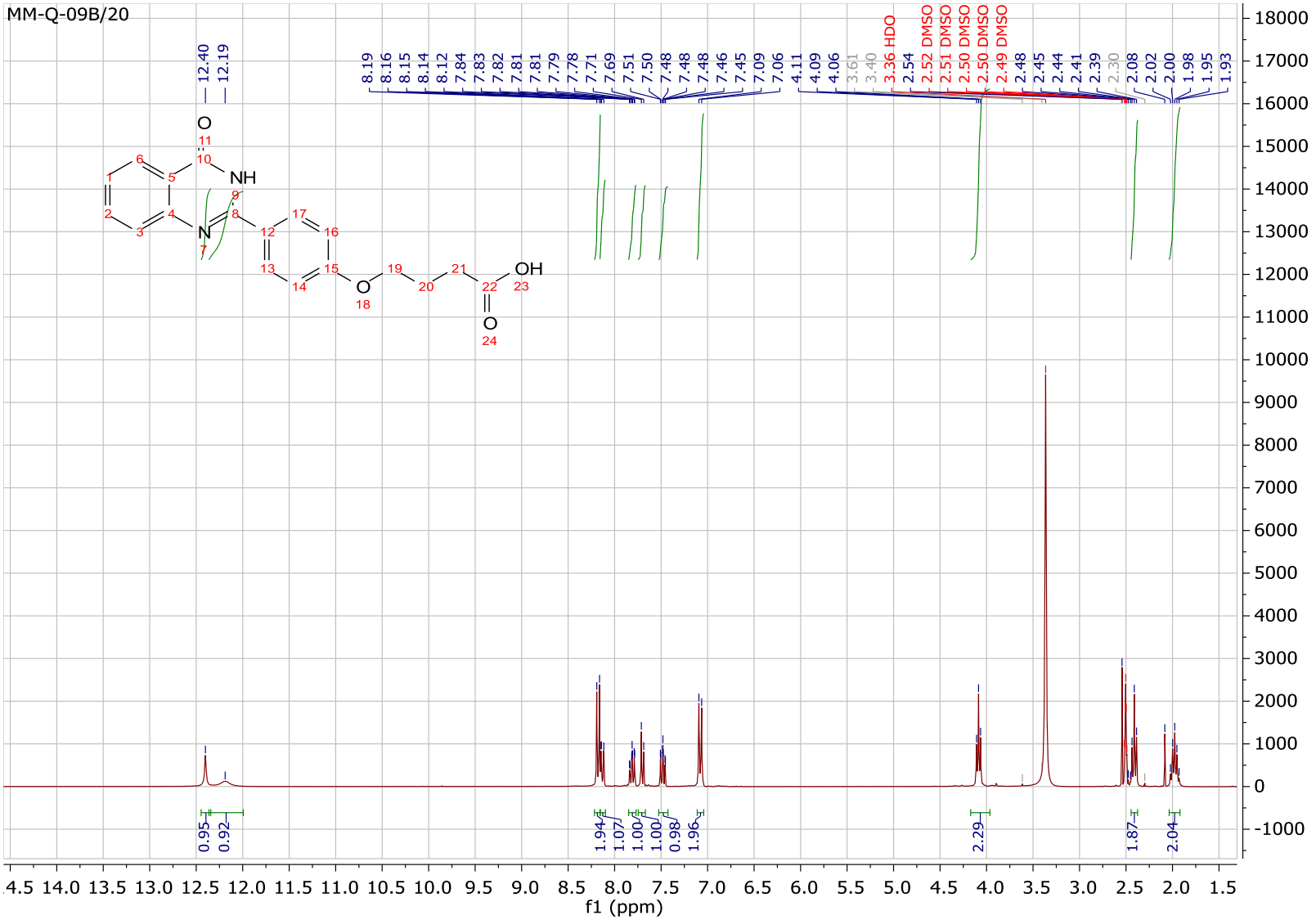

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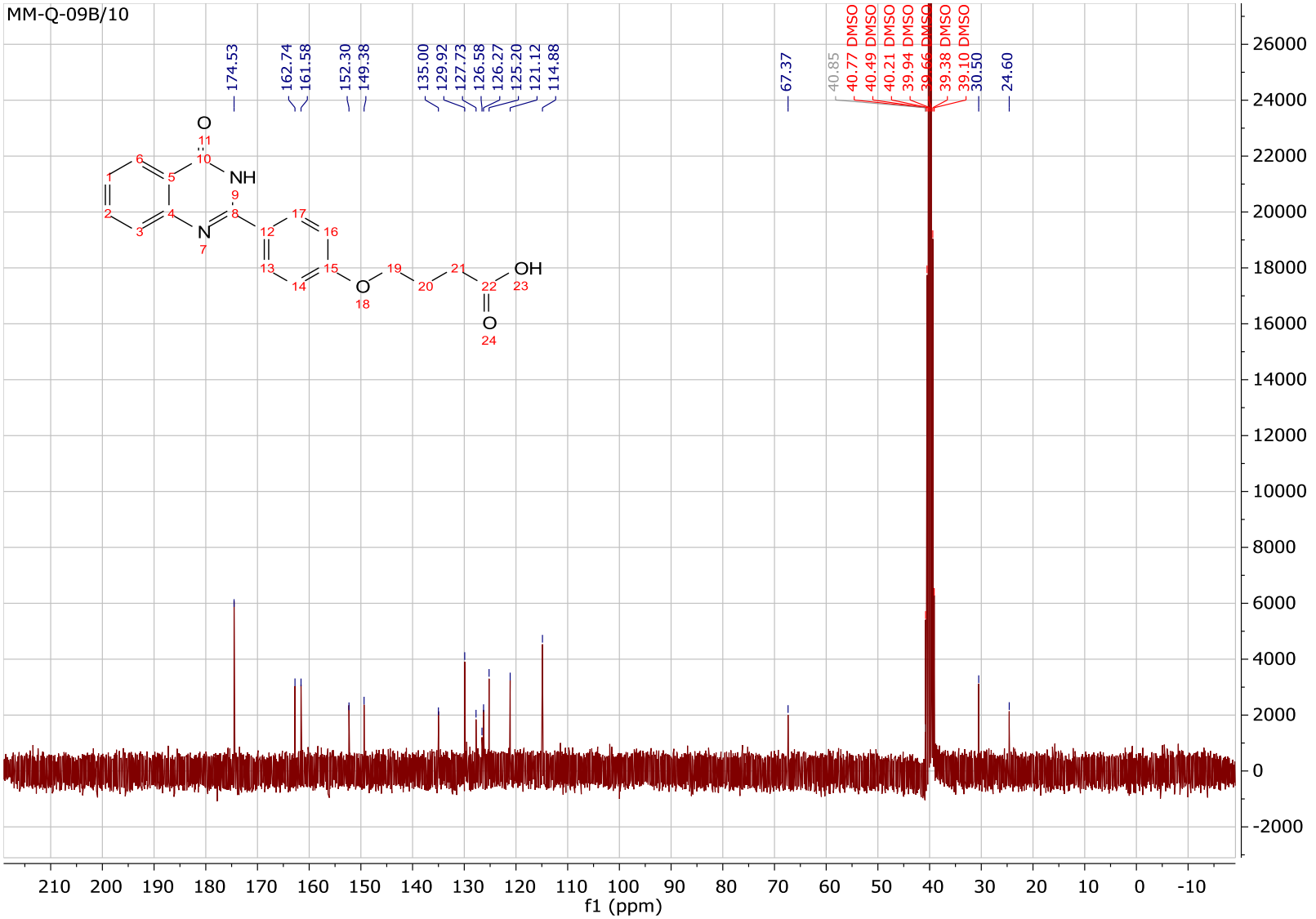
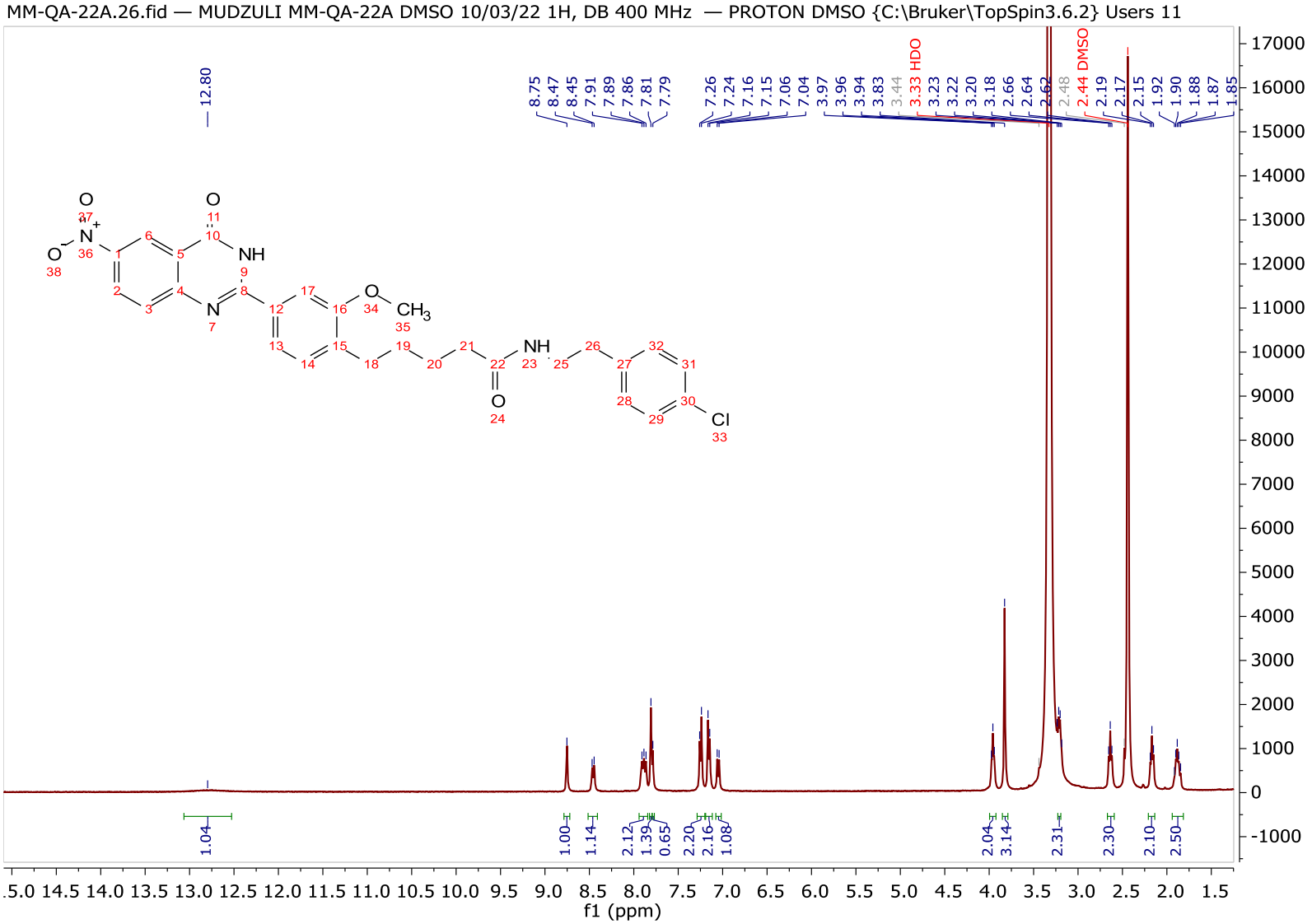
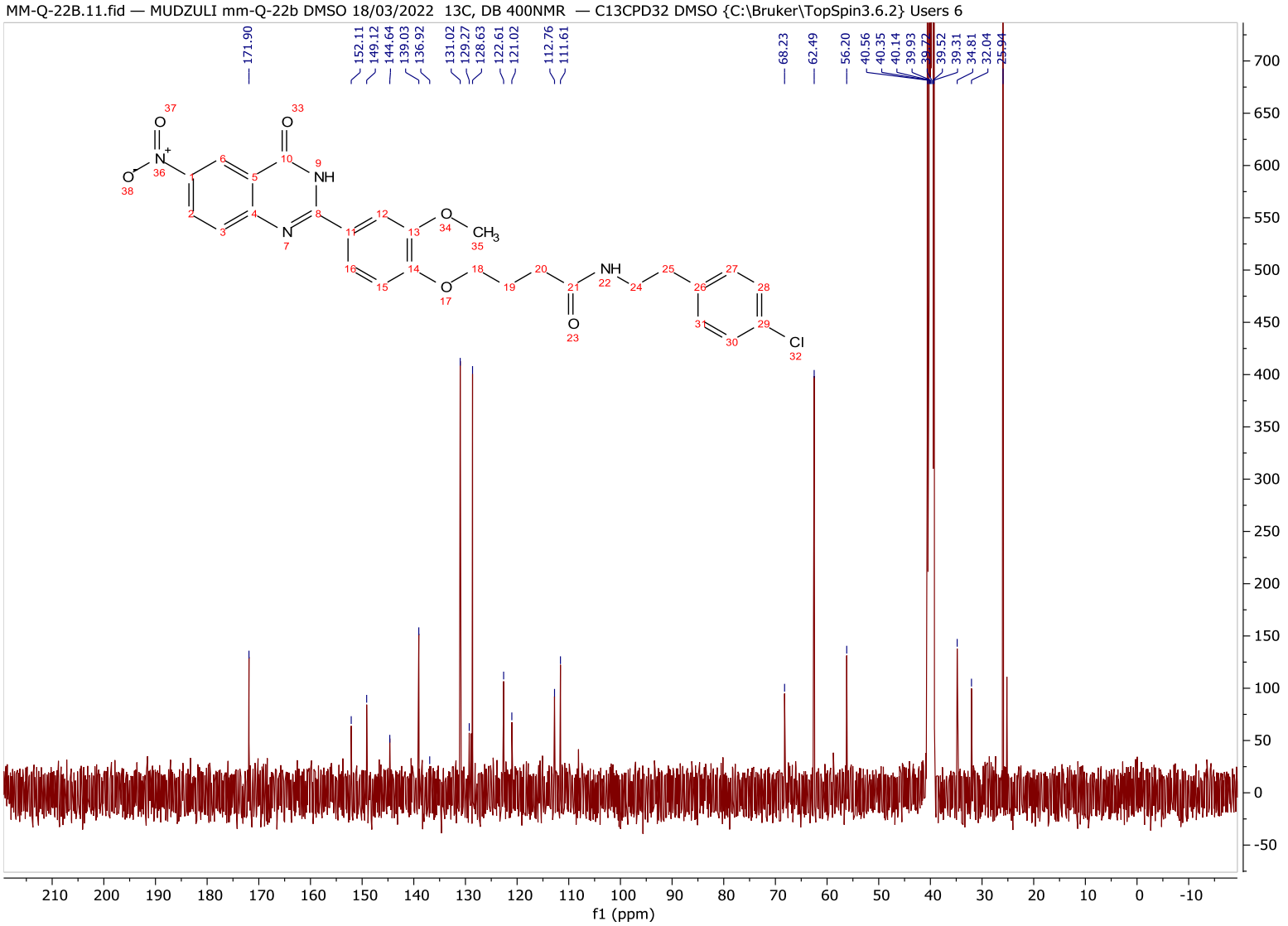


^{1}H NMR of *N*-(4-chlorophenethyl)-4-(2-methoxy-4-(6-nitro-4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanamide (32t)



^{13}C NMR of *N*-(4-chlorophenethyl)-4-(2-methoxy-4-(6-nitro-4-oxo-3,4-dihydroquinazolin-2-yl)phenoxy)butanamide (32t)