APPLICATION OF LACCASE ENZYMES IN ORGANIC SYNTHESIS

MUDZULI MAXWELL MAPHUPHA

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

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ABSTRACT

The use of enzymes as catalysts in various synthetic procedures appears to be an economical and profound way of providing selective processes in synthetic organic chemistry. Enzymes provide alternative and sustainable processes and have helped to avoid limitations encountered when using traditional heterogeneous and homogeneous catalysts; this includes the use of toxic substances, use of expensive heavy metals, extensive use of harmful organic solvents, harsh reaction conditions, and also poor selectivity of many catalysts.

Laccases are oxidoreductase enzymes capable of catalysing oxidation reactions of several low molecular weight organic compounds such as polyphenols, aminophenols, methoxyphenols, polyamines, and lignin-related molecules. The catalytic process of these enzymes occurs though a one-electron oxidation and water is released as the only by-product.

In this project we investigated the range and limitations of applications of laccase enzymes in organic synthesis. The project focus was on method development for cross-coupling reactions of Carbon, Nitrogen, Oxygen, and Sulphur substituted aromatic compounds. The laccase facilitated synthesis of five classes of compounds; biaryl compounds, benzoxazoles, benzimidazoles, benzothiazoles, and aminobenzoquinones, was investigated. The research explored the synthesis of biaryl compounds from simple substituted phenol substrates. The optimal reaction conditions for the synthesis of biaryl compounds from simple phenols were investigated. A condensation reaction between 2-aminophenol and aryl aldehyde derivatives was performed with the aim of synthesising 2-arylbenzoxazole derivatives; however various aminophenol derivatives were formed as the phenolic Schiff base failed to cyclise. Alternatively, when including the laccase-mediator ABTS, dimerization of 2-aminophenol to 2-amino-3H-phenoxazin-3-one (**4**) occurred.

A chemo-selective method for the synthesis of 2-aryl-1H-benzimidazoles from condensation of 2-phenelynediamine and aryl aldehydes was developed using laccase as an oxidising catalyst. Optimal

conditions for synthesising 2-aryl-1H-benzimidazoles were identified while using acetate buffer (0.1 M, pH 4.5), acetonitrile as a co-solvent and the commercial laccase preparation Novoprime base 268.

A modern and practical laccase-catalysed route suitable for the synthesis of 2-arylbenzothiazoles was developed. To the best of our knowledge, the laccase catalysed method for preparation of 2-arylbenzothiazole derivatives derived from condensation–dehydration reaction of 2-aminothiophenol with aryl-aldehydes has not been reported before. The method described is green, effective and simply requires a facile work-up routine, utilising solvents such as acetonitrile and DMF as co-solvents.

Finally, factors limiting yields for the synthesis of aminobenzoquinones were investigated by varying the reaction conditions. The laccase catalysed nuclear diamination of aromatic hydrobenzoquinones with aliphatic and aromatic amine molecules was investigated under mild reaction conditions using commercial laccases from Novozymes (Suberase®, Denilite® II Base, and Novoprime Base 268). Conducting the reactions under dilute conditions, sequential addition of enzyme and substrate over time and using Novoprime Base 268 as our laccase increased the yields to up to 100%.

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LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
DCM	Dichloromethane
DDQ	2,3-Dicloro-5,6-dicyano-1,4-benzoquinone
DMF	N,N-dimethylformamide
DMSO	Dimethyl Sulfoxide
EPR	Electron Paramagnetic Resonance
EtOAc	Ethyl acetate
GSTP1	Glutathione S-transferase P
HBT	N,N'-Bis-(1H-tetrazol-5-yl)-hydrazine
HRMS	High resolution mass spectroscopy
ILs	Ionic Liquids
IR	Infrared
KDa	Kilo Dalton
LTD4	Leukotriene D4
MDF	Medium-Density Fibreboards
MS	Mass Spectroscopy
NMR	Nuclear Magnetic Resonance
OLEDs	Organic Light Emitting Diodes
OMW	Olive Mill Wastewater

PARP	Poly Ribose Polymerase
PCC	Pyridinium Chlorochromate
SCD	Stearoyl-coenzyme A δ-9 desaturase
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
TLC	Thin Layer Chromatography

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Chapter 1

INTRODUCTION

1.1. General Introduction

In the present period, using enzymes to address modern challenges experienced in complex synthetic organic chemistry and biotechnology has proven valuable. Over the years, enzyme-catalysed reactions have become one of the alternate sustainable reaction processes and have helped to minimize the release of hazardous substances into the environment [1]. Laccases (benzenediol:oxygen oxidoreductases, EC1.10.3.2) are well recognized oxidoreductase enzymes belonging to the family of blue multi-copper-containing oxidases. These enzymes bear a distinctive redox ability to catalyse the oxidation reactions of several low molecular weight organic substrates utilising atmospheric oxygen as the electron acceptor in the process while releasing water as the by-product [2]. The substrates catalysed by laccases include a variety of organic and inorganic substrates such as polyphenols, aminophenols, methoxyphenols, polyamines, and lignin-related molecules and this is done through a process of one-electron oxidation [1, 3]. Because of their high level of selectivity and their effectual and environment friendly processes, laccases have been shown to have a large and increasing variety of industrial applications ranging from textile to the pulp and paper industries and from food industries to bioremediation processes [4, 5].

1.2. Distribution of Laccases in nature

In nature laccases are utilized in a number of biological processes. They carry out a variety of cellular processes in fungi; these include pigment production, sporulation and delignification [6]. The laccases commonly described in literature were isolated from fungi, but they have also been reported to be widely distributed in a range of many plants, insects and bacteria [2]. Reports indicate their presence in lichens [7]; oysters [8]; metagenome libraries of bovine rumen; and sponges [9]. In 1883 Yoshida first discovered laccase as an element of the resin ducts in the sap of the Japanese lacquer tree *Rhus vernicifera*, since then its biochemistry, structure, activity, and applications have been studied considerably [1, 4]. 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 [3]. It is believed that laccases play an important role in plant species as they are primarily involved in lignin formation and this occurs through a radical-based mechanism [2].

A few years after Yoshida's discovery of plant laccase, a French pharmacologist and bacteriologist Gabriel Bertrand discovered the first fungal laccase in 1896, and since then the majority of the laccases characterised to date have been isolated from fungi. The wood-rooting fungi are the most common laccase producers; more than 100 laccases isolated were fungal laccases and the laccase from wood-rotting fungi are one of the most frequently purified [1]. The sources of fungal laccase include ascomycetes, deuteromycetes, and basidiomycetes fungi, where the wood-rotting fungi *Trametes versicolor*, *T. hirsuta* (*C. hirsutus*), *T. ochracea*, *T. villosa*, *T. gallica*, *Cerrena maxima*, *Coriolopsis polyzona*, *Lentinus tigrinus*, and *Pleurotus eryngii* are the common laccase producers and also the most reported in literature [1-3]. In fungi, laccases carry out a variety of procedures such as degrading lignin molecules, mutagenic processes, stress defence, and also melanin synthesis [10, 11].

Laccase activity has been observed in bacteria including *Azospirillum lipoferum*, *Bacillus subtilis*, *Marinomonas mediterranea*, *Streptomyces griseus*, and *Escherichia coli* [2-3]. Their role in bacteria includes processes such as copper homeostasis in *Escherichia coli* [12], morphogenesis, and cell pigmentation [13].

More 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* [3, 4].

1.3. The structure of Laccase

Laccases are intra or extracellular glycosylated proteins which are often found as isozymes that oligomerize to form different protein complexes where less saccharide are found in bacterial and fungal enzymes than in plant enzymes [1, 3]. Normally the monosaccharides found in the carbohydrate polymers include hexoamines, glucose, arabinose, galactose, and fucose [3]. The average molecular weight observed for the glycosylated protein monomers is usually 50-130 KDa [2]. It is believed that glycosylation serves an important role in the molecular structure of laccase as it is responsible for the

stability of the enzyme. In addition it contributes towards proteolytic susceptibility, copper retention, and protection against enzyme inactivation by free radicals, and secretion [2, 3].

Laccase in its purified form generally exhibits a characteristic blue colour; this is due the extreme electronic absorption of the Cu-Cu linkages [4]. The active site of laccase is composed of four copper atoms which are classified into three redox sites (T1, T2 and T3) using spectroscopic techniques such as UV/Vis and electron paramagnetic resonance (EPR) [2-3].

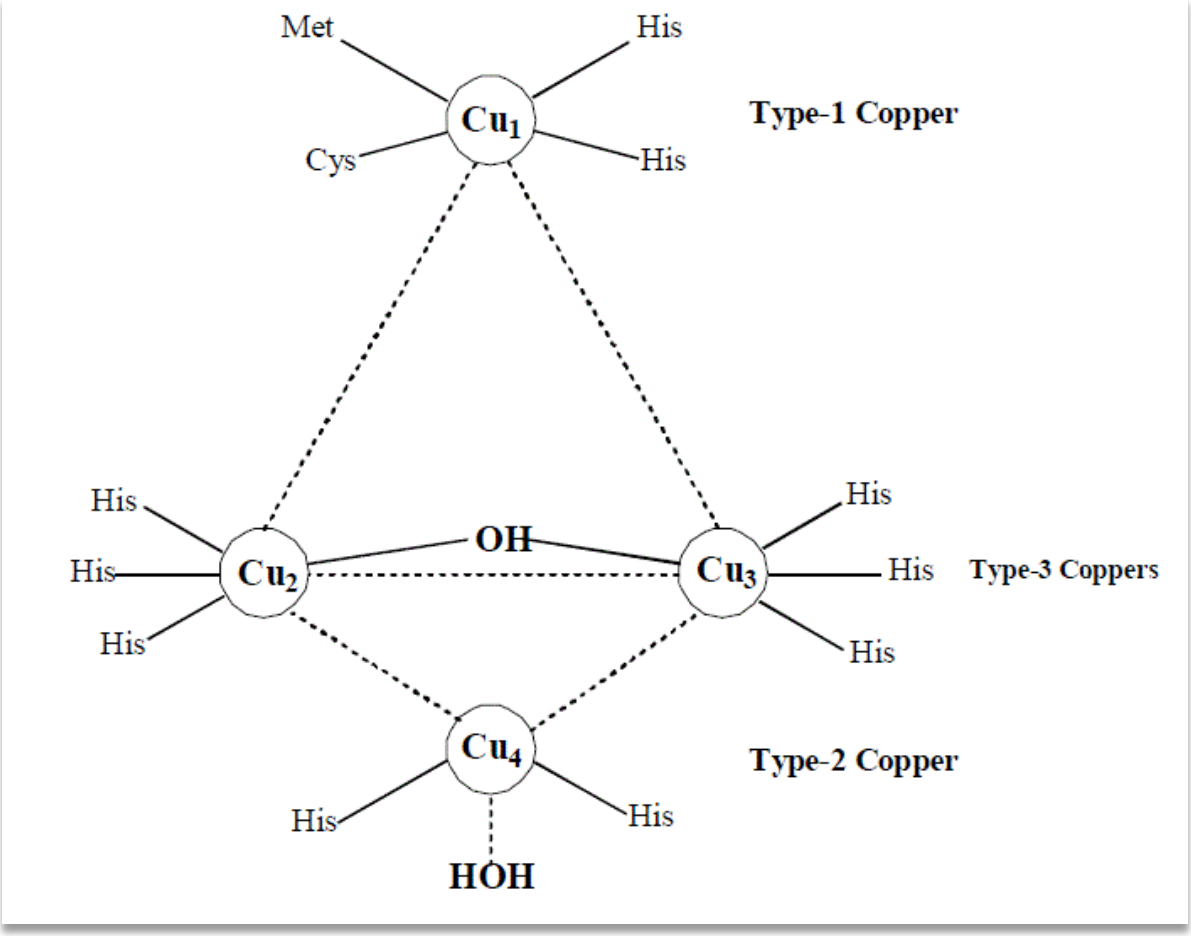


Figure 1: Laccase CotA Active site from *Bacillus subtilis* [1].

The type 1 copper atom (T1) gives the enzyme its typical intense blue colour due to its strong electronic absorption at 600 nm and it is detectable by EPR spectroscopy [3]. The observed strong absorption of the T1 copper atom is mainly associated with the copper-cysteine bond since the T1

copper forms some kind of a trigonal coordination with a single cysteine and two histidine ligands. In fungal laccases the axial ligand(s) is phenylalanine or leucine and in bacterial laccases (CotA) it is methionine [2]. Copper of the first type has been found to be the site with a high redox potential of about +740 mV, hence it is the site where oxidation of the substrate takes place [2, 3]. Copper of the second type does not exhibit any colour; shows weak absorption in the visible region but it can be detected by EPR. On the other hand copper of the third type is EPR undetectable and this is because it is comprised of two copper atoms which exhibit a form of anti-ferromagnetism due to the bridging hydroxyl ligand coupling them, hence they are diamagnetic and cannot be detected on EPR [2].

Together the type two (T2) and the type three (T3) copper atoms form what is known as a tri-nuclear cluster; which appears to be the site where atmospheric oxygen binds and four-electron reduction to water occurs [2, 3]. Studies show that the type 2 copper site is bound to two nitrogen atoms (histidines) and in the type 3 site copper atoms are coordinate with six histidine ligands [2]. The type 2 copper centre is believed to provide an important structural purpose of stabilizing the type 3 copper centre and other studies show that the methionine ligand help to stabilize the type 1 copper centre [3].

1.4. Mode of action of laccases

The enzyme catalysis of laccases takes place through the attack of phenolic compounds resulting in oxidation of alpha carbon, cleavage of aryl-aryl and alpha-beta bonds [14]. The mechanism leads by the reduction of one molecule of atmospheric oxygen to water, followed by a one electron oxidation of the substrate [15]. As a consequence of this oxidation an oxygen-centered free radical is produced, which is generally unstable and more likely to undergo a second enzymatic catalysis reaction to be converted into a quinone molecule [15, 6]. In the laccase catalytic mechanism there are three key steps that have been identified which are as follows [2]:

(i) Copper of first type (T1) is reduced by the substrate,

(ii) Electron transfer from T1 copper site to the tri-nuclear site (T2&T3), and

(iii) Reduction of molecular oxygen by the tri-nuclear centre.

The electron transfer from the substrate to the tri-nuclear cluster is carried out at the T1 copper site which is a distance of about 13Å from the tri-nuclear cluster. The laccase catalytic mechanism occurs at the copper sites, where atmospheric oxygen molecule is reduced into water through a four-electron reduction process [2]. When the completely reduced tri-nuclear cluster (T2&T3) interacts with molecular oxygen two intermediates are formed; which are identified as peroxide intermediate and native intermediate. 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 raptures into the native intermediate. The latter is believed to serve a significant purpose in the catalytic cycle of the enzyme by acting as oxygen radical throughout the reaction with molecular oxygen [2, 16].

1.5. Laccase Mediator systems

Laccases have a broad spectrum of substrate specificity and they play an important biological function in the biodegradation of lignin; however these green catalysts appear to possess a lower redox potential compared to other lignin degrading ligninolytic fungi (ligninolytic peroxidases) and therefore their oxidation capabilities are restricted to phenolic related compounds [17]. This implies that other non-phenolic substrates possessing a higher redox potential cannot be oxidized by these enzymes.

Nevertheless when the enzyme is applied together with what is called a laccase-mediator; one can overcome this obstacle and increase the oxidation aptitude of the enzyme; leading to the oxidation of nonphenolic lignin moiety substrates [3, 17]. Using laccases in the presence of a low molecular weight compound as redox mediators can potentially expand the substrate specificity of the enzyme and enhance its redox capacity towards obstinately uncooperative compounds. High molecular weight and bulky compounds such as lignin polymers usually don't interact effectively with the laccase's active site due to steric hindrance. Therefore the redox mediator plays a role of shuttling electrons; providing the oxidation of more complex compounds [17]. The idea behind using mediator systems was to utilise low molecular weight compounds that, when oxidised by the enzyme, can generate stable radical species and diffuse out of the enzymatic pocket allowing the enzyme to oxidize the high oxidation target compound without deactivating the enzyme itself [3]. Laccase mediator systems also turn out to be economical and environmentally friendly; hence they are utilized in environmental and industrial

applications where they are used in the oxidation of aromatic methyl groups, bleaching of textile dyes, oxidation of benzyl alcohols and polycyclic aromatic compounds [3, 17].

1.6. Applications of Laccases in Industries

There is a continuous growing chain of research for the use of laccases for various industrial applications [9]. Laccases have been applied in numerous applications such as paint, furniture, food, pulp and paper, textile, cosmetic, biofuel, and nanobiotechnology related industries [2, 9].

1.6.1. Paint Industry

The frequently used binding agents in the paints and coatings industry are polyester compounds consisting of unsaturated fatty acids known as alkyd resins [18]. Alkyd resins are usually prepared through a polymerization reaction of polyalcohols, dicarboxylic acids or anhydrides and unsaturated fatty acids and chemically dried through cross-linking of the unsaturated fatty acid scaffold catalysed by transition metal inorganic complexes [9, 18]. In search for efficient, less toxic and environmental friendly alternative catalysts, laccase-mediated systems were used to cross-link the alkyd resins as a replacement for transition metal catalysts [18].

1.6.2. Furniture Industry

In the furniture industry, laccases-mediated systems have been used in the production process of medium-density fibreboards (MDF) [9]. Traditionally MDFs are prepared by cross-linking lignocellulosic fibres melded with a synthetic resin in the presence of moisture under high temperatures and pressure [9, 19]. Formaldehyde is conventionally used as a binding agent for gluing lignocellulosic products; however for ecological and economic reasons alternative binding agents such as laccase-mediated systems have been employed for activating lignin on the fibre surfaces of wood during the pilot-scale production of MDF in a dry process [9, 20]. The boards produced using laccase mediated systems had comparable mechanical properties to the conventional methods and met all expected European standards for wood-based panels [20].

1.6.3. Food Industry

In the food industry laccases are utilized because of their ability to promote both homo- and hetero-polymerisation processes [9]. They are mainly used for stabilisation of wine and beer, fruit juice

processing, betterment of quality of various drinks, modifying colours of food or beverages, baking, deoxygenation of perishable oil products, and waste water treatment [2, 9]. Wines, beers, and fruit juices contain a high concentration of phenolic compounds; laccases can be used to measure the amount of phenolic compounds present in the drinks in the form of biosensor [2]. A number of laccase based biosensors have been developed to detect the presence of polyphenols in drinks such as tea and wine [21, 22]. On the other hand, commercial laccase Suberase® from Novozymes has been utilized in the wine industry for cleaning and polymerization of phenols (2,4,6-trichloroanisole) found in cork stoppers in bottled wine; as these compounds are known to add an unpleasant flavour to the wine giving it a bitter taste [2]. In the baking industry, laccase can be used to control rheological properties of dough; white-rot fungus laccase *Trametes hirsuta* was used to increase resistance of dough and reduced dough extensibility in flour and gluten dough through cross-linking of esterified ferulic acid on arabinoxylan fraction of dough [2, 23]. Laccases are also applied in bioremediation processes for waste water treatment [2]. Various laccases have been used for the removal of phenols in waste-water; this include: laccase from *Cerrenaunicolor* used for treatment of olive mill wastewater (OMW) in the oil industry [24], fungal laccase (*Trametes pubescens*) phenolic compounds and colour for the treatment of distillery wastewater from fermentation of sugarcane molasses for the production of ethanol [2].

1.6.4. Pulp and Paper Industry

In the pulp and paper industry, separating lignin from wood pulp is a very important process [2, 9]. The conventional way of doing it is through what is known as the "bleaching" process; which involves the use of chlorine-containing reagents which are harmful to the environment [2, 25]. Because laccases have been described as one of the enzymes responsible for wood decay by white rot fungi in nature; therefore they can be applied in delignification of wood fibre processes in the pulp and paper industry [2]. Applications of laccase-mediator-systems (Lignozym®-process) was described as an effective method to delignify wood in pulp and paper processes using mediators containing groups such as NO, NOH or HRNOH [26]. Laccase from *Pycnoporus cinnabarinus* was used along with various natural mediators (phenols syringaldehyde, *p*-coumaric acid, and acetosyringone) to bleach flax fibres [27].

1.6.5. Textile Industry

Laccases have been shown to have a large and increasing variety of applications in the textile industry ranging from decolourization of dyeing effluent to bleaching of denims [28, 29]. The ability of laccase to oxidize a variety of phenolic and non-phenolic aromatic organic compounds can be utilized to promote homo and/or hetero-coupling reactions producing a range of characteristic colours for different valuable dyes for textiles [9, 30]. Laccases catalysed the oxidative coupling reaction of colourless 1,2-dihydroxybenzene and 2,5-diaminobenzenesulfonic acid to generate a polymeric dye, which was then used to dye cotton fabrics [30]. CotA laccase from *Bacillus subtilis* was used to catalyse the oxidative dimerization of *ortho, meta*, and *para*-disubstituted aromatic amines to yield substituted phenazine and phenoxazinone dyes [31]. An enzymatic method for grafting water insoluble phenolic compounds such as lauryl gallate found on wool fabric was developed using laccase as a biocatalyst [32].

1.6.6. Cosmetic Industry

In the cosmetic industry laccases are being explored in personal care products for both skin and hair products [2, 9]. However, they are mainly used as oxidants and bleaching agents for hair; as a replacement for hydrogen peroxide [2, 33]. Compared to peroxide hair dyes, laccase-based dyes are preferable as they are less harsh and also easy to handle [2, 34]. There are also reports on laccases being applied in dermatological products containing proteins for skin lightening [2, 9].

1.6.7. Biofuel Industry

Natural, abundant and renewable resources such as lignocellulosic biomass are one of the important feedstocks for the production of bioethanol [9]. Bioethanol is composed of carbohydrate polymers and an aromatic polymer (lignin), and in order to utilise it, lignin must be removed to expose sugars to efficiently hydrolyse the polysaccharides [35]. Laccase is one of the biocatalysts investigated for its application mainly as a delignifying agent in biofuel production, and also as a tool for removal of fermentation inhibitors (mainly phenolic compounds) that may arise from subsequent enzymatic processes [2, 35]. A laccase from *T. versicolor* was used for detoxification of lignocellulose to effectively remove phenolic compounds for the production of bioethanol [2, 36]. A recent study presented laccases from *Ganoderma lucidum* as a great tool for processing of lignocellulose for bioethanol

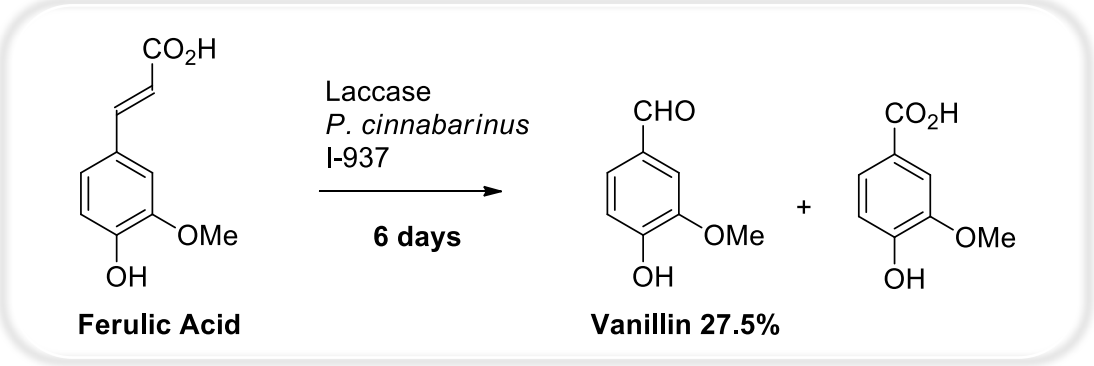
production. This laccase was able to remove up to 84% of phenolic compounds present in corn stover hydrolysate, and when added prior the cellulase hydrolysis step, ethanol productions were improved by 10% [37].

1.7. Laccases in organic synthesis

Laccases have proven useful in Green Chemistry as biocatalysts for organic transformations in a variety of bond formation reactions (C-C, C-O, C-N, and C-S) under mild and environmentally benign reaction conditions [2, 8]. Ranging from detoxification of phenolic pollutants, the transformation of antibiotics, to the dimerization of steroid hormone 17β-estradiol; laccases have been used to synthesize stable complex pharmaceutical products [2, 38].

1.7.1. Oxidative decomposition

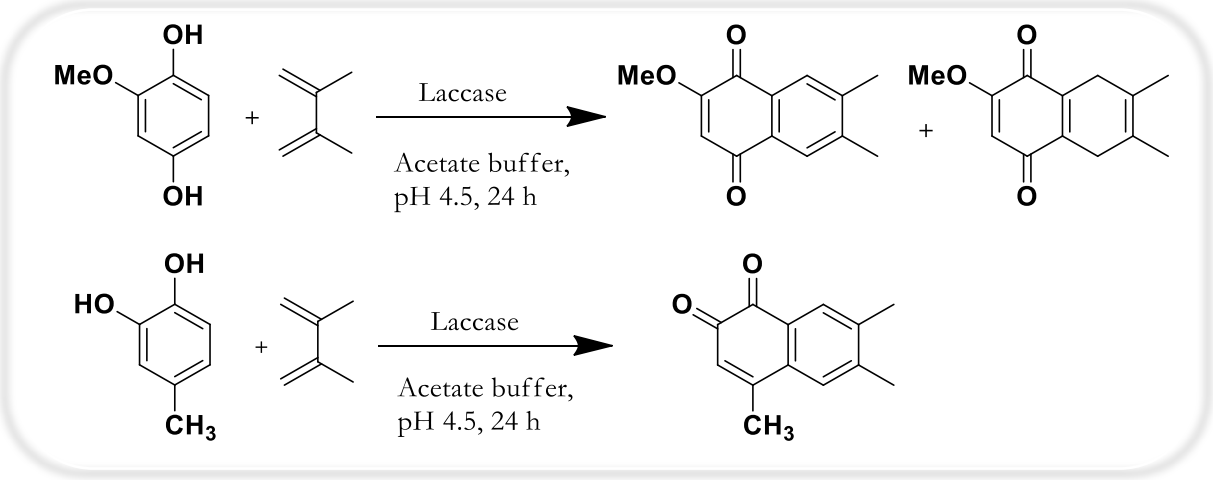
White rot fungus, *Trametes sp.* in a medium containing glucose and ethanol under aerobic conditions was used to convert ferulic acid into coniferyl alcohol [39]. The oxidative decomposition of ferulic acid in those conditions yielded other products along with coniferyl alcohol such as dihydroconiferyl alcohol, coniferylaldehyde, vanillyl alcohol, vanillic acid, 2-methoxyhydroquinone and 2-methoxyquinone [2, 39]. Years later another oxidative metabolism of ferulic acid was reported, where laccase from white-rot fungus *Pycnoporus cinnabarinus* I-937 was used to transform ferulic acid into vanillin (**Scheme 1**) [2, 40].



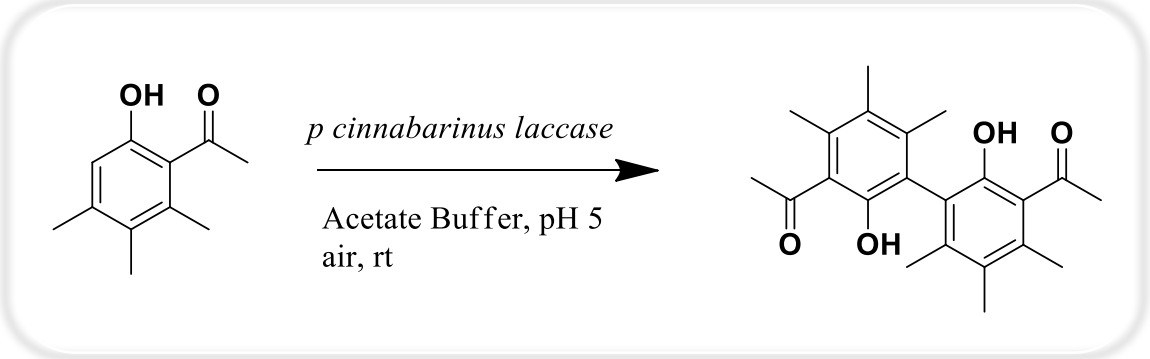
Scheme 1: A biotransformation of ferulic acid by laccase [2]

1.7.2. Dimerisation and Trimerisation (C-C bond formation)

All organic molecules consist of a carbon-carbon bond skeleton; consequently the understanding of carbon-carbon bond formation reactions is a key step in synthetic organic chemistry. Only a few biocatalysis routes have been accounted for in literature for the synthesis of C-C bonds [40]. However there are a few reports on laccase enzymes utilized as biocatalysts for C-C bond formation reactions. Examples of novel C-C bond formation reactions include intermolecular Diels-alder reactions between dienes and quinones to form *o*- and *p*-naphthaquinones catalysed by laccase from *Trametes villosa*; the reactions were conducted at 70°C, 3°C and room temperature in an open vessel using an acetate buffer (pH 4.5) (**Scheme 2**) [41]. Laccase-catalysed oxidative dimerization (C-C coupling) of salicylic esters under aerobic conditions at room temperature was also achieved (**Scheme 3**) [42].

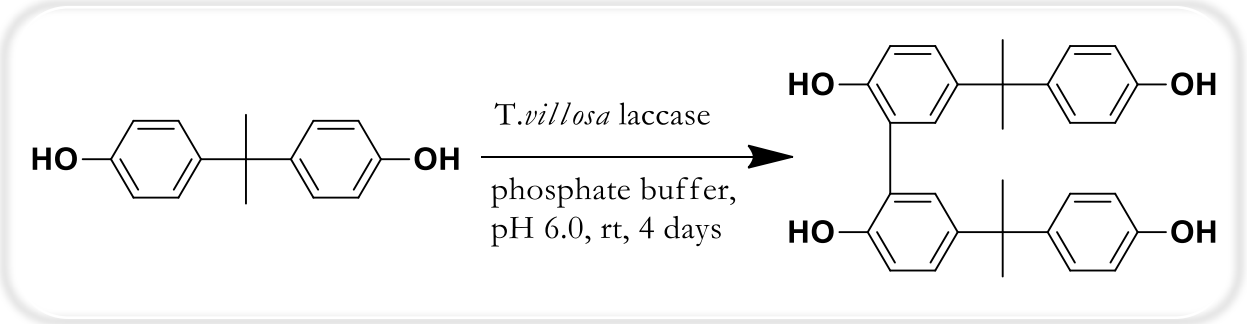


Scheme 2: Laccase catalysed synthesis of naphthaquinones Diel-Alder reactions [2]



Scheme 3: Laccase catalysed self-coupling reactions of salicylic ester [42]

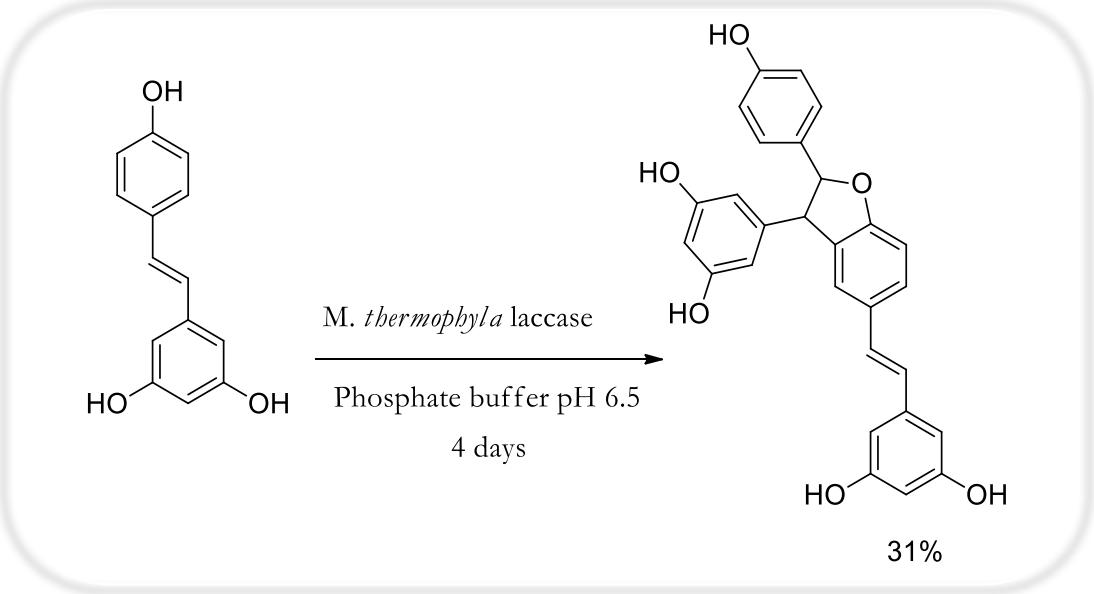
Another report shows the synthesis of dispiropyrimidinone derivatives via domino reactions catalysed by laccase from *A. bisporus* using a phosphate buffer (pH 6) at room temperature and the yields obtained were between 77% and 90% [43]. A number of functionalised biaryls have been synthesised through laccase-catalysed self-coupling reactions such as the biotransformation of 5,6,7,8-tetrahydronaphthalen-2-ol were the effect of organic solvents on product formation was investigated using various aromatic solvents such as benzene and toluene [44]. Another laccase-catalysed C-C bond formation dimerization reaction was reported on a self-coupling reaction of bisphenol; the reaction was conducted in a phosphate buffer (pH 6) at room temperature using laccase from *T. villosa* (**Scheme 4**) [45].



Scheme 4: Laccase-catalysed self-coupling reaction of bisphenol [46]

Depending on the type of substrate, solvent or co-solvent used, and also the type of laccase source; formation of dimeric and/or trimeric products is highly affected [46]. Tranchimand *et al.* (2006) reported the effectiveness of using biphasic solvent systems during the laccase catalysed bio-transformation to form liganans from ferulic acids and sinapinic acids [47]. The products obtained from biphasic systems were more stable and in greater yields and they were easy to separate with good product selectivity [46, 47]. Other studies show the influence of the type of laccase source on the formation and ratio of dimer products; where two laccases, (*Pycnoporus coccineus* (fungus) and *Rhus vernicifera* Stokes (tree)) were used in the biocatalytic oxidation of isoeugenol and coniferyl alcohol. The reactions were conducted in acetone-water system and it was found that the oxidation of the substrates by *P. coccineus* laccase was faster than that of the *R. vernicifera* Stokes laccase [46, 48]. Another report on the influence of laccase source towards yield of reaction products was on the dimerization 3,5,4'-trihydroxystilbene catalysed by laccases from *Myceliophtora thermophyla* and *Trametes pubescens* in *n*-BuOH saturated with phosphate buffer (pH 6.5) [49]. The product yield obtained using *M. thermophyla* laccase

(**Scheme 5**) was significantly higher (31%) than that obtained from the oxidation by the *T. pubescens* laccase (18% yield) [2, 49].

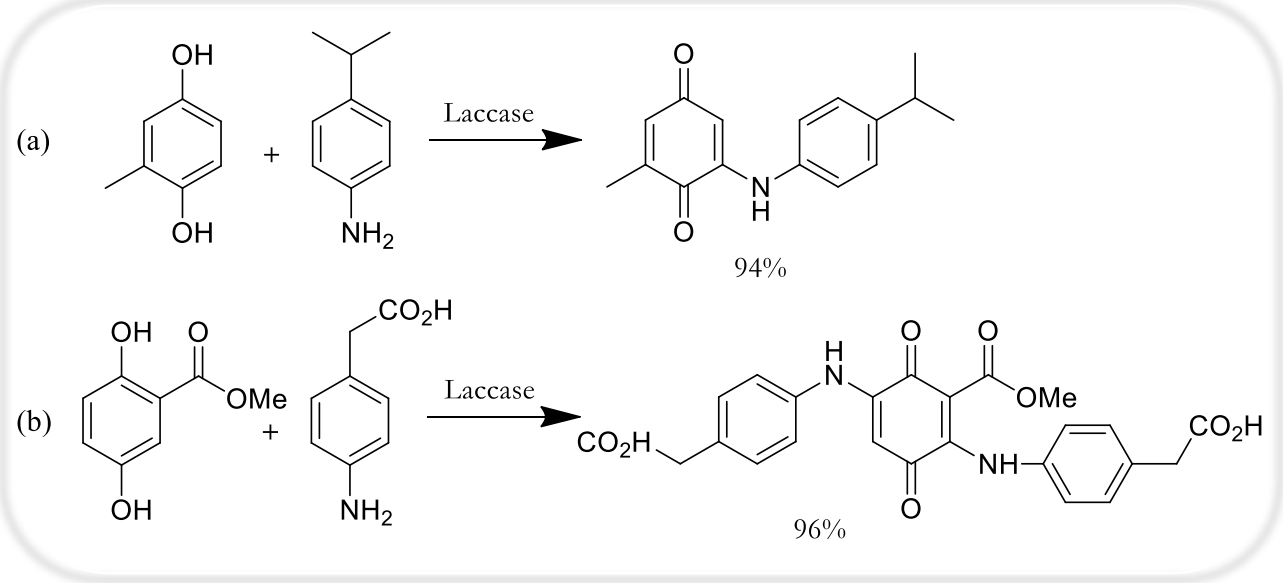


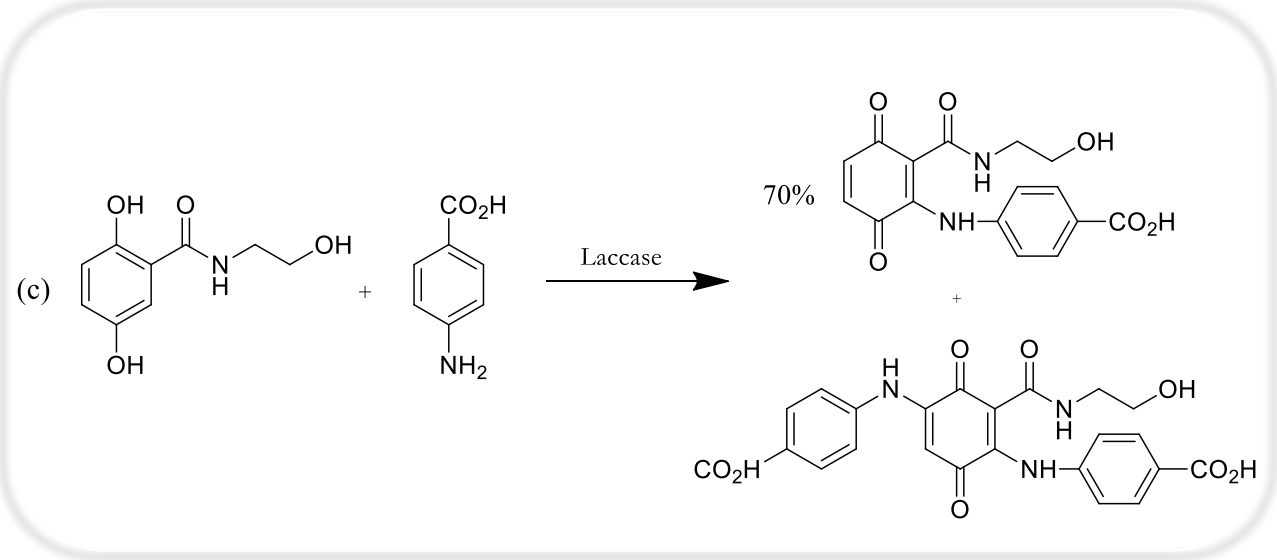
Scheme 5: Laccase-catalysed dimerization of 3,5,4'-trihydroxystilbene [46]

There is a number of studies reported in the literature that have investigated how laccase mediators play a role in laccase reactivity in organic synthesis and it was demonstrated that they can improve reaction yields and selectivity [46]. Ganachaud *et al.* (2008) demonstrated this effect while investigating the laccase-catalysed trimerisation of indole to afford 2,2-bis(3'-indolyl)indoxyl as one the products [50]. The mediators explored were ABTS, TEMPO, and HBT and it was found that TEMPO was the most efficient; reducing reaction times from 48 to 24 h and improved the yield from 23% to 51% [46, 50].

1.7.3. Nuclear amination (C-N bond formation)

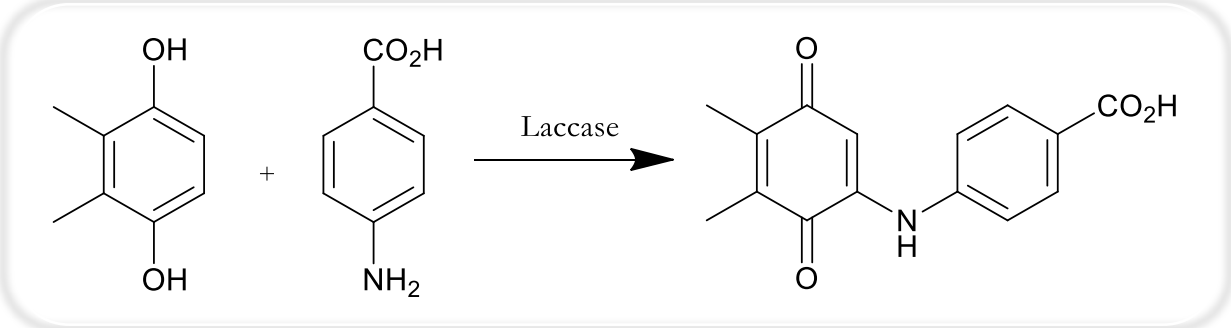
Laccases in amination reactions are becoming one of the most important synthetic routes to complex organic molecules. The use of these enzymes to catalyse C-N bond cross-coupling reactions represents an effective procedure for the synthesis of significant and important (C-N) biological molecules in mild reaction conditions [51]. There have been various novel examples of laccase-catalysed amination reactions reported in literature and amongst the products synthesised are novel antibiotics [51, 52]. Since 2005 the synthesis and development of aminoquinone molecules has been studied using fungal laccases [2]. Depending on reaction conditions such as molar ratio of substrates, solvents and co-solvents, type of buffer system used, concentration of laccase, reaction time, temperature, and pH of solution; the formation of mono- or di-aminobenzoquinones products was extremely influenced. It has been reported that different laccases tend to show different reaction courses and good to very good yields of aminoquinone products can be obtained using alkylated quinones and primary aromatic amines [53]. Niedermeyer *et al.* (2005), conducted a study on the formation of mono-aminated and di-aminated benzoquinones using two fungal laccases, and one of the findings was 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 6**) [2, 53].



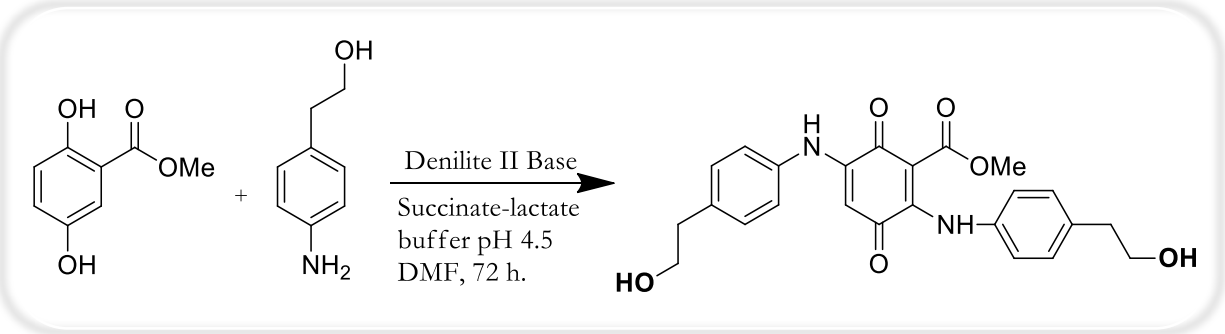


Scheme 6: (a) Monoamination (b) Diamination (c) Mono- and di-amination of *p*-hydroquinone catalysed by laccase [2]

In 2007 there was another study on laccase ability to initiate nuclear amination of *para*-hydroquinones with primary amines using laccase from *Myceliophthora thermophila* and laccase C from *Trametes sp.* for the formation of monoaminated and diaminated quinone products (**Scheme 7**) [2, 54]. Another study on nuclear deamination on *p*-hydroquinones with aliphatic and aromatic amine substrates was conducted using commercial laccase Denilite® II base from Novozymes. The reactions were done at room temperature and at 35 °C in the presence of an immobilized laccase, succinate-laccase buffer (pH 4.5), and a co-solvent to afford aminated *p*-benzoquinone products. It was observed that regardless of the reaction conditions, the reactions favoured the formation of diaminated products over monoaminated products but the products obtained were in very low yields (**Scheme 8**) [2, 55].



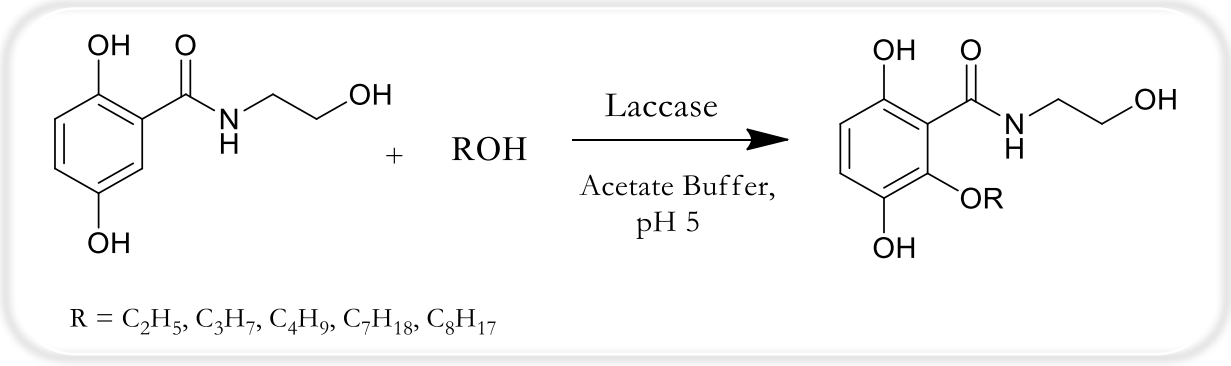
Scheme 7: Monoamination of *p*-hydroquinone with primary amine catalysed by laccase [2]



Scheme 8: Diamination of *p*-hydroquinone with primary amine catalysed by laccase [2]

1.7.4. Oxidative Coupling (C-O bond formation)

Fungal laccases have been used to catalyse carbon-oxygen bond formation through C-O bond cross-coupling reactions of dihydroxylated aromatic substrates with aliphatic alcohols under mild reaction conditions. It has been proven that fungal laccase can catalyse the cross coupling reaction between methanol and other alcohols with dihydroxylated aromatic compounds [56]. A fungal laccase from *T. villosa* obtained from Novo Nordisk (Bagsvaerd, Denmark) was used to catalyse the cross-coupling reaction of dihydroxylated aromatic substrates with alcohols in an acetate buffer (pH 5) to form homomolecular and heteromolecular products (**Scheme 9**) [2, 56].



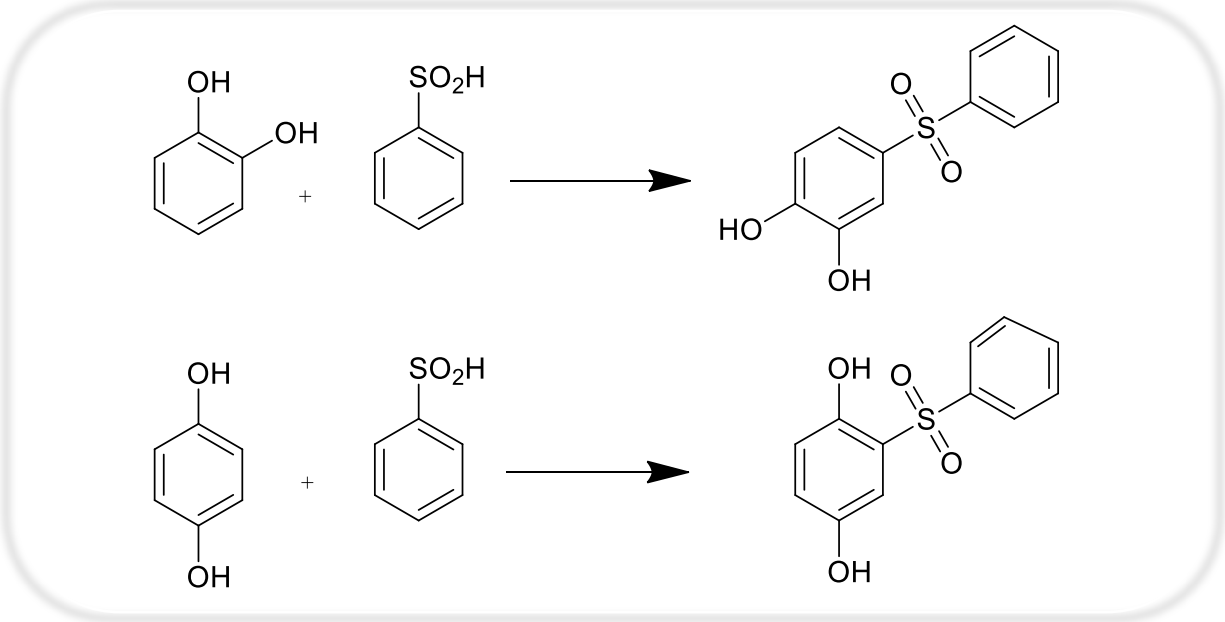
Scheme 9: Laccase-catalysed C-O bond cross-coupling reaction [2]

However oxidative coupling of phenolic substrates carried out in the presence of a solvent, an alcohol or in aqueous solution may afford a couple of undesired side reactions as these reactions tend to form homo-molecular hybrid molecules such as dimers and trimers and also the fact that aromatic and aliphatic alcohols can be candidates of laccase catalysis [56, 57]. As a result of the formation of homo-molecular products during the synthesis processes of these cross-coupling reactions the yields obtained

for the desired heteromolecular products are affected to a large extent [56]. This is evident that there is a need to develop optimal conditions for these reactions in order to minimize the formation of side products such as homomolecular products as well as the occurrence of heteromolecular coupling between solvent systems and laccase substrates leading to undesired product formation.

1.7.5. Thio-bond formation (C-S bond formation)

At this point in time, there is a limited number of reports in literature on the laccase-catalysed C-S bond formation reactions [2]. Although these compounds possess a number of biochemical and biotechnological benefits, the amount of research towards this kind of synthesis has not been addressed to a greater extent. Regardless of the extent of research; a couple of researchers have reported on a number of synthesis of C-S bond formation reactions, these include the laccase-catalysed reaction of *p*-hydroquinones with aromatic thiols through 1,4-Michael addition reaction to form multiple C-S bonds in variable reaction conditions and it was discovered that laccases are convenient catalysts for the formation of heteromolecular thiolated products at environmentally favourable conditions (**Scheme 10**) [58]. Another study was conducted on one-pot laccase synthesis of 1,4-naphthoquinone-2,3-bis-sulfides using aryl and alkyl thiols at different pH values and it was found that the yields of the product were highly affected by pH of solution, solubility of substrates, nucleophilicity, and mole ratio of thiol to laccase substrate. Another factor causing low yields of products was laccase inhibition by thiols, therefore development of laccases which are resistant to thiol inhibition is a novel way to improve C-S bond formation reactions [59].



Scheme 10: Laccase catalysed C-S bond formation reaction [2]

Substances such as ionic liquids (ILs) have been investigated as alternative solvents or co-solvents for biocatalytic reactions of many enzymes including laccases [60]. Three types of water soluble ILs (1-ethyl-3-methylimidazolium 2-(methoxyethoxy) ethylsulfate, 1-ethyl-3-methylimidazolium methanesulfonate, and1-ethyl-3-methylimidazolium ethylsulfate) have been used to investigate the activity and stability of the commercially available laccase Denilite® II base at various pH levels, and they were found to show good stability of the enzyme and also were able to inhibit enzyme activity loss for a period of 7 days [61]. On another report it was shown that ILs can be an alternative solution for solubility problems encountered during laccase biocatalysis involving poorly water soluble substrates [62].

1.8. Benzoxazoles

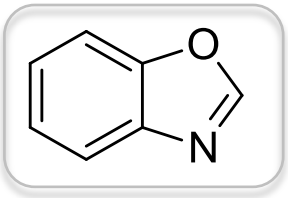


Figure 2: Benzoxazole

Benzoxazoles are important heterocyclic organic compounds consisting of a benzene-fused oxazole ring structure (**Figure 2**). They are frequently encountered as structural units in natural products and are often used as starting material for synthesis of larger pharmaceutical drugs and organic molecules [63]. The benzoxazole scaffold is found in many significant chemotherapeutic and biologically active compounds and they are widely used in medicinal applications [64]. Due their potent biological properties, benzoxazoles have received great attention in chemistry and biochemistry [65]. Various examples of medicinal application include: amyloidogenesis inhibitors [66], estrogen receptor-b agonists [67], Human GSTP1-1 inhibitors [68], melatonin receptor agonists [69], anti-HIV-1, anticancer, antimicrobial agents [70], anti-tuberculosis agents [71], anti-bacterial [72], anti-fungal [73], Rho kinase inhibitors (**Figure 3**) [74].

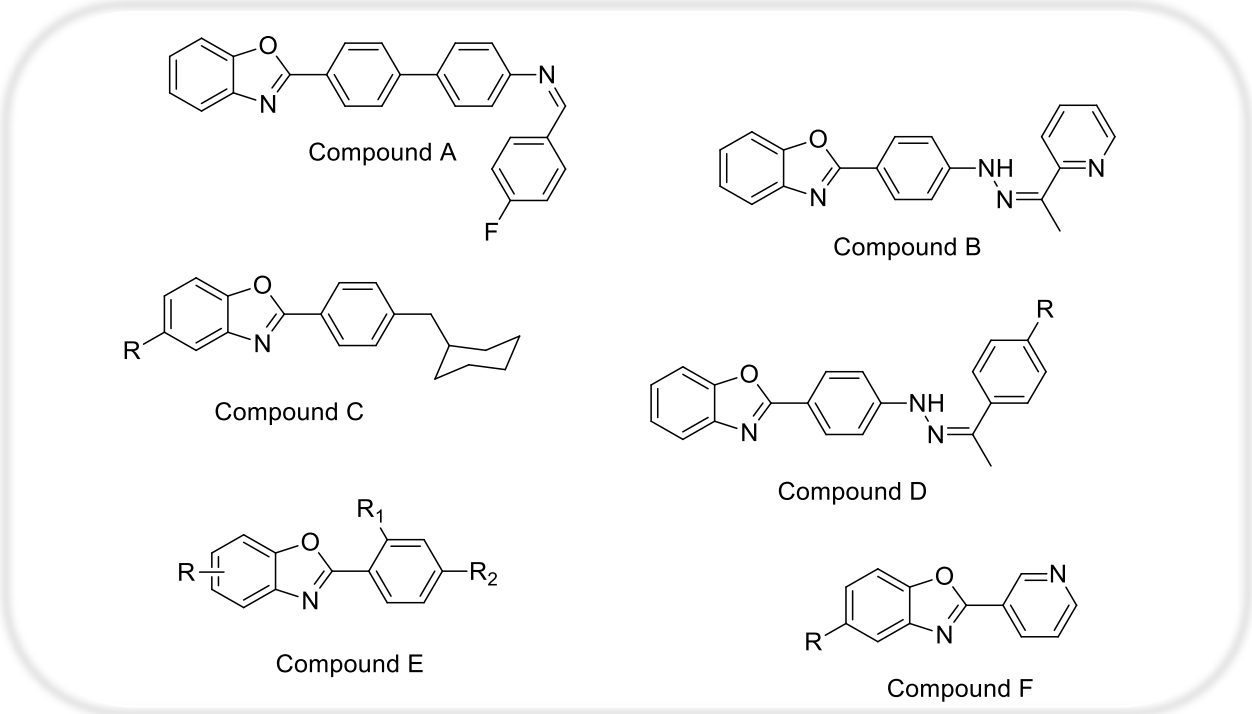


Figure 3: Examples of pharmaceutical important benzoxazoles [3, 9]

Furthermore, they also possess numerous industrial applications; some of them have been utilized in fluorescent sensors for application in two-photon microscopy [75]. Substitution around the benzoxazole nucleus results in luminescent characteristics; were they can be used in organic light emitting diodes (OLEDs) as fluorescent whitening agent dyes [65]. In addition benzoxazoles have been utilized as dye releasers in instant colour chromatography [76], dye lasers [77], as ligands in asymmetric synthesis [78] and polymer synthesis [79] (**Figure 4**).

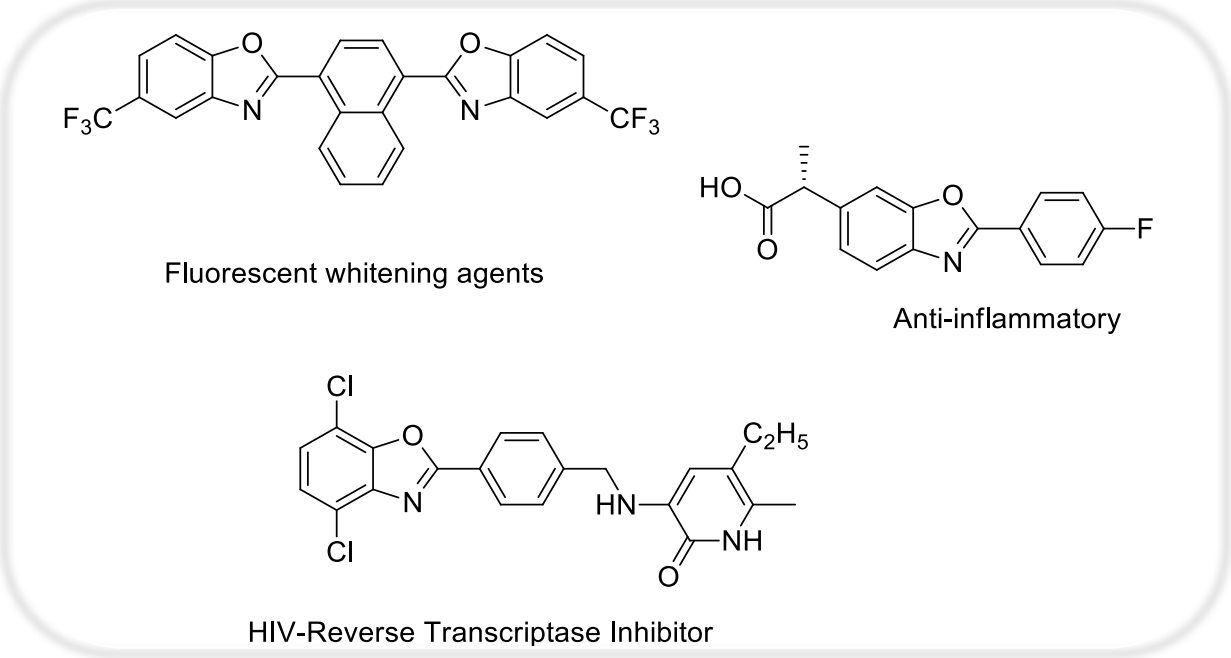


Figure 4: Compounds bearing benzoxazole nucleus

There are various methods reported for the synthesis of benzoxazoles; these include 2-aminophenol with carboxylic acids and their derivatives (amines, alcohols, acyl halides, and nitriles) catalysed by strong acids or microwave conditions. Another involves cyclization of Schiff bases derived condensation–dehydration reaction of 2-aminophenol with aldehydes in the presence of an oxidizing agent; oxidants include ThClO4, $Pb(OAc)_4$, $Mn(OAc)_3$, DDQ, PCC-supported silica gel, Dess-Martin reagent, NiO_2, and many more [80]. Other methods include cyclisation of 2-halo-N-acylanilines, reaction of 2-aminophenol with carbon–carbon triple bonds, or reaction of bromoaniline with acyl halides [65, 81]. However there are many setbacks associated with the majority of the reported methods for the synthesis of benzoxazoles. These include the use of toxic and expensive reagents, harmful organic solvents, high reaction temperatures, longer reaction times, and tedious work-up procedures [80].

1.9. Benzimidazoles

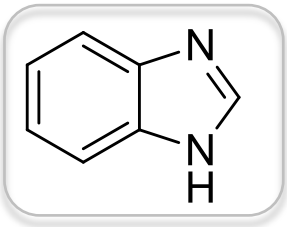


Figure 5: Benzimidazole

Benzimidazoles are a class of heterocyclic aromatic compounds composed of a phenyl ring fused with an imidazole ring system (**Figure 5**). One of the prominent natural occurring derivatives of benzimidazoles is *N*-ribosyl-dimethyl benzimidazole; a component of inhibitors such as factor Xa (Fxa) inhibitors, poly (ADP-ribose) polymerase (PARP) inhibitors, 5-HT3 antagonists, and 5-lipoxygenase inhibitors, and also serves as one of the cobalt ligands in vitamin B12 [82]. Benzimidazoles consisting of different substituent(s) around their core are associated with a wide range of biological and pharmacological activities such as anti-tumour, anti-ulcer, anti-fungal, anti-hypersensitive, anti-rhino, anti-allergic properties, as well as neuropeptide Y Y1 receptor antagonists [83, 84]. Furthermore, through blocking the secretion of gastric acid in the stomach, substituted benzimidazoles can act as gastric H+ /K+ ATPase inhibitors [85].

Because of their remarkable biological activities, benzimidazoles have been studied for more than a century, and amongst other important heteroaromatic compounds, they possess a scaffold essential in many natural products and can be found in several well recognized pharmaceutical drugs [86, 87]. Some of the well-known available drugs bearing a benzimidazole scaffold include albendazole, mebendazole, omeprazole, and bendamustine (**Figure 6**) [88]. Furthermore, compounds with benzimidazole moieties have been utilized in material science applications; they are often used as membranes for fuel cells and also in organic light-emitting diodes [89]. In this regard, synthetic procedures directed towards the synthesis of compounds containing the benzimidazole core have become a subject of interest, and various methods for the synthesis of these heterocycles are being formulated [83, 88].

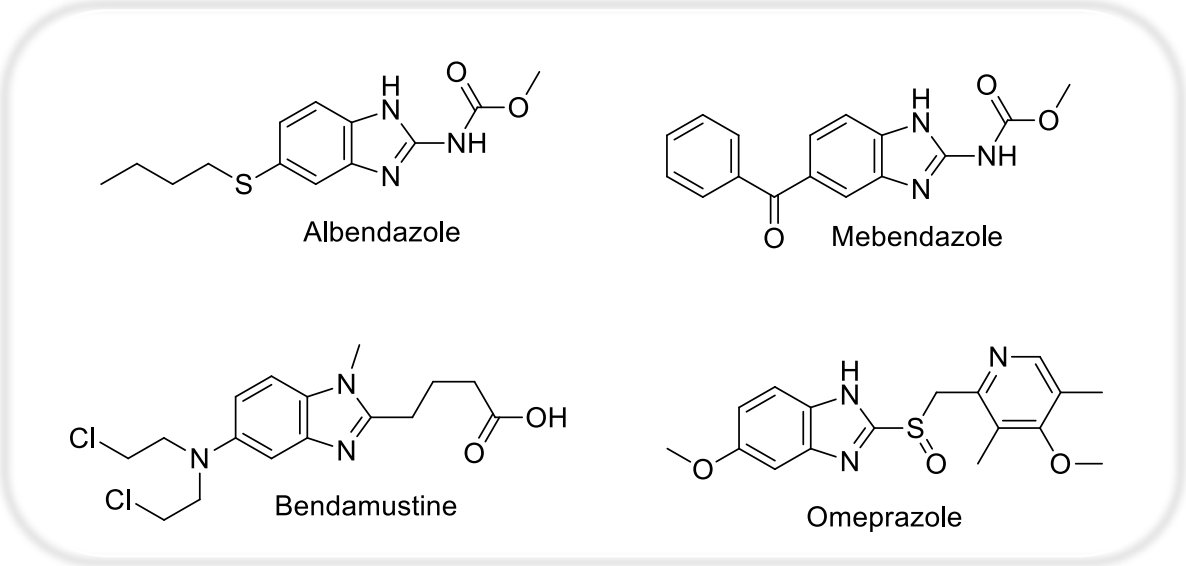


Figure 6: Examples of biological active benzimidazole derivatives [88]

The classical methods for the synthesis of benzimidazoles involve condensation reactions of 1,2-phenylenediamines with carboxylic acids and their derivatives in the presence of strong acids, ionic liquids, nanomaterials and zeolites [88, 90]. Another is oxidative cyclisation of Schiff bases obtained from condensation reactions between 1,2-phenylenediamines and aromatic aldehydes [88, 89]. A number of oxidants have been used in the synthesis of benzimidazoles and their derivatives, including iodine, peroxides, 1,4-benzoquinone, nitrobenzene, cupric acetate, and (bromodimethyl)sulfonium bromide, and recent advances include procedures such as microwave assisted synthesis, solid phase synthesis and enzyme catalysts [83, 86]. However, most of the available methods are at a disadvantage because they make use of harsh reaction conditions such as high temperatures and organic solvents, and some require the use of toxic reagents [83, 91]. The major drawback with the synthesis of benzimidazoles is the chemo-selectivity during the reaction process; they usually end up with the formation of a mixture of two products (2-substituted and 1,2-disubstituted benzimidazoles) [90].

In this sense, various methodologies have been formulated to work around the chemo-selectivity issue to selectively form either 2-substituted or 1,2-disubstituted benzimidazoles [90]. Chari *et al.* (2011) demonstrated the importance of the presence of oxygen in the system for these reactions to occur and also showed that the presence of an oxidizing agent promotes selectivity towards the formation of the mono-substituted benzimidazoles [92]. Fan *et al.* (2015) developed a highly chemo-selective method to

obtain different substituted benzimidazoles under mild reaction conditions [90]. In 2011, an enzymatic catalysed reaction to afford benzimidazole derivatives was reported; where laccase from *Agaricus bisporus* was used to catalyse the condensation reactions of *o*-phenylenediamine and various aldehydes to obtain 2-substituted benzimidazoles [82]. The reactions were performed using phosphate and acetate buffers as solvent systems, but due to solubility issues of organic substrates, methanol was used as a co-solvent to yield substituted 2-aryl-1H-benzimindazoles. However, under these reaction conditions, selectivity was still an issue; the reactions led to formation of the di-substituted side product (compound **4**) (**Figure 7**). Reaction conditions were varied to aid selectivity towards formation of mono-substituted benzimidazoles by changing the ratio of buffer to solvent. But this did not result in significant improvement, compound **4** was still formed and other side products as well [82].

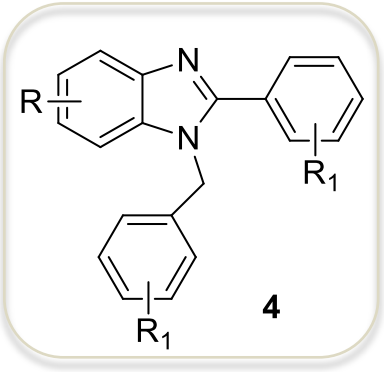


Figure 7: 1-benzylated benzimidazole

1.10. Benzothiazoles

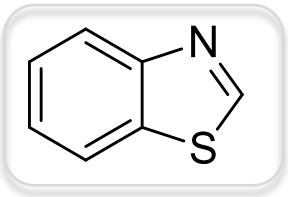
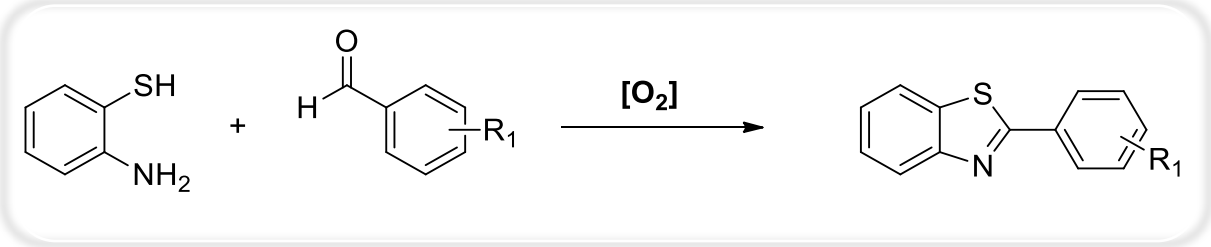


Figure 8: Benzothiazole

Benzothiazoles are an important class of bicyclic aromatic heterocyclic compounds composed of a benzene ring fused to a thiazole ring system, where a thiazole ring is a five membered ring made up of one sulphur and one nitrogen in the ring (**Figure 8**) [93, 94]. The heterocyclic scaffold of benzothiazoles can be readily substituted with various functional groups and it is known to possess incredible pharmacological and therapeutic properties. They have found numerous applications in medicinal chemistry as antitumor, anticancer, anti-HIV, antiviral, antimicrobial, antibacterial, anthelmintic, and anti-diabetic, anti-allergic, and anti-inflammatory agents [93-99]. Compounds bearing the benzothiazole nucleus have been presented in a number of applications; during the 1950s, compounds with the 2-aminobenzothiazole scaffold were intensively investigated; some displaying activity against cancer cells and they were also utilized in biochemical and electrophysiological applications [93, 94].



Scheme 11: Laccase-catalysed reaction of 2-aminothiophenol with aryl-aldehydes

Other applications include use as a LTD4 antagonist, an orexin receptor antagonist, a stearoyl-coenzyme A δ-9 desaturase (SCD) inhibitor and use in industry as oxidants, industrial dyes, functional materials. Furthermore it is also used for imaging of amyloid plaques, and its an analogue for firefly luciferase responsible for light-emission (**Figure 9**) [96-104].

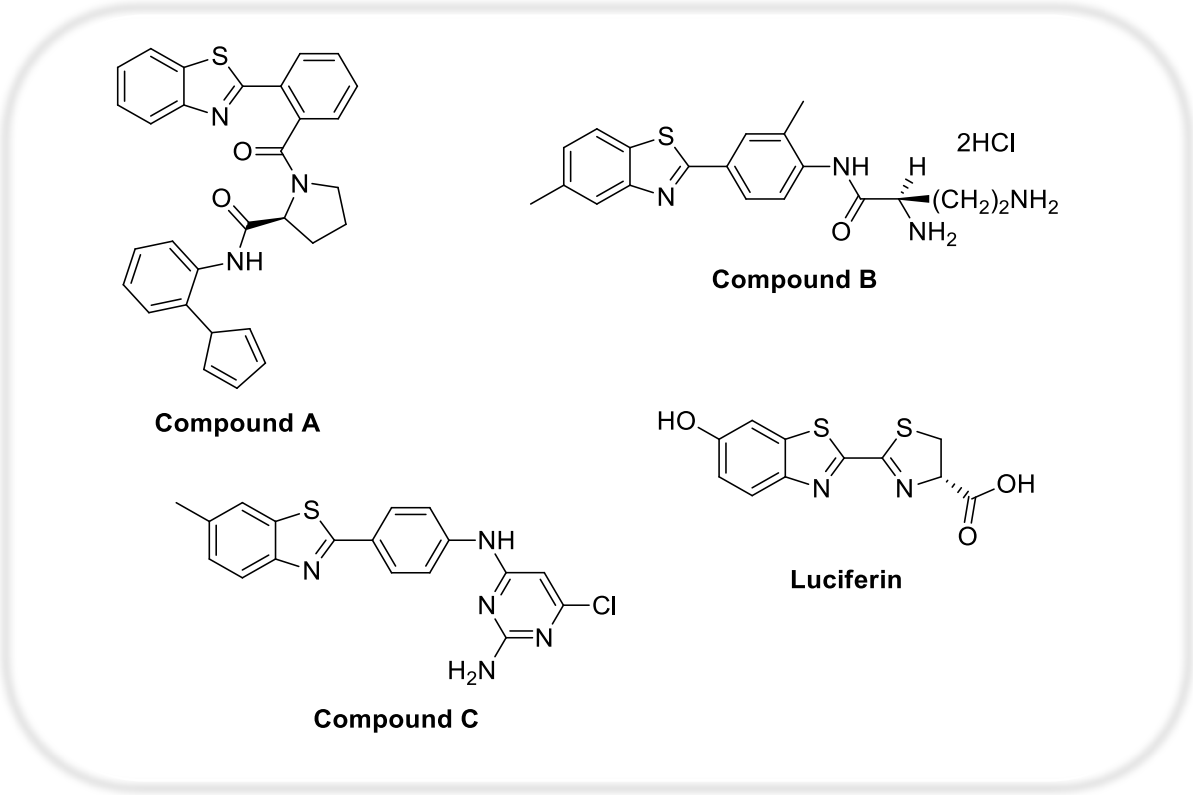


Figure 9: Examples of important benzothiazole compounds

Due to their substantial biological activity, developments of efficient synthesis methods for benzothiazoles compounds have received a significant amount of interest [93]. There are many methods already in place for preparing benzothiazole derivatives; the conversional methods include condensation–dehydration reaction of 2-aminothiophenol with carboxylic acids and their derivatives under strong acid and high temperature conditions or condensation with aldehydes under oxidative conditions (**Scheme 11**) [95, 96]. Transition-metal catalysed cross-coupling reactions of benzothiazoles and aromatic halides using aromatic boronic and carboxylic acids [97]. Even though these methods are quite efficient, they still display a number of disadvantages; these include the use of toxic oxidants, abrasive reaction conditions (high temperatures, pressure and longer reaction times), use of expensive transition metals and strong acids, and also use of environmentally unfriendly solvents [96, 97]. With a growing demand for green chemistry, the preparation of 2-aryl-benzothiazoles using environmental benign processes such as transition-metal free reactions, ambient reaction conditions, use of biocatalysts and green solvents is of paramount importance.

AIMS AND OBJECTIVES

The overall aim of this research project was to investigate the range and limitations of applications of laccase enzymes in organic synthesis. The project concentrates on method development and improvement of reaction conditions for the catalysis of bond formation in organic reactions such as oxidation, amination, and oligomerization under moderate and environmentally friendly conditions. The primary focus of this study was on the catalysis of bond formation (C-C, C-O, C-N, and C-S bonds); specifically on cross-coupling reactions of Nitrogen, Oxygen, and Sulphur based aromatic compounds. The final part of the project seeks to determine the limiting factor in the resultant yields by varying the reaction conditions.

The project consists of five sections which are the synthesis of biaryl compounds, benzoxazoles, benzimidazoles, benzothiazoles, and aminobenzoquinones. Use of laccases to synthesise biaryl bonds from substituted phenol substrates at room temperature using different organic solvents was investigated. We explore the use of laccase to catalyse both self-coupling and cross-coupling reactions of simple phenols as we identify optimal reaction conditions for these reactions. For benzoxazole derivatives we aimed at developing an effective method to catalyse the synthesis of 2-aryl-benzoxazole derivatives using laccase at room temperature; looking at factors such as solvents effects, influence of laccase origin and also the use of laccase-mediator systems. For benzimidazole derivatives we investigated the effect of different solvents or co-solvents towards the selectivity of these reactions. We also conducted a control study in different solvents/or co-solvents to determine what role does the enzyme play in these reactions. We describe how different enzymes affect the outcome of the reaction with various benzaldehydes, as we identify the optimal conditions for these reactions. For benzothiazole derivatives, while exploring the above mentioned factors, we developed a modern and practical laccase-catalysed route for the synthesis of 2-arylbenzothiazoles. To the best of our knowledge, the laccase-catalysed method for preparation of 2-arylbenzothiazole derivatives from condensation–dehydration reaction of 2-aminothiophenol with aryl-aldehydes has not been reported before. The method described is green, effective and simply requires an easy work-up routine, utilizing solvents such as acetonitrile and DMF as co-solvents.

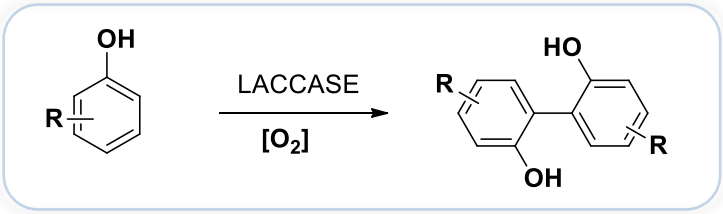
For the final section of this study we investigated improvement of yields of the monoaminated and diaminated products during amination reactions. The laccase-catalysed nuclear diamination of aromatic hydrobenzoquinones with aliphatic and aromatic amine molecules was investigated at mild reaction conditions using commercial laccases from Novozymes (Suberase®, Denilite® II Base, and Novoprime Base 268). Other factors that impact on reaction yield were explored; these include conducting reactions under dilute conditions, sequential addition of enzyme and substrate over time, and also the effect of buffer and co-solvents.

Chapter 2

RESULTS AND DISCUSSION

2.1. Dimerization of phenols

In literature, the laccase catalysed formation of biaryl compounds has been demonstrated for a variety of molecules. Examples of laccase-catalysed self-coupling reactions such as the biotransformation of 5,6,7,8-tetrahydronaphthalen-2-ol, synthesis of dispiropyrimidinone and self-coupling reaction of bisphenol have been reported [43-45]. Most of the studies in this area have been carried out on large compounds; therefore our aim was to apply the same principles using simple phenolic substrates to accomplish similar results. Various phenolic substrates were subjected to different commercial laccases from Novozymes (Suberase®, Denilite® II Base, and Novoprime Base 268) to catalyse the oxidative coupling reactions of phenols via radical mechanism to form biaryl bonds under aerobic conditions (**Scheme 12**).



Scheme 12: Laccase-catalysed oxidative dimerization of phenols

It is understood that self-coupling reactions of phenolic substrates via a radical mechanism are most likely to form oligomers and polymers [44, 45]. Polymerization of Bisphenol A was demonstrated using laccase from *T.* villosa (**Scheme 4**); some of the products obtained such as 5,5'-bis-[1-(4-hydroxy-phenyl)-1-methyl-ethyl]-biphenyl-2,2'-diol and some oligomers were insoluble and were revealed by Field-desorption mass spectrum analysis [45]. In one report the effect of different organic solvents on product formation was investigated, where relative ratio of the dimers found was significantly influenced by the nature of organic solvent used and the general yields of the dimers found were quiet low [44]. On this basis, the reactions were conducted in different solvents and/or co-solvent systems at various temperatures to account for solvent effects, using solvents such as

acetonitrile, methanol, toluene, DCM, and DMF and various substitutions around the phenolic substrates were used to limit the rate of polymerization.

Our first attempt was to perform self-coupling reactions by carrying out laccase-catalysed oxidations on a series of substituted phenol derivatives. We hypothesised that laccase primarily oxidises the phenolic moiety of the substrate, thereby generating phenoxy radicals; from these radicals, cross-coupling reactions may take place to produce biaryl bonds. Various substituted phenolic substrates (**Figure 10**) were dissolved in acetate buffer (10.0 mL, 0.1 M, pH 4.50) and DMF (10.0 mL) and were subjected to commercial laccase Suberase® from Novozymes at room temperature. The reactions were conducted for a period of 72 h under aerobic conditions and were monitored by TLC. In all attempts, no activity was observed except for 4-bromophenol and 2,4-dichlorophenol, however the products were not characterised because they were insoluble. For 4-bromophenol and 2,4-dichlorophenol, it was anticipated that since the reaction occurs through the generation of phenoxy radicals; the cross-linking reactions might have led to polymerization. However due to solubility issues, the products were not characterised. Intra *et al.* (2005) demonstrated the significant influence of solvents effect on the selectivity of laccase-catalysed oxidation of phenols [44]. On this basis, we conducted self-coupling reactions using different solvents such as methanol, toluene, acetonitrile, and DCM but no activity was observed for most of the substrates. Using toluene as a co-solvent, some activity was observed for 4-nitrophenol and 2,4-dichlorophenol, however, the reactions reversed during work-up (**Table 1**).

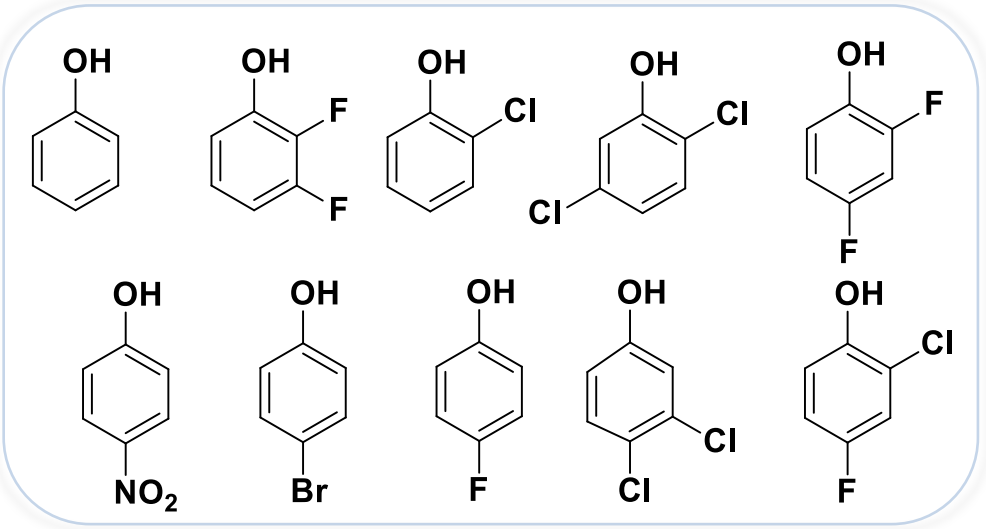
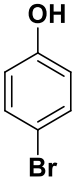
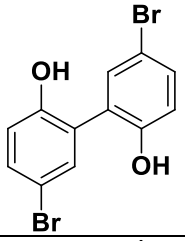
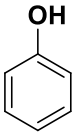
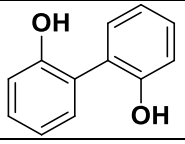
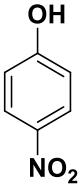
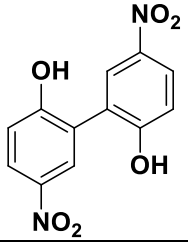
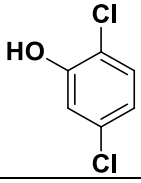
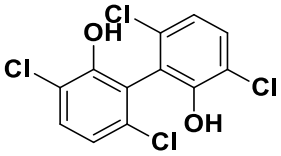
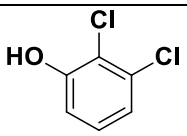
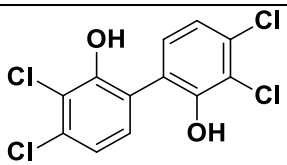
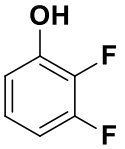
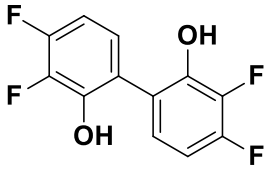
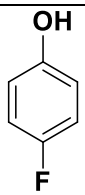
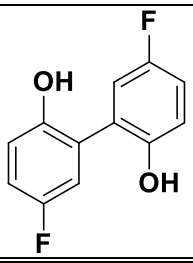


Figure 10: The phenolic substrates used in this study

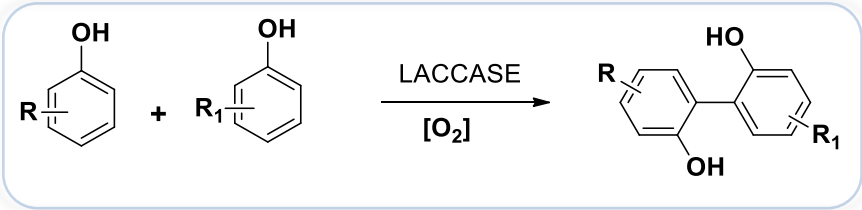
Table 1: Laccase-catalysed phenol coupling reactions in toluene

Entry	Phenol I	Product	Yield
1			Not determined[a]
2			0
3			Not determined[b]
4			Not determined[a]
5			0
6			0
7			0

a- Product was not characterised due to solubility issues.
b- Activity observed but reaction reversed during work-up

The results suggests that for the majority of the substrates the enzyme was unable to initiate the oxidation of the phenolic moiety to generate phenoxy radicals (Ph-O•). Similar results were observed with different organic solvents and other laccases. For the substrates that showed activity, major hurdles such as solubility issues and reversibility of the reaction still need to be addressed.

Our second attempt was to investigate cross-coupling reactions between various substituted phenol derivatives (**Scheme 13**). The aim here was to investigate if different functional groups have any significant influence on reactivity; thereby distinguishing if whether cross-coupling reactions between phenols have better reactivity compared to self-coupling reactions. The reactions were conducted using the same reaction conditions as in self-coupling reactions; however, no activity was observed for all the reactions. All of the tested substrates behaved the same, and the same results were obtained as in self-coupling reactions. At this stage, all the methods tested for the synthesis of biaryl compounds from phenolic substrates catalysed by laccase were not effective. Interesting results were obtained for self-coupling reactions of 4-bromophenol and 2,4-dichlorophenol, however the products were not characterized because of solubility issues. The overall difficulty encountered in these reactions is the initiation of the reaction through generation phenoxy radicals (Ph-O•) by the enzyme, and also the propensity of the reactions to favour the reverse reaction. The addition of more enzyme over time helped to overcome this; but generally it is still a major draw-back. The high loading of enzyme had no significant effect on either self-coupling or cross-coupling reactions; it seems that not enough radicals were generated to drive the reaction in the forward direction under the described reaction conditions.



Scheme 13: Laccase-catalysed oxidative dimerization of phenols

Based on the experimental data collected, it was suggested that the laccases used in this study may be unable to catalyse the oxidation of the phenolic substrates, i.e. their oxidation potency might be inadequate to generate phenoxy radicals (Ph-O•); therefore the use of mediators could help enhance the overall activity. The mediator plays a role of shuttling electrons; when oxidized by the enzyme it

can generate stable radical species which will in turn oxidise the substrate of interest [17]. Using ABTS as a mediator, various substrates were subjected to laccase in acetate buffer (0.1 M, pH 5.0) at room temperature under aerobic conditions (**Scheme 14**).

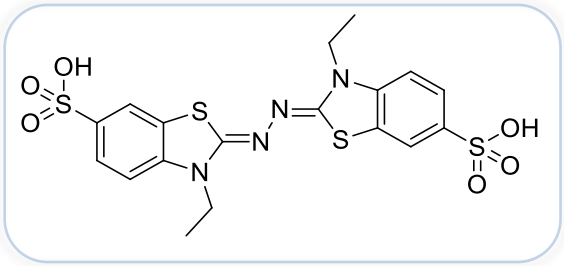
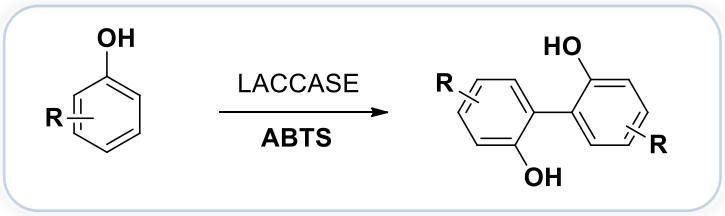


Figure 11: Structure of ABTS

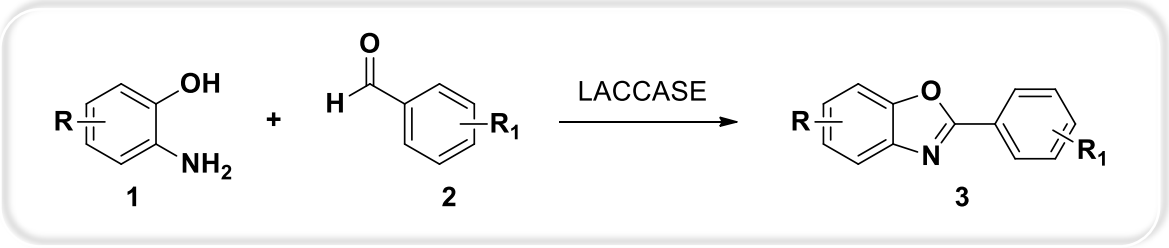
Laccase Mediator self-coupling reactions



Scheme 14: Oxidative dimerization of phenols using laccase-mediator systems

Using ABTS as a mediator; the laccase-mediator dimerization of a series of substituted phenols was investigated. The reactions were carried out using acetate buffer (5.00 mL, pH 4.50), a co-solvent (5.00 mL), Laccase (Novoprime Base 268 (80.0 mg)) and ABTS (2.00 mL) at room temperature. Activity was only observed for *p*-nitrophenol as determined by TLC; it appears there was some form of polymerisation occurring. As previously described, no form of activity was observed with most of the substrates tested. Overall, the optimal conditions for obtaining biaryl compounds using laccase were not accomplished. Cases of polymerisation were observed numerus times, however due to solubility issues the products were not characterised. On the basis of literature reports, it could be contended that the laccases used in this study did not possess enough oxidation potency to initiate the one electron oxidation of many of the presented phenols [43-45]. Where oxidation did occur, polymerisation proceeded, and the desired products were not formed. However, with further optimization of reaction conditions with different mediators in combination with other laccase enzymes, biaryl compounds may yet be obtained.

2.2. Synthesis of benzoxazole derivatives



Scheme 15: Synthesis of benzoxazole derivatives

As mentioned in Section 1.8 condensation–dehydration reaction of 2-aminophenol with aldehydes in the presence of an oxidizing agent is one of the most explored methods for preparing benzoxazoles. It is important to realise that enzymes have not yet been explored as alternative oxidants for the preparation of benzoxazoles from Schiff's bases derived from condensation of 2-aminophenol with aldehydes. In this research, a laccase-catalysed synthetic method was investigated for catalysing the synthesis of 2-substituted benzoxazoles through condensation–dehydration reaction of 2-aminophenol and aromatic aldehydes under aerobic conditions (**Scheme 15**). Reaction of 2-aminophenol (10.0 mmol, 1.08 g) with 2-Chlorobenzaldehyde (10.0 mmol, 1.41 g) was selected as the model reaction catalysed by commercial laccase, Suberase® from Novozymes at room temperature. The reaction was conducted in acetate buffer (0.1 M, pH 4.0) and a co-solvent (40%).

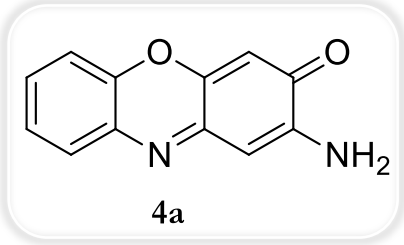
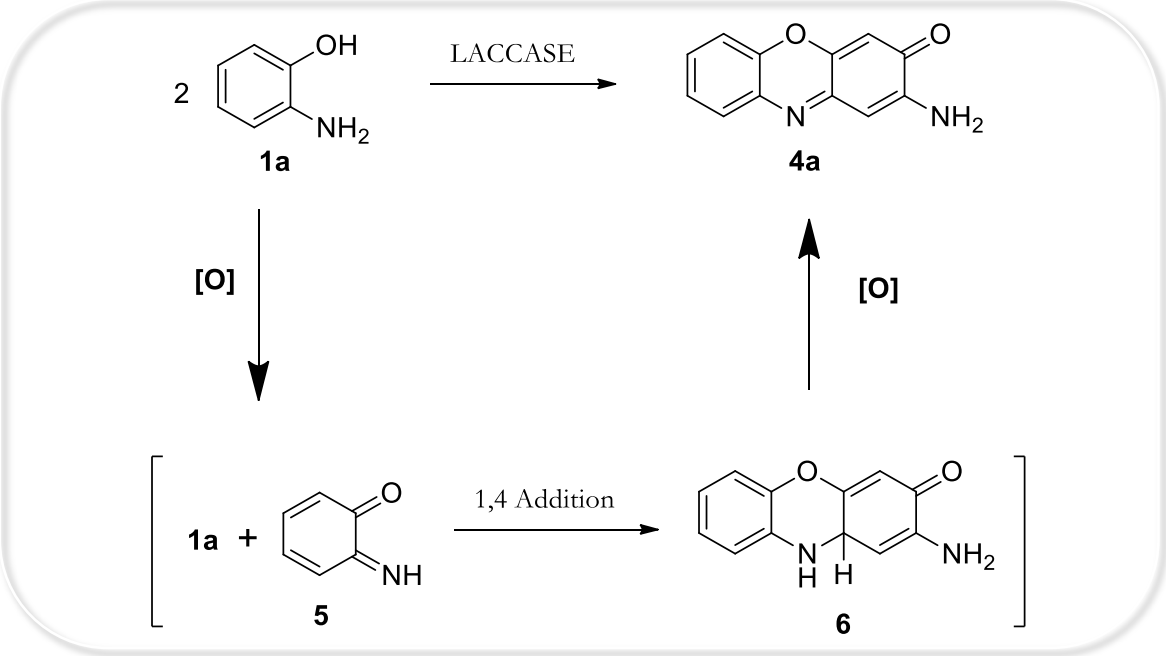


Figure 12: 2-amino-3H-phenoxazin-3-one

Sousa *et al.* (2014) reported a laccase-mediated method for dimerization of several *ortho* and *meta*, *para*-disubstituted aromatic amines into phenazine and phenoxazinone derivatives. Taking this into account, it was anticipated that the reaction was likely to be accompanied by the occurrence of dimerization; where 2-aminophenol couples with itself to form 2-amino-3H-phenoxazin-3-one (dimer **4a**) (**Figure 12**). It is assumed that in the presence of a catalytic amount of laccase, the oxidative dimerization of **1a** begins with the oxidation of 2-aminophenol (**1a**) to 6-iminocyclohexa-2,4-dienone (**5**), which reacts

with another molecule of 2-aminophenol (**1a**) through an inter- and an intramolecular 1,4-addition to yield 2-amino-10,10a-dihydro-3H-phenoxazin-3-one (**6**). The final step is another oxidation of **6** to yield 2-amino-3H-phenoxazin-3-one (**4a**) (**Scheme 16**).



Scheme 16: A laccase-catalysed aerobic dimerization of **1a** to yield 2-amino-3H-phenoxazin-3-one

For the model reaction no dimerization was observed under the described reaction conditions, however, the reaction intended did not go to completion. The formation of benzoxazole **3** is commonly understood to proceed in a sequence of steps: the first step is the condensation reaction of 2-aminophenol derivatives (**1**) with aromatic aldehydes (**2**) to yield Schiff base **7**. This is then followed by cyclization of **7** to yield a 2, 3-dihydrobenzoxazole derivative **8**, which then undergoes oxidation to give the corresponding benzoxazole **3** (**Scheme 17**). Following the proposed reaction mechanism, the step between Schiff base **7** and benzoxazoline intermediate **8** did not take place. From TLC results, the disappearance of the substrates was completed after 2 h of reaction and the solid product obtained was purified by washing with cold acetonitrile. It was observed that the reaction proceeded with the proposed mechanism except that cyclisation of Schiff base **7a** to generate 2-(2-chlorophenyl)-2,3-dihydrobenzoxazole (**8a**) does not take place. The purified product 2-((2-chlorobenzylidene)amino)phenol (**7a**) was characterized by IR, NMR (^{1}H and ^{13}C), and mass spectroscopy. As determined from proton NMR; a total of nine signals were observed in the aromatic

region. A singlet at 9.03 ppm was assigned to the alkene proton (H7'), a doublet at 8.09 ppm was assigned to H6', a multiplet integrating four protons observed at 7.35 – 7.17 ppm was assigned to H3', H4', H5', H3 protons; two triplets at 7.12 and 6.80 ppm were assigned to H5 and H4 respectively, and a doublet at 6.91 ppm was assigned to H6 proton (**Figure 13**). Elemental and mass spectra data also corresponds to the mass of Schiff base **7a**; where the mass calculated for compound **7a** $C_{13}H_{10}ClNO$: 231.68, and the mass found: [M+H]: 232.05; confirming that cyclisation into a five membered ring did not take place.

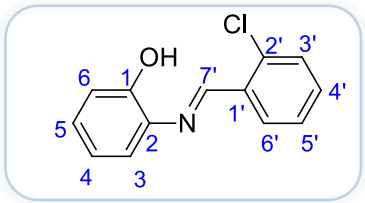
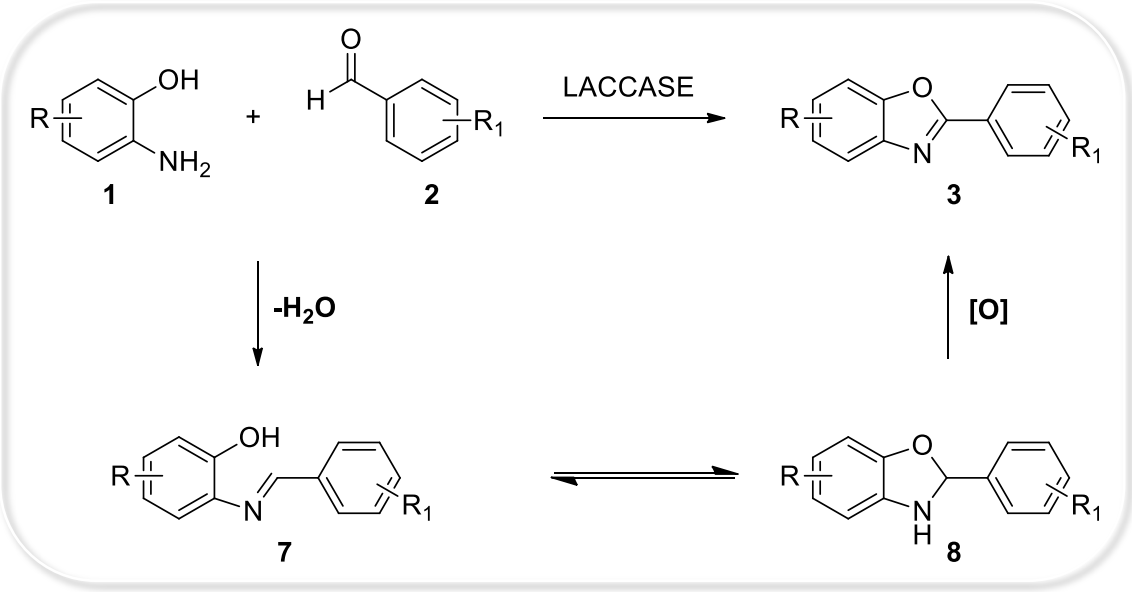


Figure 13: Schiff base **7a**



Scheme 17: Reaction of 2-aminophenol with benzaldehyde derivative to yield benzoxazoles

It has been demonstrated before that for the preparation of benzoxazoles the type of solvent used plays an important role on the yield and rate of reaction [64]. Our second approach was to investigate the role of co-solvents as we identify optimal condition to achieve benzoxazole derivatives. The laccase-catalysed reaction of 2-aminophenol (10.0 mmol, 1.08 g) with benzaldehyde (10.0 mmol, 1.06

g) in acetate buffer (0.1 M, pH 4.0) was carried out using various solvents such as methanol, ethanol, dichloromethane, DMF and acetonitrile at room temperature. In all conditions, no dimerization of 2-aminophenol was observed and it was discovered that the reaction proceeds at different rates along with yield using different co-solvents (**Table 2**). Acetonitrile (**Table 2**, entry 4) was found to be the best solvent for this transformation, using commercial laccase, Suberase® from Novozymes at room temperature. However, as before, the reaction did not go to completion; the cyclisation step from **7a** to **8a** did not occur and the product isolated was compound **7a**. Patil *et al.* (2017) also identified acetonitrile as the best solvent for the synthesis of benzoxazoles while preparing 2-phenylbenzoxazole at 60 °C using 10 mol % of TiO_2–ZrO_2 catalyst.

Table 2: Effects of co-solvent in the laccase-catalysed synthesis of 2-(2-chlorophenyl)benzoxazole (**3a**)

Entry	Solvent	Time (h)	Yield 3a (%)	Yield 7a (%)
1	Methanol	24	-	70
2	Ethanol	12	-	76
3	Dichloromethane	6	-	85
4	Acetonitrile	0.5	-	94
5	DMF	2	-	89

Using acetonitrile as the optimal co-solvent, derivatives of 2-aminophenol were treated with a series of aromatic aldehydes under aerobic conditions using commercial laccase, Suberase® from Novozymes at room temperature and the obtained results are summarized in **Table 3**. All the experiments were carried out according to the general method described below varying both the 2-aminophenol and aromatic aldehyde derivatives at room temperature. Under the described experimental conditions, no sign of dimerization was observed and as before cyclisation of Schiff base **7** did not take place for any of the substrates tested. All the products were characterized using proton and carbon NMR and high

resolution mass spectrometry, and key signals (^{1}H and ^{13}C NMR) for selected products are summarised in **Tables 4** and **5**. From proton NMR data; all the substrates showed the alkene proton singlet peak observed in a chemical shift range 8.54 – 9.16 ppm and most of the substrates showed the OH proton peak, although some did not due to solvent exchange. However the elemental and mass spectra data corresponds to the masses of the described products.

A control study was conducted using the optimal conditions without the enzyme in the reaction vessel and the results were quite similar to the ones obtained with the enzyme. However, it was observed that in the presence of a catalytic amount of laccase the reaction times are shorter compared to the reactions conducted without the enzyme. It has been shown in literature that the aromatic aldehydes with different substituents such as –Cl, –Br and –OMe groups show different reactivity; where the electron-withdrawing groups are showing greater product yields and faster reaction rates compared to aldehydes containing electron-donating groups [80]. In our case, most of the substrates showed equal ease towards the formation of products with reasonable to excellent yields. Some of the substrates containing a –OMe group showed somewhat slow reaction rates, but overall, the yields and rates were comparable to other substrates.

General method used to synthesize 2-aryl-benzoxazole derivatives

A mixture of 2-aminophenol derivative (10.0 mmol) and benzaldehyde derivative (10.0 mmol) in acetonitrile (10.0 mL) and acetate buffer (0.1 M, 10.0 mL, pH 4.0) was stirred at room temperature for 5 minutes. Suberase® (2.00 mL) was added into the mixture and the contents were stirred till completion (monitored by TLC). When the reaction completes, the product precipitates from the solution and was extracted with ethyl acetate (30.0 mL) and water (3 x 20.0 mL). After extraction, the product was concentrated under reduced pressure and washed several times with cold acetonitrile (3 x 20.0 mL) to remove excess starting material.

Table 3: Reaction of 2-aminophenols (**1a-b**) with benzaldehydes **2a–t** (1:1 ratio) in acetate buffer (pH 4.0) and acetonitrile under air at room temperature

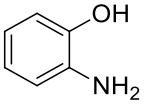
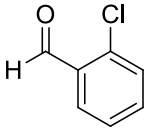
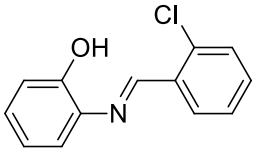
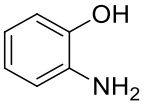
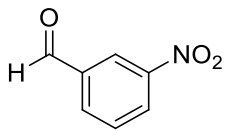
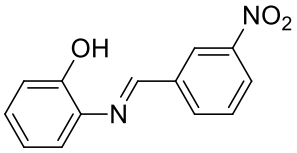
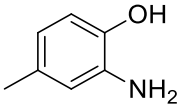
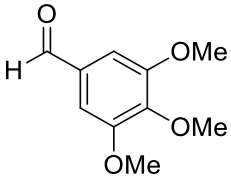
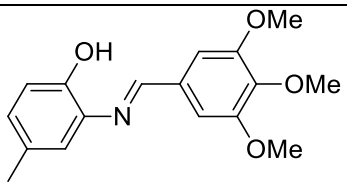
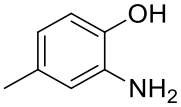
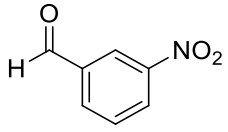
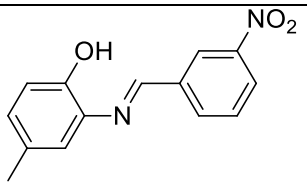
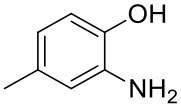
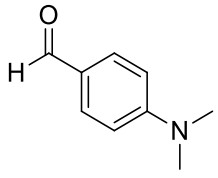
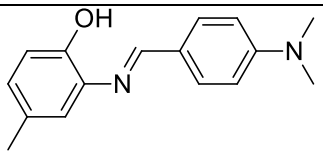
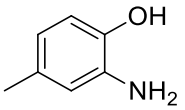
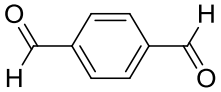
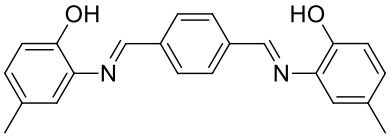
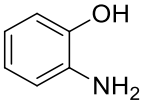
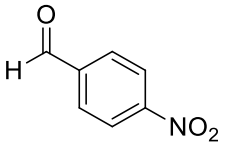
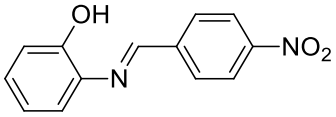
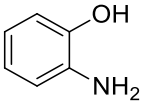
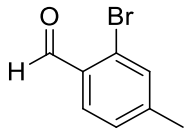
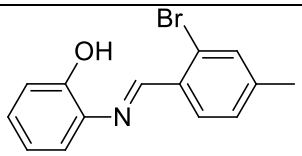
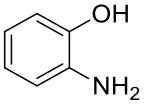
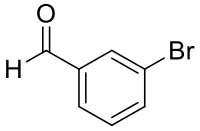
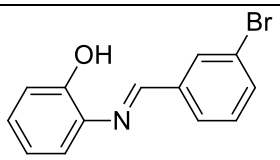
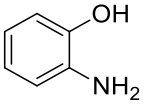
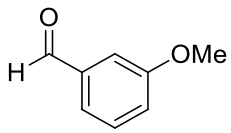
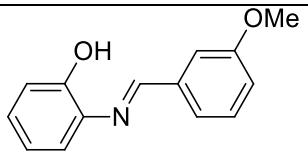
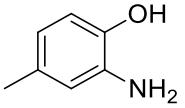
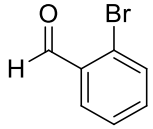
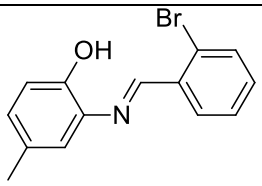
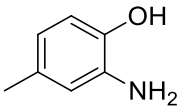
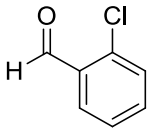
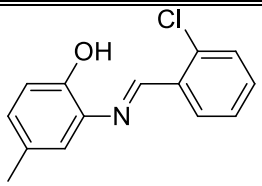
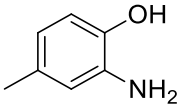
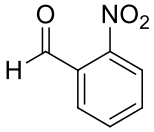
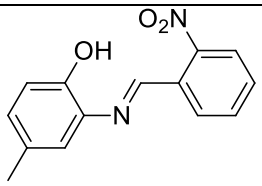
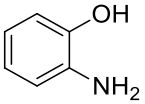
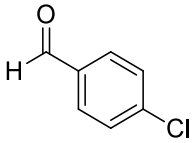
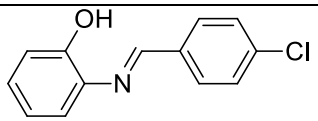
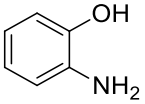
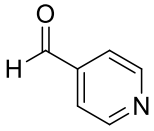
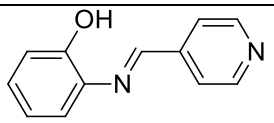
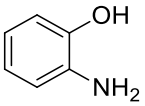
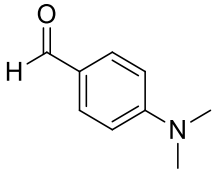
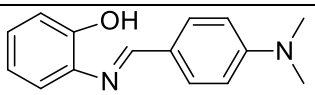
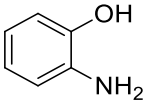
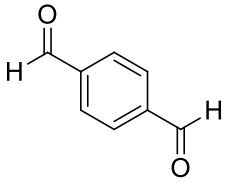
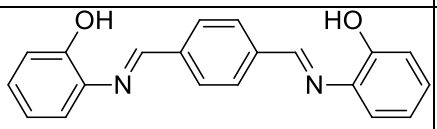
Entry	Aminophenol	Aldehyde	Product	Yield (%)	Time (h)
1				94	0.5
2				87	1
3				72	3
4				96	1
5				93	1

Table 3: Reaction of 2-aminophenols (**1a-b**) with benzaldehydes **2a–t** (1:1 ratio) in acetate buffer (pH 4.0) and acetonitrile under air at room temperature

6				97[a]	1
7				98	1
8				76	6
9				93	2
10				62	36
11				68	8

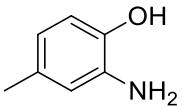
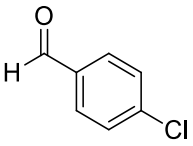
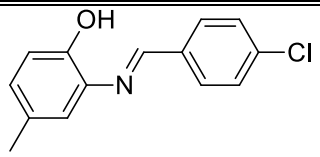
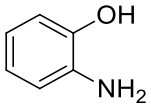
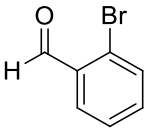
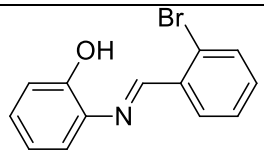
a- Two equivalents of 2-aminophenol were used

Table 3: Reaction of 2-aminophenols (**1a-b**) with benzaldehydes **2a–t** (1:1 ratio) in acetate buffer (pH 4.0) and acetonitrile under air at room temperature

12				56	2
13				48	2
14				93	2
15				95	2
16				72	18
17				98[a]	2

a- Two equivalents of 2-aminophenol were used

Table 3: Reaction of 2-aminophenols (**1a-b**) with benzaldehydes **2a–t** (1:1 ratio) in acetate buffer (pH 4.0) and acetonitrile under air at room temperature

18				94	1
19				94	2

Based on the data collected from all the experiments, it was clear that the cyclisation step from **7** to **8** was not taking place. This could be due to a number of reasons; One, it was assumed that precipitation of compound **7** from the solution during reaction could interfere with the cyclisation process. In order to account for this a biphasic system was designed, where the enzyme was in the aqueous phase and substrate in the organic phase (**Figure 14**). Two, the redox potential of the enzyme; laccase origin could factor the oxidation potency of the enzyme; therefore reactions using different laccases were conducted.

2.2.1. Biphasic Systems for laccase oxidation reaction

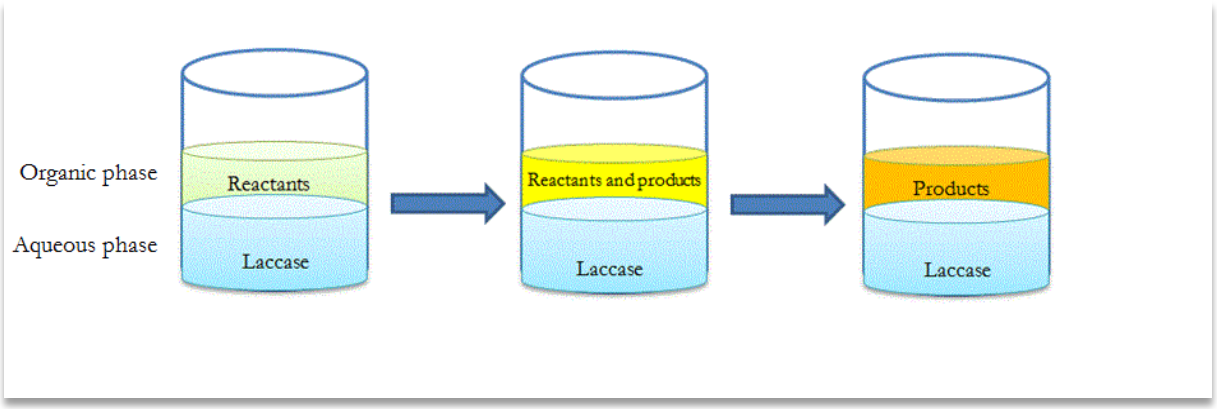
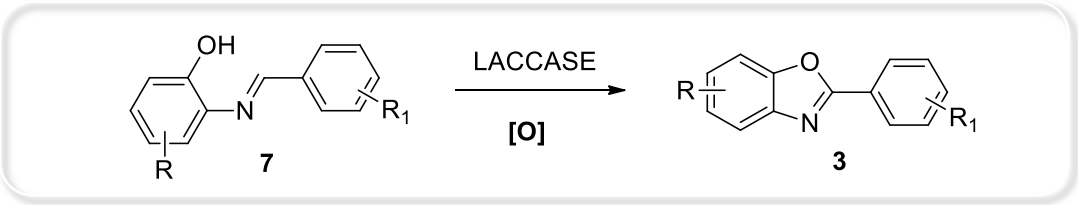


Figure 14: Biphasic system for laccase oxidation reactions.

It was assumed that the cyclisation step from Schiff base **7** to intermediate **8** was not occurring because intermediate Schiff base **7** precipitates out of solution during the reaction process. This effect causes the entire reaction medium to solidify, thereby preventing the Schiff base from cyclising. Therefore the design of biphasic systems, where the substrate will be in the organic phase and enzyme will be in aqueous phase (buffer) was suggested as a way to resolve this problem. Schiff base **7** was dissolved in an organic solvent (upper layer) and the enzyme was in the Acetate buffer (pH 4.50) (lower layer) and the contents were stirred at a lower rate for 12-36 h (**Scheme 18**).



Scheme 18: Laccase-catalysed oxidation of intermediate **7** to benzoxazole derivative

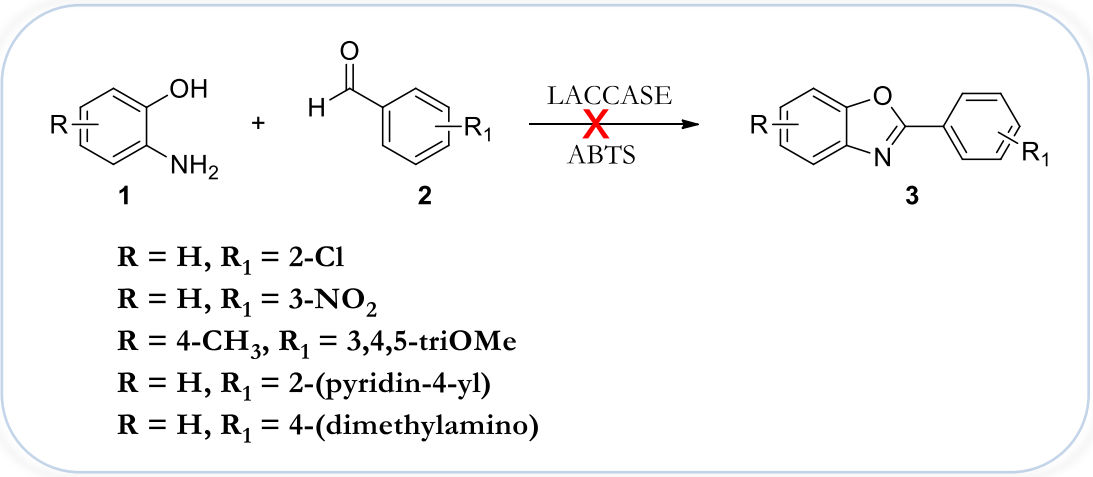
Organic solvents such as ethyl acetate, chloroform, and DCM were tested using two laccases (Suberase® and Novoprime base 268); however no activity was observed from all the substrates tested; only starting material was recovered. Cho *et al.* (2012) reported that the oxidative cyclisation from **7** to **8** is an equilibrium step and this step is the rate-determining step for the entire process. It was demonstrated that *ortho*-aminophenol is not sufficiently nucleophilic to facilitate cyclisation and it would require the use of an external nucleophile to facilitate ring closure [105].

Based on the assumption that there is equilibrium between Schiff base **7** and intermediate **8**; the overall reaction rate for the production of benzoxazoles could be increased by making use of a strong oxidant. Therefore our second approach was to test if the use of different enzymes would have any significant influence towards the formation of benzoxazoles based on their redox potential. The enzymes employed were Novozymes laccases (Suberase®, Denilite® II Base, and Novoprime Base 268). Nevertheless, the oxidative cyclization reaction did not proceed; same results as before were obtained for all the tested enzymes as depicted in **Table 3**.

As previously mentioned, when dealing with compounds possessing a higher redox potential than laccase, laccase-mediator systems can be used to overcome this obstacle and increase the oxidation aptitude of the enzyme [17]. Since we were unable to initiate the oxidative cyclisation of the substrates

to benzoxazole derivatives our next solution was to use a mediator (ABTS) to enhance the redox potential of the enzyme. Reactions of 2-aminophenols with benzaldehydes were conducted using the previously described general method together with ABTS as a mediator (**Scheme 19**). In these conditions dimerization of 2-aminophenol was observed and the products isolated were 2-amino-phenoxazin-3-one (**4a**) (54%) (**Scheme 16**) along with Schiff base **7** and traces of starting material. No traces of benzoxazoles were observed in these conditions; this confirms that the cyclisation step is indeed the rate-determining step for the whole process as reported by Cho *et al.* (2012).

2.2.2. Laccase-Mediator oxidation reactions

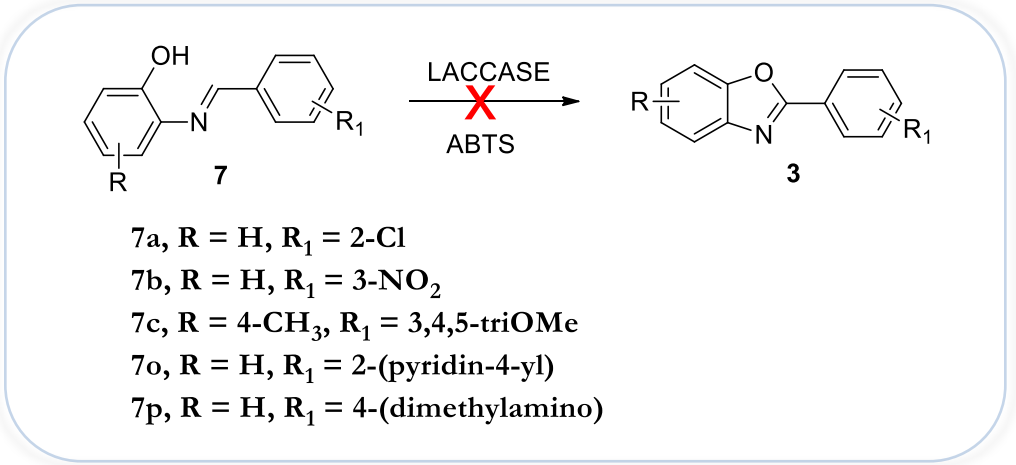


Scheme 19: Synthesis of benzoxazole derivatives catalysed by a laccase-mediator system

In order to avoid oxidation and dimerization of 2-aminophenol to form **4a**, reactions were conducted without the mediator to first generate compound **7** at room temperature. The products obtained were purified and then treated with laccase and ABTS (2.0 mL) at room temperature using different solvents and no activity was observed in all the substrates tested over a period of 72 h; only starting material was recovered (**Scheme 20**). It was determined that in the presence of a mediator the favourable reaction is the dimerization reaction of 2-aminophenol to **4a** in good yield. In literature there are numerous reports on the laccase-mediated synthesis of phenoxazinone derivatives obtained from oxidative dimerization of various substituted 2-aminophenols [31, 106]. In this project, only one phenoxazinone product (2-amino-3H-phenoxazin-3-one (**4a**)) was synthesised in a reasonable yield of 54 %. Even though synthesis of 2-aryl-benzoxazoles was not achieved, we successfully described an

easy and effective method for the synthesis of aminophenol derivatives which are important building blocks for benzoxazoles. With further optimization of the reaction conditions using laccases and also consideration of other oxidants, this method could be useful for effectively preparing benzoxazoles.

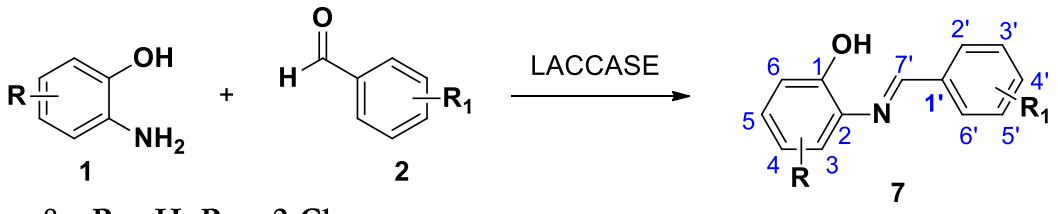
2.2.3. Laccase Mediator Systems



Scheme 20: Oxidative cyclisation of Intermediate **7** catalysed by a laccase-mediator system

As mentioned before, the Schiff base **7** products were characterized using ^{1}H and ^{13}C NMR, IR and high resolution mass spectrometry. Key signals (^{1}H and ^{13}C NMR) for selected compounds are summarised in **Tables 4 and 5**.

Table 4: Key signals in the ^{1}H NMR spectra of amino phenols



8a, R = H, R_1 = 2-Cl
8b, R = H, R_1 = 3-NO_2
8c, R = 4-CH_3, R_1 = 3,4,5-triOMe
8o, R = H, R_1 = 2-(pyridin-4-yl)
8p, R = H, R_1 = 4-(2,3-dihydrobenzo[d]oxazol-2-yl)

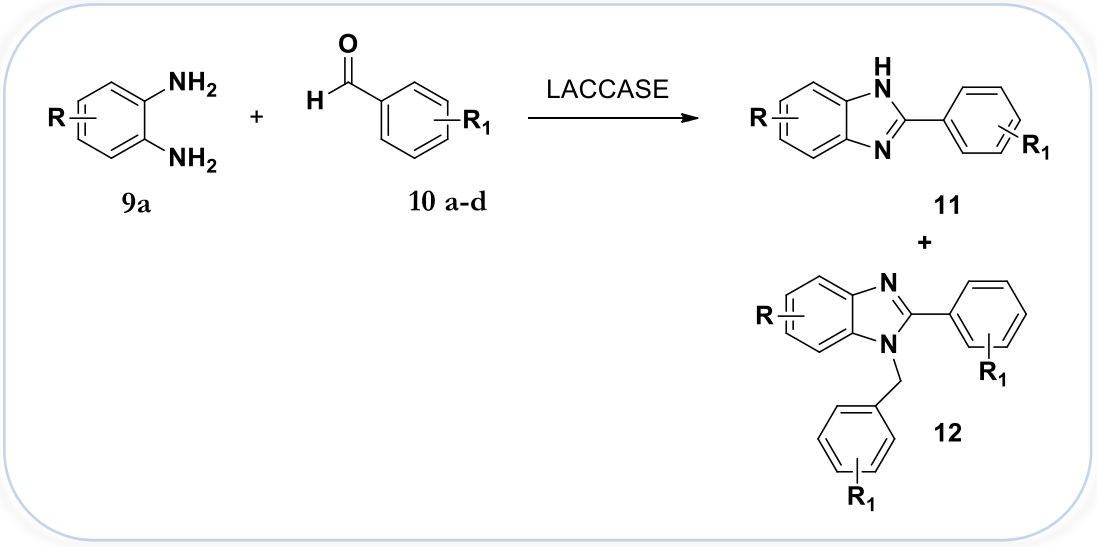
Compound	Aromatic region (ppm)	OH (ppm)	Aliphatic region (ppm)
7a	9.03 (1H, s, H7'); 8.09 (1H, d, H3'); 7.12 (1H, t, H5); 6.80 (1H, t, H4)	Not observed	-
7b	8.78 (1H, s, H7'); 8.74 (1H, s, H2)	7.15 (1H, s, OH),	-
7c	8.55 (1H, s, H7'); 7.14 (2H, s, H6', H2'); 7.07 (1H, s, H3)	Not observed	3.93 (9H, s, 3 x OMe); 2.31 (3H, s, CH_3)
7o	8.77 (2H, d, H5', H4'); 8.69 (1H, s, H7'); 7.75 (2H, d, H3', H5')	7.25 (2H, m, H4, OH)	-
7p	8.55 (1H, s, H7'), 7.80 (2H, d, H2', H6'),	7.24 (1H, s, OH)	3.07 (6H, s, 2 X CH_3)

Table 5: Key signals in the ^{13}C NMR spectra of amino phenols

Compound	Aromatic region (ppm)	-OMe (ppm)	-CH_3 (ppm)
7a	153.43 (C-7'), 152.55 (C-1), 136.21 (C-2), 135.37 (C-2'), 132.89 (C-1'), 132.32 (C-4')	-	-
7b	153.90 (C-7'), 152.71 (C-1), 148.80 (C-3'), 137.48 (C-2), 134.47 (C-1'), 133.96 (C-6'), 130.13 (C-5'), 129.96 (C-5)	-	-
7c	156.82 (C-7'), 153.55 (C-3'), 149.80 (C-5'), 141.28 (C-1), 135.36 (C-4'), 131.37 (C-2), 129.41 (C-1'), 119.71 (C-4)	61.02 (OMe-12); 56.25 (OMe-10)	20.69 (CH_3)
7o	154.33 (C-7'), 152.87 (C-3', C-5'), 150.69 (C-1'), 142.40 (C-1), 134.43 (C-2), 130.41 (C-6', C-2')	-	-
7p	157.15 (C-7'), 152.70 (C-4'), 151.89 (C-1), 136.65 (C-2), 130.55 (C-1'), 127.36 (C-2', C-6'), 124.05 (C-5), 119.93 (C-4)	-	40.14 (2 X CH_3)

2.3. Synthesis of benzimidazole derivatives

Laccase catalysed the synthesis of benzimidazole derivatives from reactions of aldehydes with derivatives of *o*-phenylenediamine using molecular oxygen as an oxidant. Leutbecher *et al.* (2011) discovered the laccase catalysed domino reaction between *o*-phenylenediamine with various aromatic aldehydes in aerobic conditions using a phosphate buffer and obtained 2-aryl-1H-benzimidazoles in good to excellent yields. It was discovered that when the reaction was carried out in the absence of laccase, otherwise under identical reaction conditions chemo-selectivity problems were encountered; where the reaction led to the formation of 2-substituted and 1,2-disubstituted benzimidazoles. However, in the presence of laccase chemo-selectivity was improved tremendously. Because most of the benzaldehyde substrates were not soluble in the buffer alone, methanol was used as a co-solvent to enhance the solubility of the substrates. However, it was under these reaction conditions where other reactions took place; reactions resulted in the formation of 2-substituted benzimidazole (**11**) and 1,2-disubstituted benzimidazole (**12**) as a side product and sometimes the latter could be isolated as the only product (**Scheme 21**) [83]. Although this protocol has been proven effective, the main disadvantage of this method is chemo-selectivity. Therefore it is important to develop a new laccase-catalysed synthetic methodology that is both effective and chemo-selective.



Scheme 21: Laccase catalysed reaction of 1 and 2 in acetate buffer (pH 4.5) using various co-solvents.

It has been shown that in the presence of an oxidizing agent the reaction favours the formation of product **11** over product **12** [92]. Following this, our aim was to first identify how and why the 1-

benzylated side product was formed and if its formation could be controlled and possibly be eliminated. Our initial approach was to investigate the selectivity of these reactions by varying the types of buffers and co-solvents used in the synthesis of these compounds. The co-solvents used in this study were methanol, ethanol, DMF, and acetonitrile and the buffers were acetate and phosphate at different pH values. To explore the role of various solvents towards selectivity, we investigated the reaction between *o*-phenylenediamine (**9a**) with benzaldehydes **10a–d** (Figure **15**) (1:2 ratio) in acetate buffer (pH 4.5) using laccase Suberase® from Novozymes at room temperature (**Scheme 21**). The reaction times for these reactions varied from 2 to 24 h; it was observed that for the majority of the substrates, the mono-substituted product (**11**) forms first and then followed by the 1-benzylated product (**12**). Therefore 24 h of reaction time was selected for the model studies to further increase the yield of compound **12**. Inferior results were obtained while using methanol and ethanol as co-solvents, as they both showed a great deal of competitive formation of products **11** and **12**. Good chemo-selectivity was observed with DMF and acetonitrile; with DMF we were able to minimize the formation of the 1-benzylated product, however some of the substrates resulted in the formation of side product **12** (**Table 6**). The results obtained compare to the ones reported in literature; using methanol as a co-solvent promoted the formation of product **12** and DMF promoted the formation of product **11** under the described reaction conditions [83, 88].

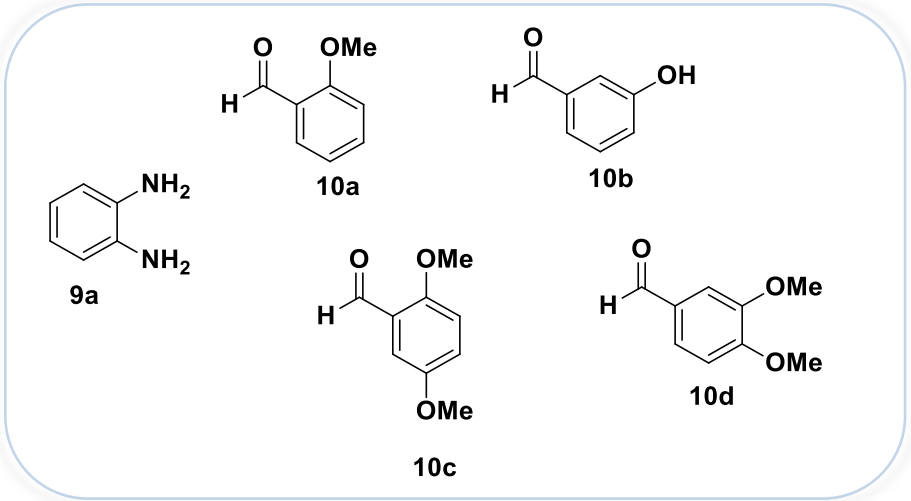


Figure 15: *O*-phenylenediamine and aromatic aldehydes used in this study

The relative ratios of products **11** and **12** obtained by laccase-catalysed oxidation were profoundly influenced by the type of organic solvent used. Similar results were obtained using a different laccase

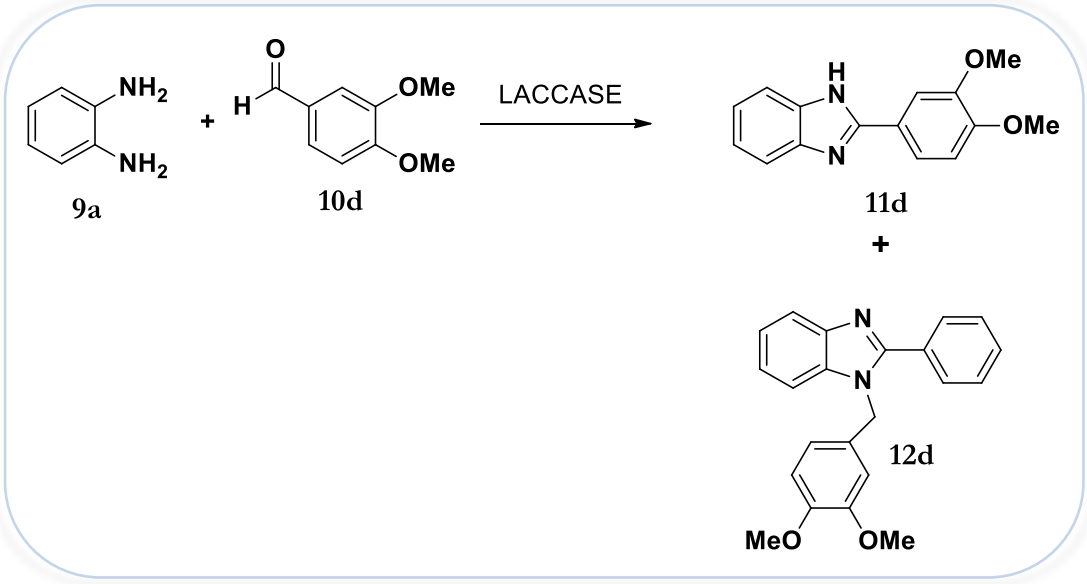
(Denilite® II base from Novozymes) which indicates the significance of the nature of the organic solvent for the laccase oxidation of phenolic Schiff bases to form 2-benzimidazoles.

Table 6: Reaction of *o*-phenylenediamine (**9a**) with benzaldehydes **10a–d** (1:2 ratio) in acetate buffer (pH 4.5) and a co-solvent under air at r.t., 24 h to afford product **11** and/or **12**

Entry	Aldehyde	Co-solvent	11 yield (%)	12 yield (%)
1	**10a**	Methanol	59	20
2	**10a**	Ethanol	-	13
3	**10a**	DMF	72	-
4	**10a**	Acetonitrile	62	-
5	**10b**	Methanol	-	-
6	**10b**	Ethanol	-	97
7	**10b**	DMF	-	98
8	**10b**	Acetonitrile	-	82
9	**10c**	Methanol	10	90
10	**10c**	Ethanol	25	70
11	**10c**	DMF	-	94
12	**10c**	Acetonitrile	-	56
13	**10d**	Methanol	34	66
14	**10d**	Ethanol	-	-
15	**10d**	DMF	67	23
16	**10d**	Acetonitrile	59	36

Our second approach was to explore the possible effect of the type of laccase used on selectivity of these reactions. When Fan *et al.* (2015) developed a highly chemo-selective method for either 2-benzimidazoles or 1, 2-benzimidazoles; it was demonstrated that the presence of a strong oxidizing agent such as hydrogen peroxide influences the production of the 2-substituded benzimidazoles over the 1,2-disubstituted benzimidazoles [92]. This implies that the redox potential of the laccase used

strongly influences the chemo-selectivity of the reactions. Therefore, using laccases (Suberase®, Denilite® II Base, and Novoprime Base 268) from Novozymes, we investigated the role of enzyme type on these reactions. The reaction between *o*-phenylenediamine (**9a**) with benzaldehydes **10d** was chosen as a model reaction and it was performed using an acetate buffer (pH 4.5) and acetonitrile at room temperature (**Scheme 22**), and the results are shown in **Figure 16**.



Scheme 22: Laccase-catalysed reaction of **9a** and **10d** in acetate buffer (pH 4.5) using acetonitrile as a co-solvent.

From the findings, it clearly indicates that the specific laccase influences the chemo-selectivity of benzimidazole derivatives to a great extent, based on the redox potential of each enzyme. Novoprime Base 268 (uncertain origin) presented the best oxidation results compared to the two laccases preparations from *Myceliophthora thermophile* (Suberase® and Denilite® II Base) for the formation of 2-(3,4-dimethoxyphenyl)-1H-benzimidazole (**11d**). The reaction conducted using Suberase® as our laccase afforded a yield of 59% of the 2-substituted benzimidazole **11d** and 36% was the 1,2-disubstituted benzimidazole **12d**. When using Denilite® II Base as our laccase, the ratio was 57% compound **11d** and 30% compound **12d**. And finally with Novoprime Base 268 we were able to produce 78% of compound **11d** and compound **12d** was not formed in these reaction conditions.

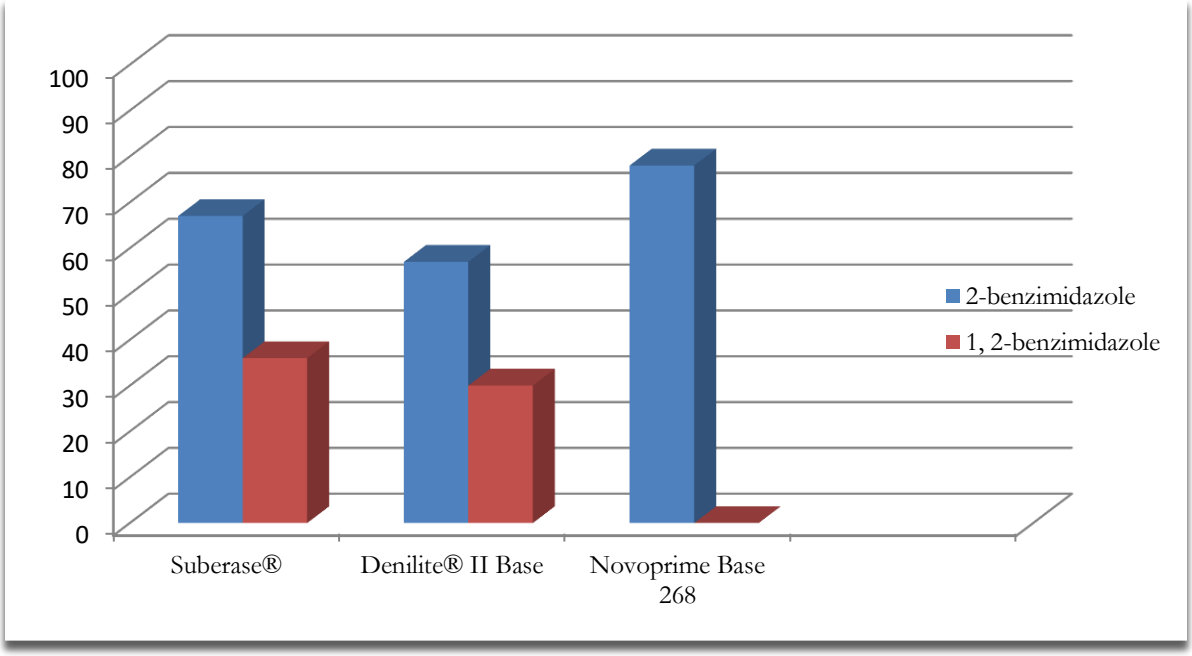


Figure 16: Suberase® and Denilite® are laccases from *Myceliophthora thermophile*. Novoprime – *uncertain origin*

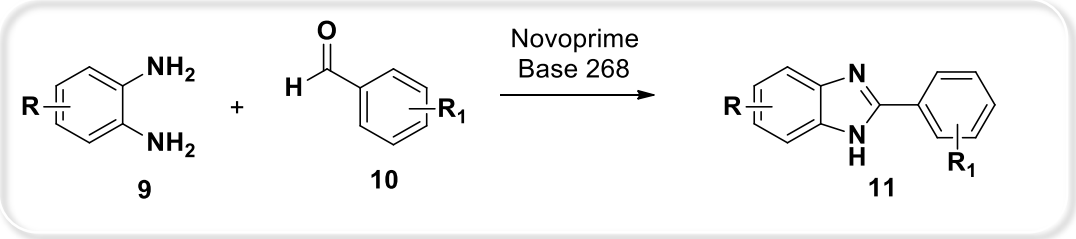
Finally, in order to identify the optimal conditions for these reactions we also considered the effects of using different buffers and pH towards the reaction selectivity. Leutbecher *et al.* (2011) investigated the effect of different buffers using acetate and phosphate buffers while varying buffer/co-solvent ratios. It was shown that the ratios of buffer/co-solvent affect chemo-selectivity and consequently the yield of the products formed [83]. In this study the buffer and pH studies were conducted using phosphate buffer (0.1 M, pH 6.0; 7.15; 7.5) and acetate buffer (0.1 M, pH 3.0; 4.0; 4.5; 5.0) systems using DMF and acetonitrile as co-solvents at room temperature. Reactions at lower pH values (3.0; 4.5; 5.0) occurred at faster rates than reactions carried out at higher pH values (7.15; 7.5).

Using the discovered optimal conditions (General method), derivatives of *o*-phenylenediamine were treated with a series of aromatic aldehydes using commercial laccase, Novoprime Base 268, from Novozymes at room temperature and the obtained results are summarised in **Table 7** below.

General method used to synthesize 2-aryl-1H-benzimidazole derivatives

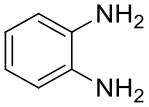
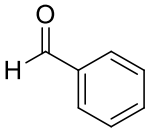
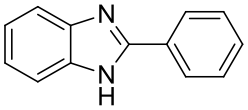
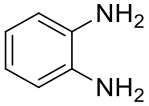
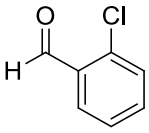
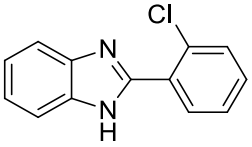
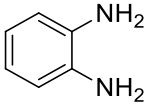
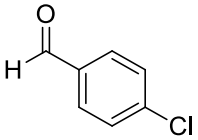
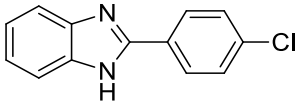
A mixture of *o*-phenylenediamine (10.0 mmol, 1 equiv) and benzaldehyde derivative (10.0 mmol, 1 equiv) in acetonitrile (10.0 mL) and acetate buffer (10.0 mL, pH 4.50) was stirred at room temperature for 5 minutes. Laccase (Novoprime Base 268 (0.105 g)) was added to the mixture and the contents

were stirred until reaction completes (monitored by TLC). The product precipitates from the solution as the reaction proceeds and after completion, the product was extracted with ethyl acetate (30.0 mL) and water (3 x 20.0 mL) and concentrated on a rotary evaporator. The product was washed several times with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material.



Scheme 23: Synthesis of 2-aryl-1H-benzimidazoles

Table 7: Reaction of *o*-phenylenediamines (1 equiv) and benzaldehyde derivatives (1 equiv) in acetonitrile and acetate buffer (pH 4.0) at room temperature

Entry	Amine	Benzaldehyde	Product	Yield (%)	Time (h)
1				92	2
2				94	2
3				88	4

a- Two equivalents of *o*-phenylenediamines were used

Table 7: Reaction of *o*-phenylenediamines (1 equiv) and benzaldehyde derivatives (1 equiv) in acetonitrile and acetate buffer (pH 4.0) at room temperature

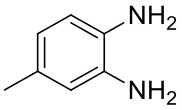
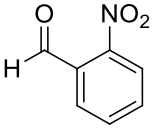
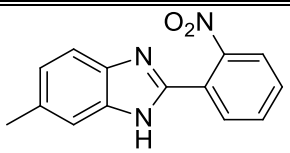
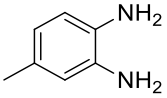
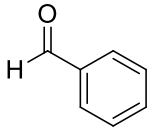
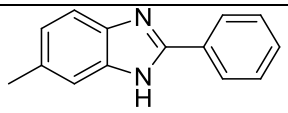
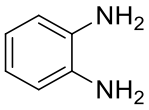
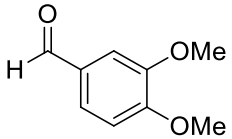
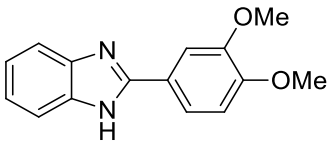
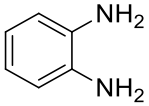
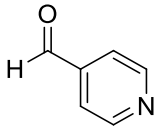
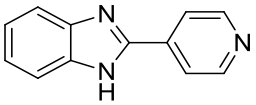
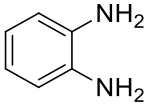
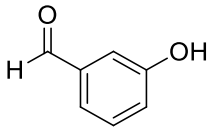
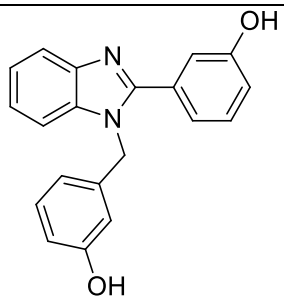
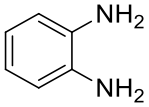
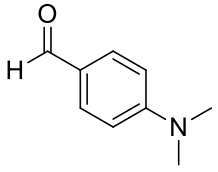
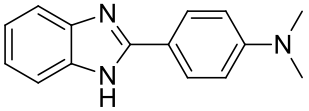
4				56	8
5				79	8
6				78	8
7				60	2
8				86	4
9				64	8

Table 7: Reaction of *o*-phenylenediamines (1 equiv) and benzaldehyde derivatives (1 equiv) in acetonitrile and acetate buffer (pH 4.0) at room temperature

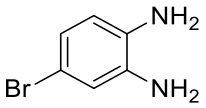
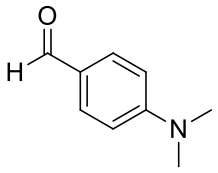
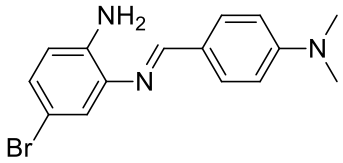
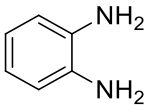
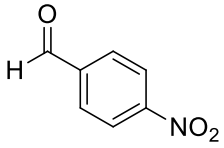
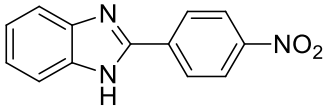
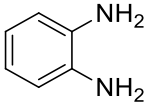
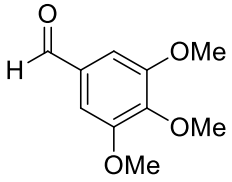
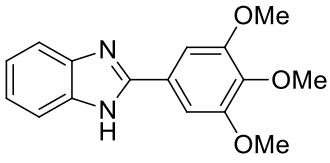
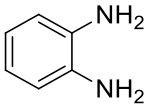
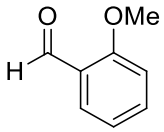
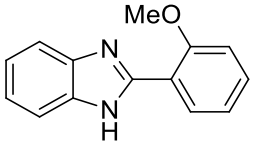
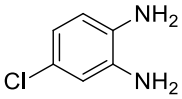
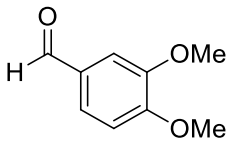
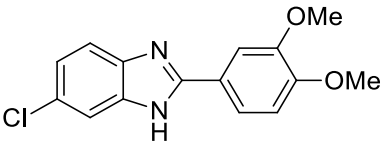
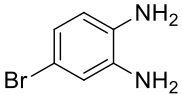
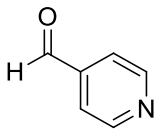
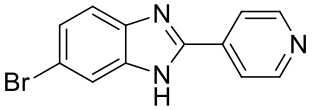
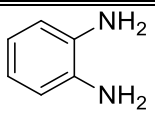
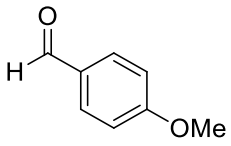
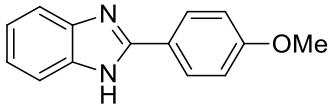
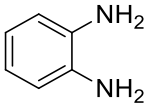
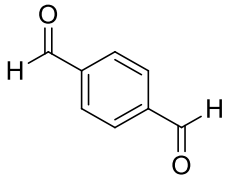
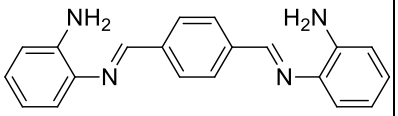
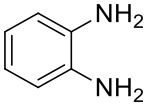
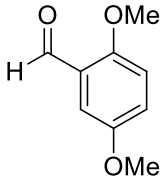
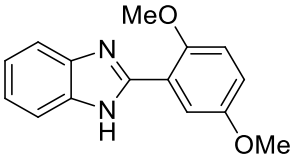
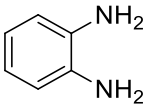
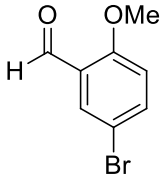
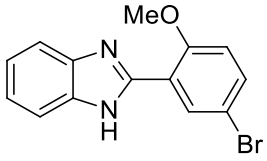
10				98	12
11				95	2
12				89	24
13				69	24
14				93	2
15				94	4

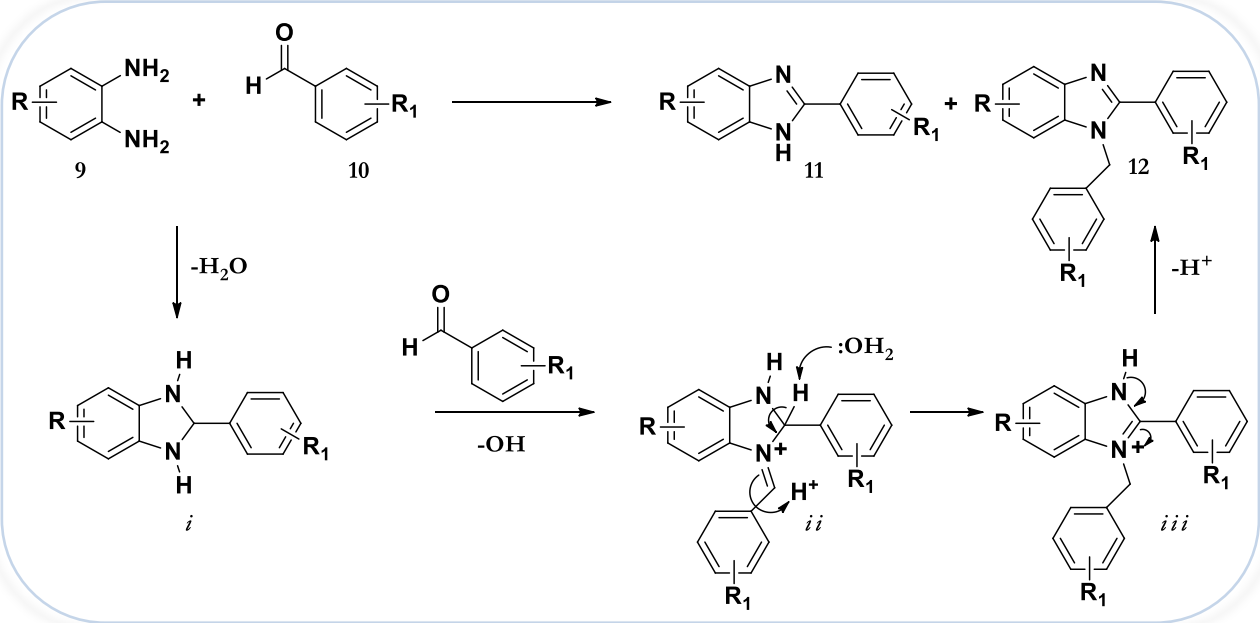
Table 7: Reaction of *o*-phenylenediamines (1 equiv) and benzaldehyde derivatives (1 equiv) in acetonitrile and acetate buffer (pH 4.0) at room temperature

16				68	24
17				98[a]	12
18				76	24
19				90	3

a- Two equivalents of *o*-phenylenediamines were used

As shown in **Table 7** above, 2-aryl-1H-benzimidazoles were obtained in good to excellent yields from different aromatic aldehydes. It is clear the method applies for a variety of aryl aldehydes containing both electron-donating and electron-withdrawing substituents around the ring. The structures of the obtained products were characterized using proton and carbon NMR and the keys signals for selected compounds are summarised in the **Tables 8** and **9** bellow. Similar to benzoxazole derivatives, some of the substrates did not cyclise further to the final product (**11** and/or **12**) (**Table 7**, entry 10 and 17). The formation of the 1, 2-disubstituted benzimidazole product was anticipated in this case, taking into

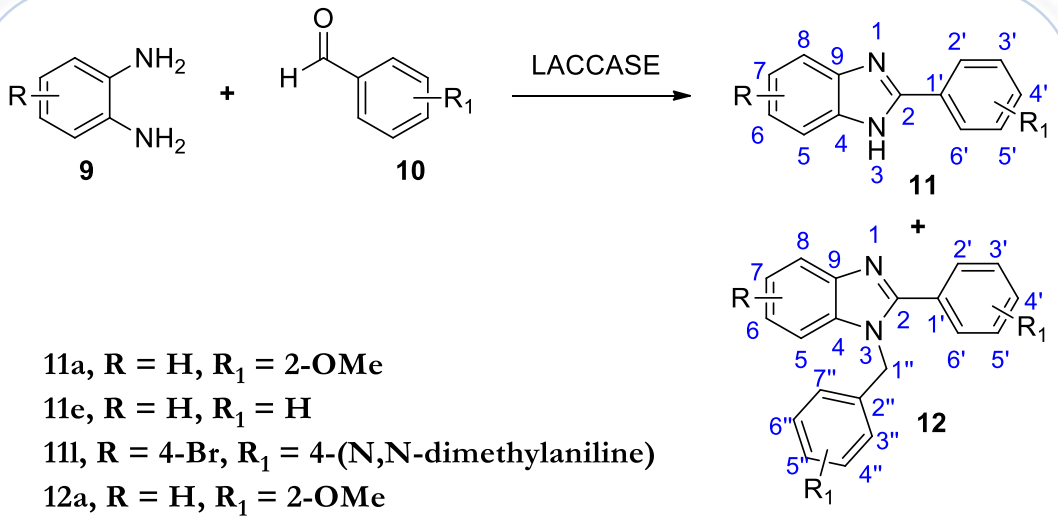
account the fact that it does not require an external oxidant in order to form. As shown in **Scheme 24** below; after the condensation of the diamine and the aromatic aldehyde, the subsequent 'intermediate' benzimidazoline ***i*** may condense further with another molecule of benzaldehyde to afford another 'intermediate' iminium ion ***ii***. Successively, this intermediate may tautomerize to the most stable form ***iii*** and reshuffle to give out the resultant disubstituted benzimidazole product **12** [88].



Scheme 24: Synthesis of the 1, 2-disubstituted benzimidazole product

In summary, our results correspond to literature reports; we have shown that the absence of an external oxidant and the use of organic solvents such as ethanol and methanol promotes the formation the 1,2-disubstituted product [83, 88]. We have demonstrated that using acetonitrile or DMF in the presence of a catalytic amount of laccase (Novoprime base 268) promotes the formation of the mono-substituted benzimidazole. Our method is simple, selective and can afford 2-aryl-1H-benzimidazoles in good to excellent yields (Key signals of some of the products obtained are summarized in **Tables 8** and **9**).

Table 8: Key signals in the **^{1}H NMR** spectra of 2-aryl-1H-benzimidazoles



Compound	Aromatic region (ppm)	NH (ppm)	Aliphatic region (ppm)
11a	8.33 (1H, d, H6'), 7.63 (2H, dd, H5, H8), 7.48 (1H, t, H4'), 7.24 (1H, d, H7)	12.11 (1H, s, NH)	4.03 (3H, s, OMe)
11e	8.19 (2H, d, H2', H6'), 7.68 (1H, d, H8), 7.53 (4H, m, H5, H7, H3', H5')	12.89 (s, 1H, NH)	-
11l	8.43 (1H, s, H7'), 7.78 (2H, d, , H2', H6'), 6.95 (1H, d, H6), 6.85 (1H, s, H3)	5.31 (2H, s, NH)	3.00 (6H, s, N-CH_3)
12a	7.67 (1H, d, H6'), 7.52 (1H, t, H5'), 7.42 (1H, d, H7"), 7.36 (1H, d, H3'), 7.27 – 7.12 (4H, m, H6, H7, H8, H4'), H4"),	-	5.21 (2H, s, H1")
12b	9.77 (1H, d, H6'), 9.43 (1H, s, H2'), 7.73 (1H, d, H8), 7.39 (1H, d, H5), 7.33 (1H, q, H5'), 7.23 (3H, td, H6, H7, H6"), 7.17 – 7.06 (2H, m, H1")	-	5.49 (2H, s, H1")

Table 9: Key signals in the **^{13}C NMR** spectra of 2-aryl-1H-benzimidazoles

Compound	Aromatic region (ppm)	-OMe (ppm)	-CH_2, -CH_3 (ppm)
11a	157.25 (C-2), 149.43 (C-2'), 143.20 (C-9), 135.19 (C-4), 131.70 (C-6'), 130.23 (C-4'), 122.51 (C-5'), 121.96 (C-1'), 121.34 (C-3')	56.25 (OMe)	-
11e	151.69 (C-2), 144.29 (C-9, C-4), 135.47 (C-1'), 130.65 (C-4'), 130.29 (C-3', C-5'), 129.40 (C-2'), 126.90 (C-6')	-	-
11l	157.60 (C-7'), 152.76 (C-4'), 145.55 (C-1, C-2), 136.21 (C-1'), 130.76 (C-2', C-6'), 124.66 (C-5), 119.01 (C-6), 118.80 (C-3)	-	Not observed[a]
12a	157.53 (C-3"), 156.83 (C-2'), 152.31 (C-2), 143.35 (C-4), 135.68 (C-9), 132.39 (C-6'), 132.02 (C-4'), 129.17 (C-7"), 127.94 (C-5"), 124.64 (C-2"), 122.66 (C-7), 122.03 (C-6), 120.98 (C-5'), 120.57 (C-6"), 120.00 (C-1')	55.75 (OMe), 55.73 (OMe)	43.25 (C-1")
12b	156.09 (C-3'), 155.91 (C-4"), 151.69 (C-2), 140.98 (C-4), 136.71 (C-9, C-2"), 134.25 (C-1'), 129.58 (C-5'), 128.19 (C-6"), 120.96 (C-7), 120.52 (C-6), 117.87 (C-6')	-	45.81 (C-1")

a- For the exact structure, refer to compound **11l** in Section 4.7.11

2.4. Synthesis of benzothiazole derivatives

It has been mentioned that condensation–dehydration reaction of 2-aminothiophenol with aryl aldehydes under oxidative conditions is one of the traditional methods for preparing benzothiazoles. However, with an increasing demand for the development of green processes, the preparation of 2-arylbenzothiazoles using environmentally friendly reagents such as biocatalysts is of great importance. Herein we report an effective method for the one-pot synthesis of 2-arylbenzothiazoles from condensation–dehydration reaction of 2-aminothiophenol with aryl-aldehydes in the presence of a catalytic amount of a commercially available laccase (Suberase® from Novozymes). To the best of our knowledge, a laccase-catalysed synthesis of 2-arylbenzothiazoles from condensation–dehydration reaction of 2-aminothiophenol with aryl-aldehydes has not been reported before. The model reaction selected was the oxidative condensation between 2-aminothiophenol (**13**) and benzaldehyde (**14a**) under aerobic conditions at room temperature. The laccase-catalysed cross-coupling reaction between **13** and **14a** to afford 2-phenyl-benzothiazole (**15a**) (**Figure 17**) in 85% yield was performed in an acetate buffer (0.1 M, pH 4.0) using acetonitrile (50%) as a co-solvent (**Scheme 24**, **Table 7**, entry 4).

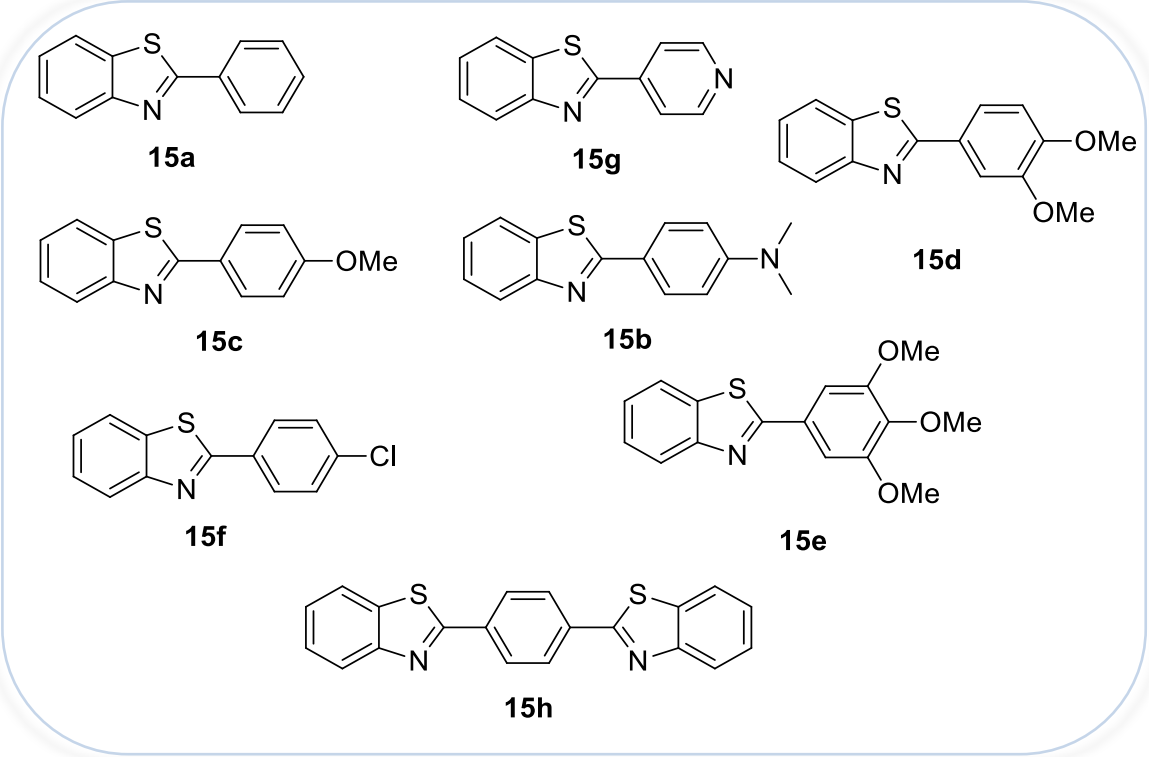
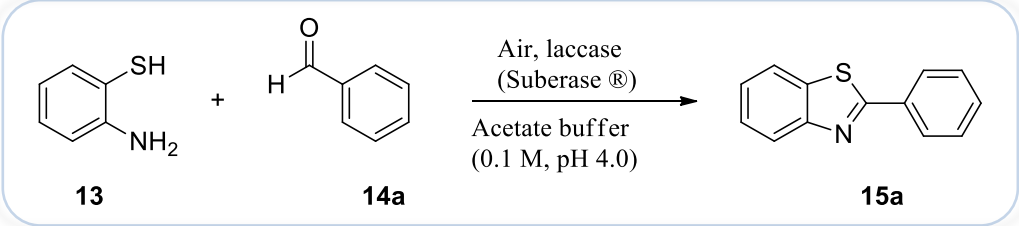


Figure 17: Products **15a–h** of the laccase-catalysed domino reaction between **13a** and **14a–g**.

The effect of organic solvents used when preparing benzothiazoles has been demonstrated in literature; solvents such as ethanol, acetonitrile, hexane, chloroform, DMSO and water have been investigated [106, 107]. Sayyahi *et al.* (2015) identified ethanol as the best solvent for preparing 2-arylbenzothiazoles under reflux conditions [107]. Gao *et al.* (2014) described water as the optimal solvent using KI catalyst [108]. Therefore, in order to identify optimum reaction conditions we explored the effect of using various co-solvents. 2-Aminothiophenol (**13**) and benzaldehyde (**14a**) were reacted in the presence of a laccase (Suberase®, from Novozymes) in acetate buffer (pH 4.0) using various solvents at room temperature (**Table 10)**.



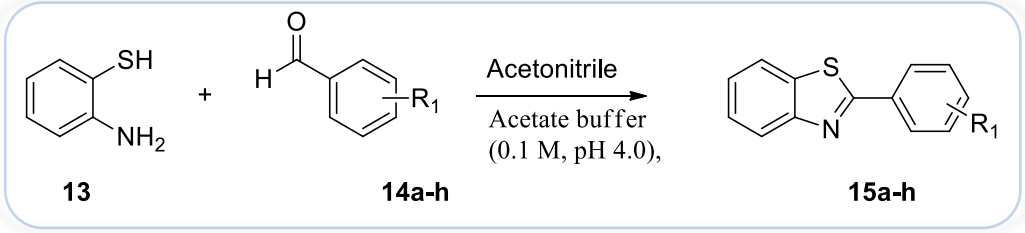
Scheme 25: Laccase-catalysed synthesis of 2-phenyl-benzothiazole

Table 10: Laccase-catalysed synthesis of 2-phenyl-benzothiazole (**15a**) by reaction of 2-aminothiophenol (**13**) and benzaldehydes **14a** in acetate buffer (0.1 M, pH 4.0) using various co-solvents

Entry	Aldehyde	Co-solvent	Time (h)	Product	Yield (%)
1	**14a**	Methanol	24h	**15a**	58
2	**14a**	Ethanol	24h	**15a**	55
3	**14a**	DMF	24h	**15a**	78
4	**14a**	Acetonitrile	2h	**15a**	85
5	**14a**	DCM	24h	**15a**	65

Acetonitrile gave the best yield under the described reaction conditions. When the reaction completes the product precipitates out of solution and any remaining starting material can be washed with cold acetonitrile to yield pure product. While using methanol and ethanol as co-solvents, the reaction did not go to completion after 24 h of reaction and the product (**15a**) (yellow oil) was difficult to isolate from the starting material (2-aminothiophenol (**13**)) as they both have almost indistinguishable R_f values (40% EtOAc/hexane). Sayyahi *et al.* (2015) obtained excellent yields using ethanol and the reaction did not go to completion while using acetonitrile, when using the ionic liquid [bmim][$FeCl_4$] as a catalyst [107]. However, we obtained inferior results while using laccase as an oxidising agent in ethanol, while conversely acetonitrile gave an excellent yield. In order to purify compound **15a** from the starting material, the product was dried overnight under high-vacuum to completely dry-out the oily product to almost solid form, and cold acetonitrile was added to propel precipitation of the product. As mentioned before, another amount of cold acetonitrile (3 x 20.0 mL) was used to wash the starting material off the product.

In order to elucidate the role of the enzyme as a catalyst, control reactions were conducted using benzaldehydes **14a–h** under air in acetate buffer (0.1 M, pH 4.0)/acetonitrile (50:50) in the absence of laccase (**Scheme 26**). However only two substrates resulted in the formation of a fused product in very low yield (<10%) and no activity was observed for the remaining substrates (**Table 11**). Conversely, quantitative yields of the same substrates were obtained in the presence of an enzyme.



Scheme 26: Laccase-free synthesis of benzothiazoles

Table 11: Reaction of 1 equiv of 2-aminothiophenol (**13**) with 1 equiv of benzaldehydes 14**a–h** in acetate buffer (0.1 M, pH 4.0)/acetonitrile (50:50), for 24 h in the absence of laccase

Entry	Aldehyde	Product	Yield (%)
1	**14a**	**15a**	-
2	**14b**	**15b**	8
3	**14c**	**15c**	-
4	**14d**	**15d**	-
5	**14e**	**15e**	7
6	**14f**	**15f**	-
7	**14g**	**15g**	c
8	**14h**	**15h**	-

c - Two equiv of 2-aminothiophenol (**13**) were used and no product was obtained

At this point, the most efficient method presenting the highest catalytic activity for preparing 2-arylbenzothiazoles was using acetonitrile and acetate buffer (pH 4.0), similar to the method described for 2-aryl-benzimidazole. Therefore to study the range and limitations of the optimised procedure 2-aminothiophenol was treated with a series of aryl-aldehydes in the presence of a catalytic amount of laccase to yield 2-arylbenzothiazole derivatives in good to excellent yield at room temperature (General method) and the results obtained are summarized in **Table 12**. Similar reactivity was observed for benzaldehydes with a variety of substituent groups; both electron-withdrawing and electron-donating at various positions showed comparable yields and reaction rates.

General method used to synthesize 2-aryl-benzothiazole derivatives

A mixture of 2-aminothiophenol (1.63 g, 15.0 mmol) and benzaldehyde derivative (1.06 g, 10.0 mmol) in acetonitrile (10.0 mL) and acetate buffer (10.0 mL, pH 4.0) was stirred at room for 5 minutes. Suberase® (2.00 mL) was then added into the mixture and the contents were stirred for 1 h. When the reaction completes, the product precipitates from the solution and was extracted with ethyl acetate (30.0 mL) and water (3 x 20.0 mL) and concentrated on a rotary evaporator. The product was washed several times with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material.

Table 12: Reaction of 2-aminothiophenol (1.5 equiv) and benzaldehyde derivatives (1 equiv) in acetonitrile and acetate buffer (pH 4.00) at room temperature

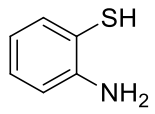
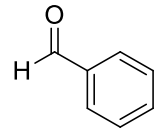
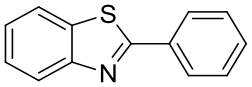
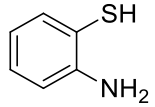
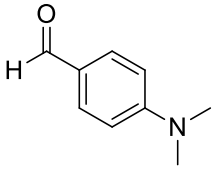
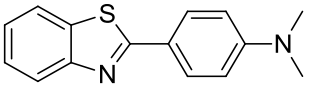
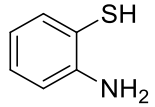
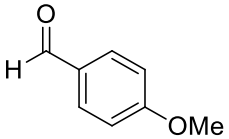
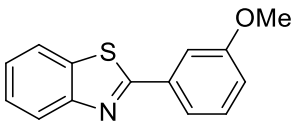
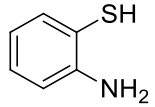
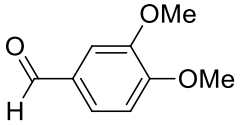
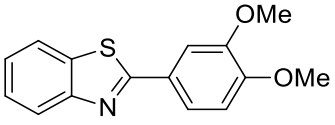
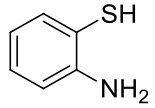
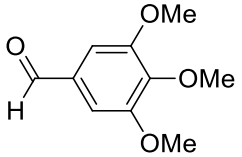
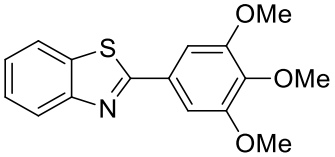
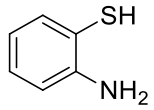
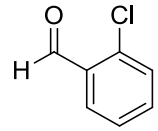
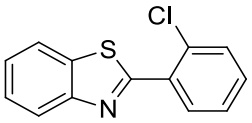
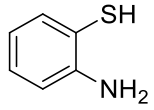
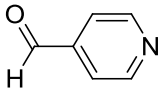
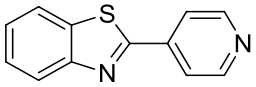
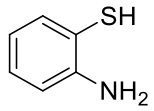
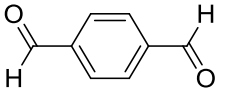
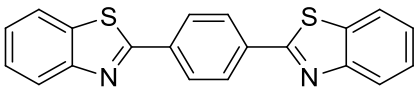
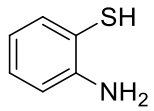
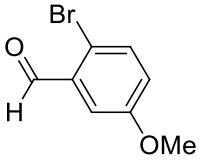
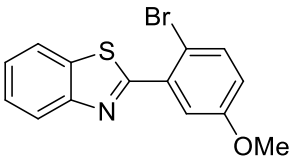
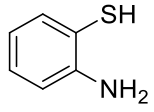
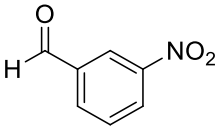
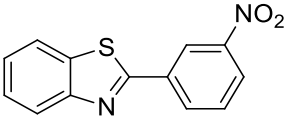
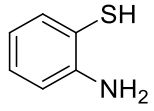
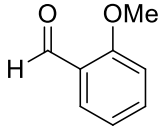
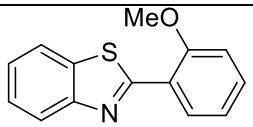
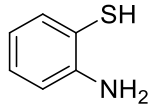
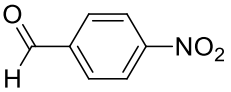
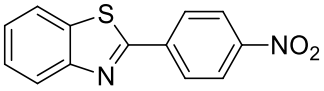
Entry	Amine	Benzaldehyde	Product	Yield (%)	Time (h)
1				85	24
2				56	24
3				68	24
4				73	24
5				65	24

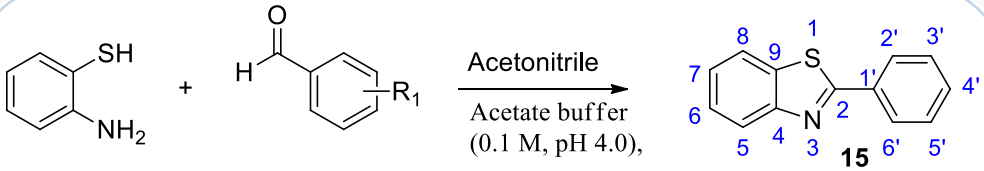
Table 12: Reaction of 2-aminothiophenol (1.5 equiv) and benzaldehyde derivatives (1 equiv) in acetonitrile and acetate buffer (pH 4.00) at room temperature

6				56	24
7				88	24
8				87[a]	24
9				76	24
10				48	24
11				74	24
12				84	24

a- Two equivalents of 2-aminothiophenol were used

We have successfully developed a simple and efficient method for the synthesis of 2-arylbenzothiazole derivatives using laccase as a catalyst at room temperature. The structures proposed are consistent with analytical data from the literature. Key signals for selected **^{1}H** and **^{13}C NMR** spectra are shown in **Tables 13** and **14**.

Table 13: Key signals in the **^{1}H NMR** spectra of 2-aryl-benzothiazoles



13 **14a-h** **15**

15a, R_1 = H
15b, R_1 = 4-(N,N-dimethylaniline)
15c, R_1 = 4-OMe
15g, R_1 = 2-(pyridin-4-yl)
15i, R_1 = 2-Br, 5-OMe

Compound	Aromatic region (ppm)	Aliphatic region (ppm)
15a	8.15 – 8.04 (3H, m, H8, H2', H6'), 7.91 (1H, d, H5), 7.50 (4H, m, H6, H7, H3', H5')	-
15b	8.03 – 7.91 (2H, m, H2', H6'), 7.84 (1H, d, H5), 7.44 (1H, t, H6), 7.30 (1H, t, H7)	3.01 (6H, d, N-CH_3)
15c	8.04 (3H, d, H5, H2', H6'), 7.88 (1H, d, H8), 7.47 (1H, t, H6), 7.35 (1H, t, H7)	3.88 (3H, s, OMe)
15g	8.78 (2H, d, H3', H5'), 8.13 (1H, d, H5), 7.99 – 7.87 (3H, m, H8, H2', H6')	-
15i	8.68 (1H, d, H5), 8.10 (1H, d, H8), 7.93 (1H d, H3'), 7.57 – 7.46 (2H, m, H6, H6')	4.04 (3H, s, OMe)

Table 14: Key signals in the **^{13}C NMR** spectra of 2-aryl-benzothiazoles

Compound	Aromatic region (ppm)	-OMe (ppm)	-CH_3 (ppm)
15a	154.17 (C-2), 135.08 (C-4), 133.65 (C-1'), 130.97 (C-9), 129.03 (C-2', C-6'), 127.57 (C-5'), 126.32 (C-3'), 125.19 (C-4')	-	-
15b	168.79 (C-2), 154.43 (C-4'), 152.20 (C-4), 134.56 (C-9), 128.87 (C-6'), 127.71 (C-2'), 125.97 (C-6), 125.29 (C-7), 124.18 (C-1')	-	40.48 (N-CH_3) 40.18 (N-CH_3)
15c	167.85 (C-2), 161.94 (C-4'), 154.24 (C-4), 134.87 (C-1'), 129.12 (C-9), 126.47 (C-6'), 126.19 (C-2'), 124.79 (C-6), 122.83 (C-7)	55.46 (OMe)	-
15g	165.10 (C-4), 153.99 (C-2), 150.77 (C-3', C-5'), 140.49 (C-1'), 135.23 (C-9), 126.82 (C-6), 126.20 (C-7), 123.94 (C-8)	-	-
15i	161.43 (C-2), 156.22 (C-5'), 152.00 (C-4), 136.20 (C-1'), 134.12 (C-9), 131.88 (C-3'), 126.11 (C-6), 124.93 (C-7), 124.05 (C-8)	56.03 (OMe)	-

2.5. Synthesis of aminobenzoquinones

There are a limited number of reports on the synthesis of aminobenzoquinones derived from amination of hydrobenzoquinones by aliphatic and aromatic amine using chemical oxidants. This is because there are a number of problems encountered when using chemical oxidants, these includes issues such as susceptibility of amino groups to oxidation and hydrolysis [2]. Other problems encountered using chemical oxidants include the issue of selectivity between the formation of monoaminated and diaminated products [109]. Chemical oxidants that have been successfully used to achieve amination of *p*-hydroquinones include sodium iodate, cupric acetate and silver (I) oxide [2, 110]. It has been demonstrated in literature that laccases from different origins have a great influence on reactions [46, 48]. In this section we investigated how different laccases can be used to develop ideal conditions for the production and improvement of yields of the monoaminated and diaminated products for the synthesis of aminobenzoquinones. The laccase catalysed nuclear amination of aromatic hydrobenzoquinones with aliphatic and aromatic amine molecules was investigated at mild reaction conditions using commercial laccases from Novozymes (Suberase®, Denilite® II Base, and Novoprime Base 268). Other factors that impact on reaction yield were explored; these include conducting reactions under dilute conditions, sequential addition of enzyme and substrate over time, and also the effect of buffer and co-solvents.

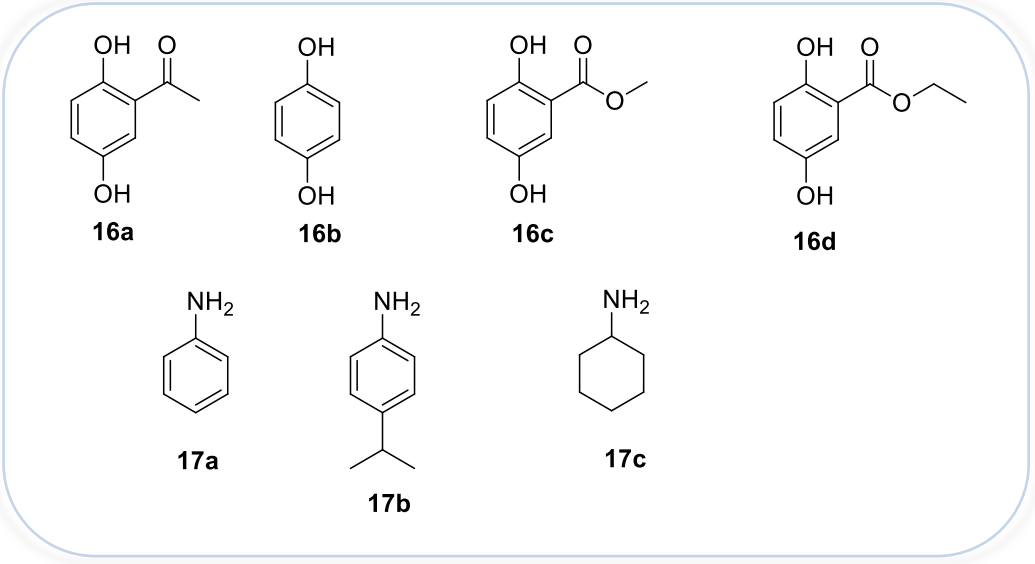
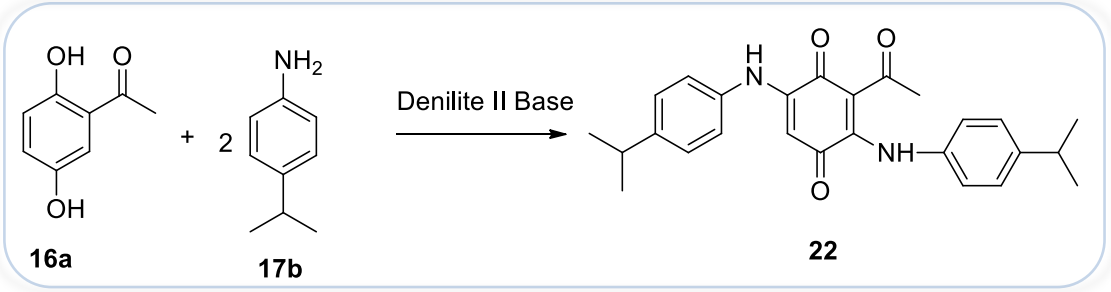


Figure 18: Examples of *p*-hydrobenzoquinones and primary amines used in this study

Wellington *et al.* (2010) demonstrated a laccase-catalysed synthesis of diaminobenzoquinones using the commercial laccase, Denilite® II Base (on an inert support) from Novozymes. The report describes an efficient protocol for preparing diaminobenzoquinones derived from *p*-dihydroxylated benzoic acid derivatives and aryl and alkyl primary amines [55]. A drawback of this method was that the yields of the products produced were exceedingly low. It was suggested that factors such as the number of equivalents of primary amines, effect of co-solvent and solubility of substrates and also laccase activity affected the formation of products. Following from this, our first approach to solve this problem was to investigate the effect of different co-solvents on the solubility of the substrate and how they impact the yields. Since it has been shown in a number of reports that nuclear amination reactions of *p*-hydroquinones by primary amines favour the formation of diaminated products over monoaminated products; reactions were conducted using two equivalents of the primary amine [2, 55]. Following from Wellington *et al.* (2010) similar substrates were chosen (**Figure 18**), and we focused on the ones which resulted in lower yields and developed methods to make improvements.

For the investigation of solvent effects on reaction yields, the laccase-catalysed reaction of 2,5-dihydroxyacetophenone (10.0 mmol, 1.52 g) and 4-isopropylaniline (20.0 mmol, 2.70 g) in acetate buffer (20.0 mL, 0.1 M, pH 4.00) was chosen as the model reaction (**Scheme 27**). The reaction was carried out in the presence of various co-solvents such as methanol, DMF, and acetonitrile at room temperature and the results obtained are summarized in **Table 15**. Different reactivity and yields were observed while using different solvent systems, as reported by Wellington *et al.* (2010) [2].



Scheme 27: Laccase-catalysed reaction between 2,5-dihydroxyacetophenone and 4-isopropylaniline

Table 15: Reactions between 2,5 dihydroxyacetophenone and 4-isopropylaniline were catalysed by Denilite® II base in different co-solvent systems

Entry	Buffer/co-solvent (mL)	Co-solvent	Reaction time	Product (22) yield
1	9:1	Methanol	72 h	12%
2	10:10	Acetonitrile	72 h	17%
3	10:10	DMF	72 h	25%

The reactions between 2,5-dihydroxyacetophenone and 4-isopropylaniline were catalysed by Denilite® II base from Novozymes in various solvent systems. The main obstacle in these reactions was the solubility of the substrate in the reaction vessel, as mentioned, the various co-solvents deployed helped to enhance the solubility of the substrates. For the tested solvents, methanol and acetonitrile showed poor results; the hydroquinone substrate tends to precipitate out of solution during the course of the reaction which then prevents the reaction from moving forward. DMF showed some promising results, however to an extent; the reaction moves forward for the first 6 hours before the substrate precipitates out. Because of this factor, the reactions were diluted to lower concentrations to reduce the effect of the substrate precipitating out of solution during the reaction. The 2,5-dihydroxyacetophenone substrate was dissolved in DMF and was added dropwise to the mixture over time and this improved the yield significantly (**Table 16**). Since it is understood from literature that the role of laccase in the reaction is to oxidise the hydroquinone to a quinone [2]; the addition of the hydroquinone at a slower rate would ensure enough and fresh enzyme to oxidise the substrate. Wellington *et al.* (2010) reported that the reactions did not go to completion because the hydroquinone was not being completely converted to the quinone, therefore slow addition of hydroquinone with an increase of enzyme quantity would ensure complete conversion to quinone.

Table 16: Reactions between 2,5-dihydroxyacetophenone and 4-isopropylaniline were catalysed by Denilite® II base in different co-solvent systems under dilute conditions

Entry	Buffer/co-solvent (mL)	Co-solvent	Reaction time	Product (22) yield
1	36:4	Methanol	72 h	21%
2	20:20	Acetonitrile	72 h	28%
3	20:20	DMF	72 h	42%

Our second approach was to investigate the effect of using different types of laccases for the oxidation of the hydroquinone substrates. Using DMF as the optimal co-solvent, various commercial laccases from Novozymes (Suberase®, Denilite® II Base, and Novoprime Base 268) were tested for the model reaction (**Table 17**). Novoprime Base 268 gave the best yield for these transformations using acetate buffer at room temperature. As discussed by Wellington *et al.* (2010), the enzymes becomes denatured over time during reaction; as a consequence it was decided to increase the amount of enzymes at different time intervals to reducing the possibility of the enzyme denaturing all at once. This effect improved the yields of some the products [2].

Table 17: Reactions between 2,5-dihydroxyacetophenone and 4-isopropylaniline catalysed by various laccases under dilute conditions

Entry	Buffer/DMF (mL)	Laccase	Reaction time (h)	Product yield (%)
1	20:20	Suberase®	72	19
2	20:20	Denilite® II Base	72	42
3	20:20	Novoprime Base 268	72	45

To account for solubility issues due to precipitation of substrate and the effect of enzyme denaturing during reaction, we decided to sequentially add both enzyme and substrate slowly over time to increase the reaction yield. This method proved to be very effective and was applied to various substrates and excellent yields were obtained (**Table 18**).

Table 18: Reactions between 2,5-dihydroxyacetophenone (1 equiv) and primary amine (2 equiv) catalysed by Novoprime Base 268 under dilute conditions (using Method Z)

Entry	Hydroquinone	Primary amine	Product	Reaction time (h)	Yield (%)	Yield (%) by Wellington *et al.* (2010)
1	**16a**	**17b**	**18**	24	72	52
2	**16b**	**17a**	**19**	72	100[a]	-
3	**16b**	**17b**	**20**	72	56	32
4	**16b**	**17c**	**-**	72	**[b]	-
5	**16a**	**17a**	**-**	18	72[c]	32
6	**16c**	**17b**	**21**	12	28[d]	20
7	**16d**	**17a**	**22**	4	100	25

a- The product obtained was the mono-aminate product not diaminated
b- The actual yields were not obtained due to quinone decomposition during purification by column chromatography
c- The yield obtained was lower compared to the conversion (TLC) which was high, product decomposed during separation
d- Product was not characterised due to decomposition during purification, yield based on previous experimental data.

In summary, the combination of methods such as reactions under dilute conditions, sequential addition of enzyme and substrate over time, and using Novoprime Base 268 laccase; afforded five diaminobenzoquinones and one monoaminobenzoquinone (**Table 18**, entry 2) from the *p*-hydrobenzoquinones in excellent yields (**Figure 19**). From the data in **Table 18** it can be observed that the yields range from moderate (entry 6) to excellent (entries 1, 2 and 7) when the reactions were conducted using a combination of the described methods (Method Z). One monoaminated product 2-(phenylamino)cyclohexa-2,5-diene-1,4-dione (entry 2) was obtained using one equivalent of *p*-

hydrobenzoquinone and primary amine; from TLC results all the starting material was depleted after 72 h of reaction and only a single product was obtained. Some of the substrates were not fully characterised (entries 4 and 5) due to the effect of quinone decomposition on silica during purification by column chromatography, as detected by TLC. For product **21**, the yield obtained was 28%, but better conversion was estimated from TLC during reaction; this implies that product could have been lost during purification. Nonetheless, the yield is an improvement on Wellington *et al.* (2010) (20%). A 100% yield was obtained for the diaminated product **22** of which the highest obtained by Wellington *et al.* (2010) was 25%. Overall this method has proven to be successful for preparing both mono and diaminatedbenzoquinones at room temperature and could be applied to a much broader range of substrates.

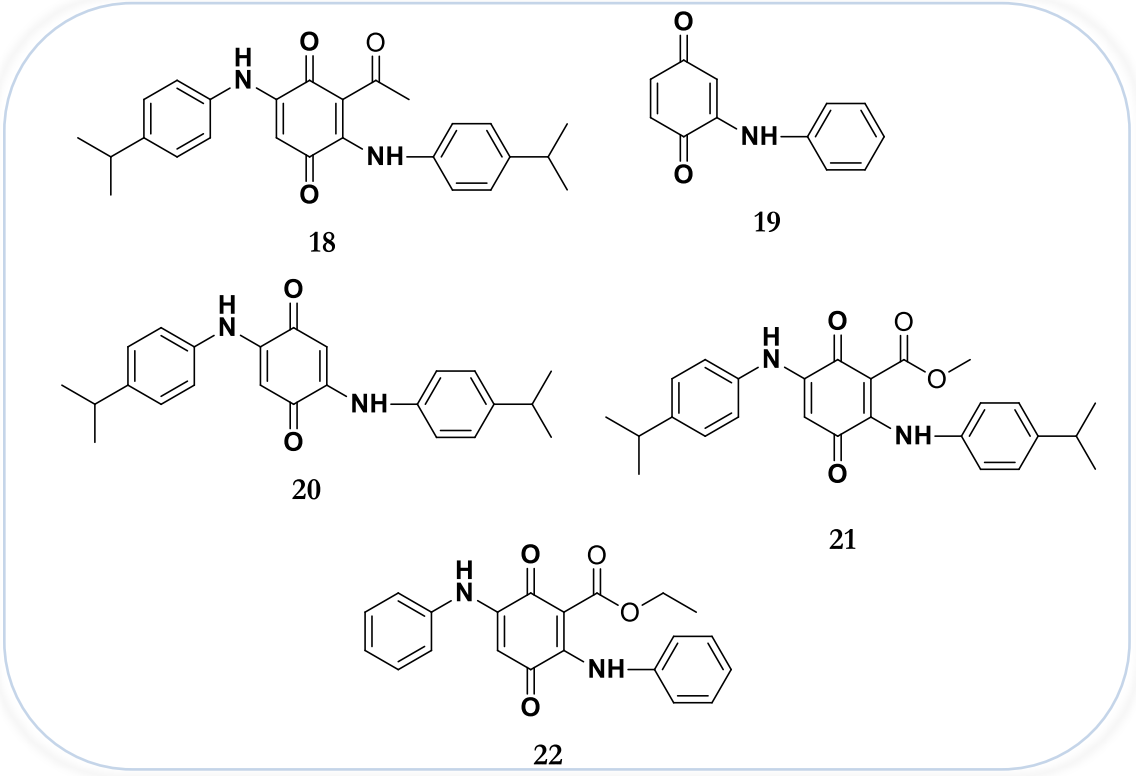


Figure 19: Structures of nuclear amination products

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Chapter 3

CONCLUSIONS AND FUTURE WORK

The overall aim of the project was to investigate the range and limitations of applications of laccase enzymes in organic synthesis. We were able to study the catalysis of bond formation of reactions such as oxidation, amination, and dimerization precisely on cross-coupling reactions of Carbon, Nitrogen, Oxygen, and Sulphur based aromatic compounds using laccase as a catalyst. In total we produced 56 compounds from four different classes.

We investigated the potential use of laccases to synthesise biaryl bonds from substituted phenol substrates, but this synthesis was not accomplished. Instances of polymerisation were encountered a few times, however due to solubility problems the products were not characterised. Based on the literature, it could be argued that the laccases used in this study did not possess enough oxidative potency to initiate phenoxy radicals to generate biaryl bonds for most of the substrates.

We investigated the synthesis of benzoxazoles; our main interest was to develop optimal conditions for the synthesis of benzoxazoles through a method that is selective and suitable for large scale preparations. We investigated the one-pot synthesis benzoxazoles from a condensation reaction between 2-aminophenol and aromatic aldehydes using laccase as an oxidizing catalyst. As the cyclisation of Schiff base **7** step did not take place, direct benzoxazole synthesis was not achieved. However we achieved the synthesis of the intermediate aminophenol derivatives, which are important building blocks for benzoxazoles, in good to excellent yields. Including ABTS as a reaction mediator under the same conditions resulted in the conversion of 2-aminophenol to 2-amino-3H-phenoxazin-3-one (**4**) at a reasonable yield. With further optimization of the reaction conditions using laccases and possibly inclusion of other oxidants, this method may yet be useful for effectively preparing benzoxazoles.

We investigated the selectivity factor for the production of 2-aryl-1H-benzimidazoles from the condensation reaction between *o*-phenylenediamine and benzaldehyde derivatives in the presence of a laccase. Laccase successfully catalysed the synthesis 2-aryl-1H-benzimindazole derivatives at room

temperature with high chemo-selectivity. The optimal conditions for conducting these reactions appear to be using Novoprime Base 268 in acetate buffer (0.1 M, pH 4.5) and acetonitrile as a co-solvent, compared to phosphate buffer systems. Good results were also obtained using DMF and acetate buffer (0.1 M, pH 4.5), however the reactions were less selective in these conditions; some substrates led to formation of the disubstituted side product. Overall we have shown that the absence of a chemical oxidant, types of organic solvents used, and the oxidative potency of laccases significantly affect the chemo-selectivity of these reactions.

We have also demonstrated that conducting reactions under dilute conditions, with gradual addition of enzyme and substrate over time, greatly improved the previously achieved reaction yields of aminobenzoquinones.

Future work

Develop optimal conditions for obtaining biaryl compounds by exploring different mediators and laccase enzymes. Further develop methods that promote ring closer such as the use of an external nucleophile and consideration of other laccase sources and mediators for the preparation of 2-aryl-benzoxazoles.

Chapter 4

EXPERIMENTAL SECTION

4.1. General

All chemicals were reagent grade materials obtained from Sigma-Aldrich (South Africa).

4.2. Materials

The solvents used for reactions were methanol, ethanol, DCM, DMF, ethyl acetate and acetonitrile and were purchased from Sigma-Aldrich (South Africa) or Merck KGaA (South Africa). Purification of some of the compounds was done using Macherey-Nagel silica gel 60 (particle size 0.063 mm to 0.20 mm) and the solvents used (ethyl acetate and hexane) were distilled before use to remove any impurities. Reactions were monitored by TLC carried out on Merck Aluminium foil backed plates coated with silica gel (60 F254) and visualization was done under UV light.

4.3. Substrates

All the substrates used were purchased from Sigma-Aldrich (South Africa) and were used as received without any further purification.

4.4. Enzymes

Myceliophthora thermophilia laccases (Suberase® and Denilite® II Base) and Novoprime Base 268 (uncertain origin) were obtained from Novozymes.

4.5. Instrumentation

All the melting point recordings of the compounds were performed on a Stuart SMP10 instrument. Bruker 300 and 500 MHz spectrometers were used to record both the ^{1}H and ^{13}C Nuclear Magnetic Resonance data using a suitable solvent at room temperature. Data processing of the spectra was done using MestreNova Software under license from Mestrelab Research, CA, USA. Bruker Tensor-27 Fourier Transform spectrometer was used to perform infrared spectroscopy.

4.6. Synthesis of benzoxazole derivatives

The following methods were used for the synthesis of benzoxazoles

Method A

A mixture of a 2-aminophenol derivative (10.0 mmol, 1 equiv) and benzaldehyde derivative (10.0 mmol, 1 equiv) in acetonitrile (8.0 mL) and acetate buffer (0.1 M, 12.0 mL, pH 4.0) was stirred at room temperature for 5 minutes. Suberase® (2.0 mL) was then added into the mixture and the contents were stirred until the reaction completes (monitored by TLC). When the reaction completes, the product precipitates from the solution and was extracted with ethyl acetate (30.0 mL) and water (3 x 20.0 mL). After extraction, the solvent was removed on a rotary evaporator to afford a solid product. The solid product was washed several times with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material.

Method B

The same as Method A, except that methanol (2.0 mL) was used instead of acetonitrile.

Method C

The same as Method A, except that ethanol (4.0 mL) was used instead of acetonitrile.

Method D

The same as Method A, except that dichloromethane (8.0 mL) was used instead of acetonitrile.

Method E

The same as Method A, except that DMF (8.0 mL) was used instead of acetonitrile.

Method F

Intermediate **8** (3.0 mmol) was dissolved in ethyl acetate (4.0 mL) and was added to a mixture containing Suberase® (2.0 mL) and acetate buffer (0.10 M, 6.0 mL, pH 4.0). The reaction mixture was stirred overnight at room temperature and was monitored by TLC.

Method G

The same as Method F, except that chloroform (4.0 mL) was used instead of ethyl acetate.

Method H

The same as Method F, except that dichloromethane (4.0 mL) was used instead of ethyl acetate.

Method I

A repetition of method F-H using Novoprime Base 268 (0.105 g) instead of Suberase®.

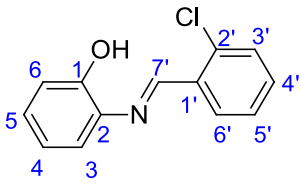
Method J

A repetition of Method A using Denilite® II Base (0.085 g) and Novoprime Base 268 (0.105 g) laccases instead of Suberase® (2.0 mL).

Method K

A repetition of Method A using ABTS (2.0 mL) and Novoprime Base 268 (0.105 g) as laccase.

4.6.1. Synthesis of (*E*)-2-((2-chlorobenzylidene)amino)phenol (8a)



4.6.1.1. METHOD A

Stirring time = 30 min. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a bright yellow solid (1.66g, 72%). R_f (40% EtOAc/hexane) 0.58. MP = 96 – 100 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 9.03 (1H, s, H7'), 8.09 (1H, d, *J* = 7.5 Hz, H6'), 7.35 – 7.17 (4H, m, H3', H4', H5', H3), 7.12 (1H, t, *J* = 7.7 Hz, H5), 6.91 (1H, d, *J* = 8.1 Hz, H6), 6.80 (1H, t, *J* = 7.6 Hz, H4). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 153.43 (C-7'), 152.55 (C-1), 136.21 (C-2), 135.37 (C-2'), 132.89 (C-1'), 132.32 (C-4'), 130.13 (C-3'), 129.49 (C-5), 128.32 (C-6'), 127.12 (C-5'), 120.19 (C-4), 116.19 (C-6), 115.18 (C-3). **HRMS** *m/z*: calculated for $C_{13}H_{10}ClNO$: 231.68, found: [M+H]: 232.05. **IR (ν_{max}/cm^{-1})**: 3069 (**Ar**C-H); 1582, 1561 (**Ar**C=C); 1122 (C-O); 710 (C-Cl).

4.6.1.2. METHOD B

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a yellow solid (1.66 g, 67%). R_f (40% EtOAc/hexane) 0.58.

4.6.1.3. METHOD C

Stirring time = 12 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a yellow solid (1.66 g, 70%). R_f (40% EtOAc/hexane) 0.58.

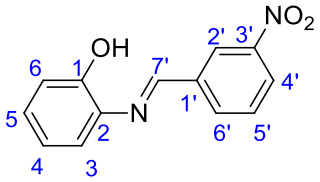
4.6.1.4. METHOD D

Stirring time = 6 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a yellow solid (1.66 g, 70%). R_f (40% EtOAc/hexane) 0.58.

4.6.1.5. METHOD E

Stirring time = 2 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a yellow solid (1.66 g, 71%). R_f (40% EtOAc/hexane) 0.58.

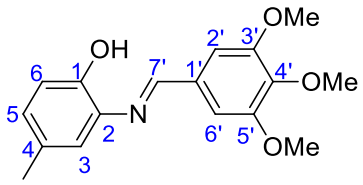
4.6.2. Synthesis of (*E*)-2-((3-nitrobenzylidene)amino)phenol (8b)



4.6.2.1. METHOD A

Stirring time = 8 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a green-yellow solid (2.098 g, 87%). R_f (40% EtOAc/hexane) 0.78. MP = 136 – 142 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)** δ 8.78 (1H, s, H7'), 8.74 (1H, s, H2'), 8.34 (1H, d, *J* = 8.2 Hz, H6'), 8.25 (1H, d, *J* = 7.7 Hz, H4'), 7.68 (1H, t, *J* = 7.9 Hz, H5'), 7.34 (1H, d, *J* = 8.0 Hz, H3), 7.30 – 7.20 (1H, m, H5), 7.15 (1H, s, OH), 7.05 (1H, d, *J* = 8.1 Hz, H6), 6.94 (1H, t, *J* = 7.7 Hz, H4). **^{13}C NMR (126 MHz, Chloroform-*d*)** δ 153.90 (C-7'), 152.71 (C-1), 148.80 (C-3'), 137.48 (C-2), 134.47 (C-1'), 133.96 (C-6'), 130.13 (C-5'), 129.96 (C-5), 125.77 (C-4'), 123.31 (C-2'), 120.31 (C-4), 115.91 (C-6), 115.58 (C-3). **HRMS** *m/z*: calculated for $C_{13}H_{10}N_2O_3$: 242.23, found: [M+H]: 243.08. **IR (ν_{max}/cm^{-1})**: 3076 (**Ar**C-H); 1582, 1510, (**Ar**C=C); 1475&1375(N-O); 1077 (C-O).

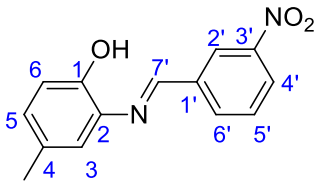
4.6.3. Synthesis of (*E*)-4-methyl-2-((3,4,5-trimethoxybenzylidene)amino)phenol (8c)



4.6.3.1. Method A

Stirring time = 3 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a dark green solid (2.098 g, 72%). R_f (40% EtOAc/hexane) 0.44. MP = 114 – 123 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.55 (1H, s, H7'), 7.14 (2H, s, H2', H6'), 7.07 (1H, s, H3), 7.00 (1H, d, *J* = 8.2 Hz, H5), 6.91 (1H, d, *J* = 8.2 Hz, H6), 3.93 (9H, s, OMe), 2.31 (3H, s, CH_3). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 156.82 (C-7'), 153.55 (C-3'), 149.80 (C-5'), 141.28 (C-1), 135.36 (C-4'), 131.37 (C-2), 129.41 (C-1'), 119.71 (C-4), 117.68 (C-5), 116.58 (C-3), 115.15 (C-6), 105.83 (C-2', C-6'), 61.02 (2 x OMe), 56.25 (OMe), 20.69 (CH_3). **HRMS** *m/z*: calculated for $C_{17}H_{19}NO_4$: 301.34, found: [M+H]: 302.13. **IR (ν_{max}/cm^{-1})**: 3008 (**Ar**C-H); 1573, 1503, 1417 (**Ar**C=C); 1371 (CH_3); 118 (C-O).

4.6.4. Synthesis of (*E*)-4-methyl-2-((3-nitrobenzylidene)amino)phenol (8d)

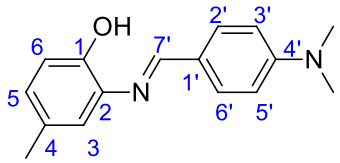


4.6.4.1. Method A

Stirring time = 1 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a dark green solid (2.459 g, 96%). R_f (40% EtOAc/hexane) 0.72. MP = 168 – 176 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.75 (1H, s, H7'), 8.72 (1H, s, H2'), 8.32 (1H, d, *J* = 8.7 Hz, H6'), 8.24 (1H, d, *J* = 7.7 Hz, H4'), 7.67 (1H, t, *J* = 7.9 Hz, H5'), 7.15 (1H, s, OH), 7.06 (1H, d, *J* = 8.2 Hz, H5), 7.01 – 6.88 (2H, m, H3, H6), 2.33 (3H, s, CH_3). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 153.50 (C-7), 150.51 (C-3'), 148.79 (C-1), 137.59 (C-2),

134.06 (C-1'), 133.88 (C-6'), 130.77 (C-4), 129.92 (C-5'), 129.60 (C-5), 125.65 (C-4'), 123.27 (C-2'), 116.29 (C-3), 115.25 (C-6), 20.77 (CH_3). **HRMS** *m/z*: calculated for $C_{14}H_{12}N_2O_3$: 256.26, found: [M+H]: 257.09. **IR (v_{max}/cm^{-1})**: 3069 (**Ar**C-H); 1588, 1575 (**Ar**C=C); 1516 &1344(N-O); 1371 (CH_3); 1075 (C-O).

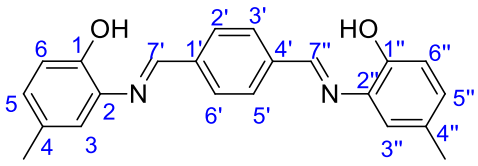
4.6.5. Synthesis of (*E*)-2-((4-(dimethylamino)benzylidene)amino)-4-methylphenol (8e)



4.6.5.1. METHOD A

Stirring time = 1 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a dark brown solid (2.359 g, 93%). R_f (40% EtOAc/hexane) 0.59. MP = 162 – 170 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.54 (1H, s, H7'), 7.79 (2H, d, *J* = 8.7 Hz, H2', H6'), 7.07 (1H, s, H3), 6.93 (1H, d, *J* = 8.2 Hz, H5), 6.87 (1H, d, *J* = 8.1 Hz, H6), 6.74 (2H, d, *J* = 8.7 Hz, H3', H5'), 3.07 (6H, s, 2 x N-CH_3), 2.31 (3H, s, CH_3). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 156.83 (C-7'), 152.65 (C-4'), 149.64 (C-1), 136.24 (C-2), 130.49 (C-4), 127.87 (C-5), 124.18 (C-2', C-6'), 116.13 (C-1'), 115.12 (C-3), 114.04 (C-6), 111.59 (C-3', C-5'), 40.17(2 x N-CH_3), 20.86 (CH_3). **HRMS** *m/z*: calculated for $C_{16}H_{18}N_2O$: 254.33, found: [M+H]: 255.15. **IR (v_{max}/cm^{-1})**: 3017 (**Ar**C-H); 1586, 1547 (**Ar**C=C); 1499 (CH_3); 1364 (C-N); 1065 (C-O).

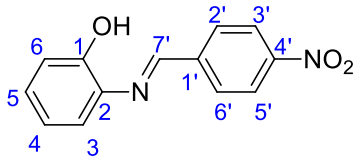
4.6.6. Synthesis of 2,2'-((*1E,1'E*)-(1,4-phenylenebis(methanylylidene))bis(azanylylidene))bis(4-methylphenol) (8f)



4.6.6.1. METHOD A

2-aminophenol (2 equiv), Stirring time = 1 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a yellow solid (3.33 g, 97%). R_f (40% EtOAc/hexane) 0.78. MP = 226 – 231 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.73 (2H, s, H7', H7"), 8.02 (4H, s, H2', H3', H5', H6'), 7.16 (2H, s, OH), 7.09 (2H, s, H3', H3"), 7.04 (2H, d, *J* = 8.2 Hz, H5', H5"), 6.93 (2H, d, *J* = 8.2 Hz, H6', H6"), 2.33 (6H, s, 2 x CH_3). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 155.37 (C-7', C-7"), 150.42 (C-1, C-1"), 138.50 (C-1', C-4'), 134.78 (C-4, C-4"), 130.13 (C-2, C-2"), 129.45 (C-2', C-3', C-5', C-6'), 129.08 (C-5, C-5"), 116.22 (C-3, C-3"), 114.91 (C-6, C-6"), 20.81 (2 x CH_3). **HRMS** *m/z*: calculated for $C_{22}H_{20}N_2O_2$: 344.41, found: [M+H]: 345.16. **IR (v_{max}/cm^{-1})**: 3023 (**Ar**C-H); 1588, 1552 (**Ar**C=C); 1371 (CH_3); 1053 (C-O).

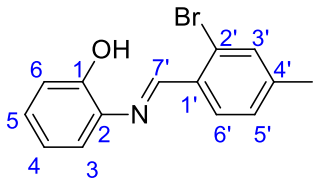
4.6.7. Synthesis of (*E*)-2-((4-nitrobenzylidene)amino)phenol (8g)



4.6.7.1. METHOD A

Stirring time = 1 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a yellow solid (3.33 g, 98%). R_f (40% EtOAc/hexane) 0.74. MP = 166 – 170 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.79 (1H, s, H7'), 8.34 (2H, d, *J* = 8.6 Hz, H3', H5'), 8.08 (2H, d, *J* = 8.6 Hz, H2', H6'), 7.36 (1H, d, *J* = 8.0 Hz, H3), 7.31 – 7.22 (1H, m, H5), 7.18 (1H, s, OH), 7.06 (1H, d, *J* = 8.1 Hz, H6), 6.95 (1H, t, *J* = 7.7 Hz, H4). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 153.81 (C-7'), 152.91 (C-1'), 149.36 (C-4'), 141.15 (C-1), 134.49 (C-2), 130.43 (C-3', C-5'), 129.28 (C-2', C-6'), 124.14 (C-5), 120.34 (C-4), 115.87 (C-6), 115.63 (C-3). **HRMS** *m/z*: calculated for $C_{13}H_{10}N_2O_3$: 242.23, found: [M+H]: 243.08. **IR (v_{max}/cm^{-1})**: 3068 (**Ar**C-H); 1588, 1513, (**Ar**C=C); 1477&1374(N-O); 1103 (C-O).

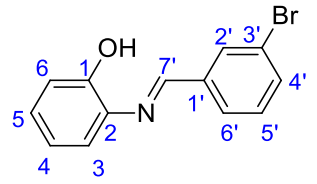
4.6.8. Synthesis of (*E*)-2-((2-bromo-4-methylbenzylidene)amino)phenol (8h)



4.6.8.1. METHOD A

Stirring time = 6 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a light green solid (2.21 g, 76%). R_f (40% EtOAc/hexane) 0.81. MP = 102 – 108 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 9.06 (1H, s, H7'), 8.10 (1H, d, *J* = 8.0 Hz, H6'), 7.47 (1H, s, H3'), 7.34 (1H, d, *J* = 7.9 Hz, H5'), 7.21 (3H, m, H3, H5, OH), 7.02 (1H, d, *J* = 8.0 Hz, H6), 6.92 (1H, t, *J* = 7.6 Hz, H4), 2.40 (3H, s, CH_3). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 155.89 (C-7'), 152.46 (C-1), 143.65 (C-1'), 135.47 (C-4'), 133.81 (C-3'), 131.61 (C-2), 129.23 (C-6'), 128.74 (C-5'), 128.56 (C-2'), 126.20 (C-5), 120.18 (C-4), 116.20 (C-6), 115.08 (C-3), 21.24 (CH_3). **HRMS** *m/z*: calculated for $C_{14}H_{12}BrNO$: 290.16, found: [M+H]: 291.07. **IR (ν_{max}/cm^{-1})**: 3029 (**Ar**C-H); 1588, 1514 (**Ar**C=C); 1373 (CH_3); 1103 (C-O); 731 (C-Br).

4.6.9. Synthesis of (*E*)-2-((3-bromobenzylidene)amino)phenol (8i)

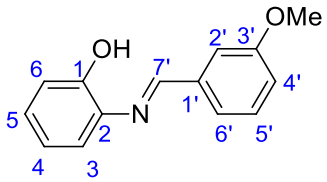


4.6.9.1. METHOD A

Stirring time = 6 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a light brown solid (2.57 g, 93%). R_f (40% EtOAc/hexane) 0.42. MP = 104 – 109 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.65 (1H, s, H7'), 8.11 (1H, s, H2'), 7.83 (1H, d, *J* = 7.8 Hz, H4'), 7.65 (1H, d, *J* = 8.0 Hz, H6'), 7.52 – 7.15 (4H, m, H3, H5, H6, H5'), 7.07 (1H, t, *J* = 8.1 Hz, H4), 6.96 (1H, s, OH). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 155.13 (C-7'), 152.50 (C-1), 137.80 (C-1'), 134.93 (C-2'), 134.37 (C-2), 131.18 (C-4'), 130.34 (C-5'), 129.47 (C-6'), 127.55 (C-3'), 123.12 (C-5), 120.16 (C-4), 115.85 (C-6), 115.27 (C-3). **HRMS** *m/z*:

calculated for $C_{13}H_{10}BrNO$: 276.13, found: [M+H]: 277.05. **IR (v_{max}/cm^{-1})**: 3038 (**Ar**C-H); 1579, 1557 (**Ar**C=C); 1473 (CH_3); 1089 (C-O); 733 (C-Br).

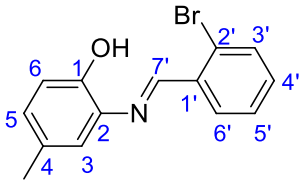
4.6.10. Synthesis of (*E*)-2-((3-methoxybenzylidene)amino)phenol (8j)



4.6.10.1. METHOD A

Stirring time = 36 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a solid (1.54 g, 68%). R_f (40% EtOAc/hexane) 0.80. MP = 116 – 119 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 9.07 (1H, s, H7'), 8.24 (1H, d, *J* = 2.3 Hz, H6'), 7.52 (1H, dd, *J* = 8.8, 2.5 Hz, H5'), 7.31 (1H, d, *J* = 7.9 Hz, H3), 7.26 (1H, s, H2'), 7.24 (1H, s, OH), 7.19 (1H, t, *J* = 7.7 Hz, H5), 7.01 (1H, d, *J* = 8.0 Hz, H6), 6.90 (1H, t, *J* = 7.6 Hz, H4), 6.85 (1H, d, *J* = 8.8 Hz, H4'), 3.91 (3H, s, OMe). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 158.53 (C-7'), 152.49 (C-3'), 151.37 (C-1), 135.73 (C-1'), 135.24 (C-2), 129.85 (C-5'), 129.04 (C-5), 126.19 (C-4), 120.05 (C-6'), 116.13 (C-6), 115.04 (C-3), 113.61 (C-4'), 113.13 (C-2'), 55.90 (OMe). **HRMS** *m/z*: calculated for $C_{13}H_{10}BrNO$: 227.26 found: [M+H]: 228.95. **IR (v_{max}/cm^{-1})**: 3003 (**Ar**C-H); 1580, 1509, (**Ar**C=C); 1120 (C-O).

4.6.11. Synthesis of (*E*)-2-((2-bromobenzylidene)amino)-4-methylphenol (8k)

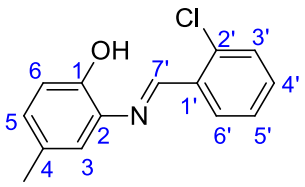


4.6.11.1. METHOD A

Stirring time = 8 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a green solid (1. 967 g, 68%). R_f (40% EtOAc/hexane) 0.79. MP = 127 – 135 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 9.08 (1H, s, H7'), 8.20 (1H, d, *J* =

7.4 Hz, H3'), 7.64 (1H, d, *J* = 8.0 Hz, H6'), 7.42 (1H, t, *J* = 7.5 Hz, H4'), 7.33 (1H, t, *J* = 8.0 Hz, H5'), 7.15 (1H, s, H3), 7.04 (1H, d, *J* = 8.2 Hz, H5), 6.92 (1H, d, *J* = 8.2 Hz, H6), 2.26 (3H, s, CH_3). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 155.46 (C-7'), 150.36 (C-1'), 134.88 (C-1), 134.36 (C-3'), 133.43 (C-4), 132.49 (C-2), 130.16 (C-6'), 129.52 (C-4'), 128.81 (C-5'), 127.73 (C-2'), 126.21 (C-5), 116.59 (C-6), 114.88 (C-3), 20.81 (CH_3). **HRMS** *m/z*: calculated for $C_{14}H_{12}BrNO$: 290.16, found: [M+H]: 291.07. **IR (ν_{max}/cm^{-1})**: 3024 (**Ar**C-H); 1580, 1558 (**Ar**C=C); 1377 (CH_3); 1114 (C-O); 733 (C-Br).

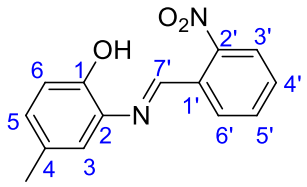
4.6.12. Synthesis of (*E*)-2-((2-chlorobenzylidene)amino)-4-methylphenol (8l)



4.6.12.1. METHOD A

Stirring time = 2 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a solid (1.37 g, 56%). R_f (40% EtOAc/hexane) 0.86. MP = 117 – 120 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 9.14 (1H, s, H7'), 8.22 (1H, d, *J* = 7.7 Hz, H3'), 7.51 – 7.32 (3H, m, H4', H5', H6'), 7.15 (1H, s, OH), 7.03 (2H, d, *J* = 6.3 Hz, H5, H3), 6.92 (1H, d, *J* = 8.2 Hz, H6), 2.33 (3H, s, CH_3). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 153.06 (C-7'), 150.37 (C-1'), 136.18 (C-1), 134.98 (C-2'), 133.03 (C-4), 132.25 (C-2), 130.15 (C-3'), 130.13 (C-6'), 129.50 (C-4'), 128.33(C-5'), 127.13 (C-5), 116.55 (C-6), 114.86 (C-3), 20.80 (CH_3). **HRMS** *m/z*: calculated for $C_{14}H_{12}ClNO$: 245.70, found: [M+H]: 246.92. **IR (ν_{max}/cm^{-1})**: 3026 (**Ar**C-H); 1582, 1580 (**Ar**C=C); 1114 (C-O); 732 (C-Cl).

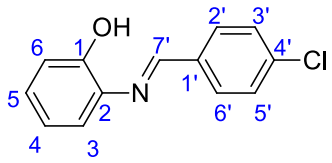
4.6.13. Synthesis of (*E*)-4-methyl-2-((2-nitrobenzylidene)amino)phenol (8m)



4.6.13.1. METHOD A

Stirring time = 2 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a solid (1.23 g, 48%). R_f (40% EtOAc/hexane) 0.70. MP = 125 – 127 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 9.15 (1H, s, H7'), 8.27 (1H, d, *J* = 7.8 Hz, H3'), 8.06 (1H, d, *J* = 8.1 Hz, H6'), 7.74 (1H, t, *J* = 7.6 Hz, H5'), 7.63 (1H, t, *J* = 7.7 Hz, H4'), 7.15 (1H, s, OH), 7.07 (1H, d, *J* = 8.3 Hz, H5), 6.99 – 6.88 (2H, m, H3, H6), 2.32 (3H, s, CH_3). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 151.54 (C-7'), 150.63 (C-2'), 149.42 (C-1), 134.32 (C-1'), 133.40 (C-4), 131.29 (C-6'), 130.92 (C-2), 130.65 (C-4'), 129.71 (C-5'), 129.48 (C-3'), 124.67 (C-5), 116.70 (C-6), 115.15 (C-3), 20.75 (CH_3). **HRMS** *m/z*: calculated for $C_{14}H_{12}N_2O_3$: 256.26, found: [M+H]: 257.09. **IR (ν_{max}/cm^{-1})**: 3044 (**Ar**C-H); 1582, 1500 (**Ar**C=C); 1086 (C-O).

4.6.14. Synthesis of (*E*)-2-((4-chlorobenzylidene)amino)phenol (8n)

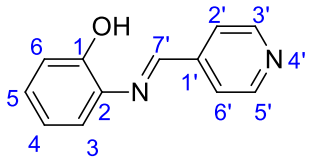


4.6.14.1. METHOD A

Stirring time = 2 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a light brown solid (2.14 g, 93%). R_f (40% EtOAc/hexane) 0.74. MP = 122 – 126 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.69 (1H, s, H7'), 7.89 (2H, d, *J* = 8.4 Hz, H3', H5'), 7.49 (2H, d, *J* = 8.4 Hz, H2', H6'), 7.33 (1H, d, *J* = 7.9 Hz, H5), 7.28 – 7.18 (2H, m, H3, OH), 7.05 (1H, d, *J* = 8.0 Hz, H6), 6.94 (1H, t, *J* = 7.6 Hz, H4). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 155.53 (C-7'), 152.39 (C-1), 137.71 (C-1'), 135.16 (C-4'), 134.34 (C-2), 129.90 (C-3', C-5'), 129.24 (C-2', C-6'), 129.21 (C-5), 120.16 (C-4), 115.81 (C-6), 115.16 (C-3). **HRMS**

m/z: calculated for $C_{13}H_{10}ClNO$: 231.68, found: [M+H]: 232.05. **IR (v_{max}/cm^{-1})**: 3299 (**Ar**C-H); 1583, 1565 (**Ar**C=C); 1095 (C-O); 752 (C-Cl).

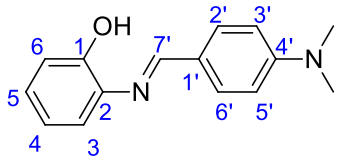
4.6.15. Synthesis of (*E*)-2-((pyridin-4-ylmethylene)amino)phenol (8o)



4.6.15.1. METHOD A

Stirring time = 2 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford an amber solid (1.88 g, 95%). R_f (40% EtOAc/hexane) 0.21. MP = 174 – 176 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.77 (2H, d, *J* = 5.6 Hz, H3', H5'), 8.69 (1H, s, H7'), 7.75 (2H, d, *J* = 5.6 Hz, H2', H6'), 7.34 (1H, d, *J* = 8.0 Hz, H5), 7.25 (2H, m, H3, OH), 7.04 (1H, d, *J* = 8.1 Hz, H6), 6.93 (1H, t, *J* = 7.7 Hz, H4). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 154.33 (C-7'), 152.87 (C-3', C-5'), 150.69 (C-1'), 142.40 (C-1), 134.43 (C-2), 130.41 (C-6', C-2'), 122.07 (C-5), 120.29 (C-4), 115.98 (C-6), 115.63 (C-3). **HRMS** *m/z*: calculated for $C_{12}H_{10}N_2O$: 198.22, found: [M+H]: 199.09. **IR (v_{max}/cm^{-1})**: 3043 (**Ar**C-H); 1577, 1558 (**Ar**C=C); 1365 (C-N); 1088 (C-O).

4.6.16. Synthesis of (*E*)-2-((4-(dimethylamino)benzylidene)amino)phenol (8p)

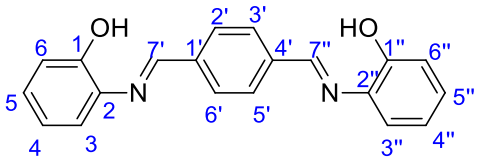


4.6.16.1. METHOD A

Stirring time = 2 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a solid (1.73 g, 72%). R_f (40% EtOAc/hexane) 0.46. MP = 123 – 124 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.55 (1H, s, H7'), 7.80 (2H, d, *J* = 8.7 Hz, H2', H6'), 7.26-7.24 (1H, m, H3), 7.12 (1H, t, *J* = 7.7 Hz, H3), 6.99 (1H, d, *J* = 8.0 Hz, H6), 6.88 (1H, t, *J* = 7.6 Hz, H4), 6.74 (2H, d, *J* = 8.7 Hz, H3', H5'), 3.07 (6H, s, 2 x CH_3). **^{13}C NMR (126**

MHz, Chloroform-*d*): δ 157.15 (C-7'), 152.70 (C-4'), 151.89 (C-1), 136.65 (C-2), 130.55 (C-1'), 127.36 (C-2', C-6'), 124.05 (C-5), 119.93 (C-4), 115.58 (C-6), 114.38 (C-5), 111.57 (C-3', C-5'), 40.14 (2 x CH_3). **HRMS** *m/z*: calculated for $C_{15}H_{16}N_2O$: 240.30, found: [M+H]: 241.33. **IR (v_{max}/cm^{-1})**: 3004 (**Ar**C-H); 1584, 1536 (**Ar**C=C); 1482 (CH_3); 1369 (C-N); 1087 (C-O).

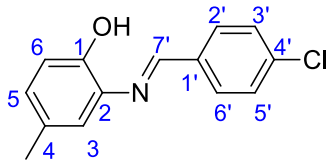
4.6.17. Synthesis of 2,2'-((*1E,1'E*)-(1,4-phenylenebis(methanylylidene))bis(azanylylidene))diphenol (8q)



4.6.17.1. METHOD A

2-aminophenol (2 equiv), Stirring time = 2 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a gold solid (3.09 g, 98%). Rf (40% EtOAc/hexane) 0.48. MP = 198 – 224 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.75 (2H, s, H7', H7"), 8.03 (4H, s, H2', H3', H5', H6'), 7.34 (2H, d, *J* = 7.9 Hz, H3, H3"), 7.26 (2H, d, *J* = 3.0 Hz, OH), 7.23 (2H, t, *J* = 7.7 Hz, H5, H5"), 7.04 (2H, d, *J* = 8.1 Hz, H6, H6"), 6.93 (2H, t, *J* = 7.6 Hz, H4, H4"). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 155.73 (C-7', C-7"), 152.63 (C-1, C-1"), 138.49 (C-1', C-4'), 135.17 (C-2, C-2"), 129.54 (C-2', C-3', C-5', C-6'), 129.15 (C-5, C-5"), 120.20 (C-4, C-4"), 115.82 (C-6, C-6"), 115.25 (C-5, C-5"). **HRMS** *m/z*: calculated for $C_{20}H_{16}N_2O_2$: 316.35, found: [M+H]: 317.13. **IR (v_{max}/cm^{-1})**: 3045 (**Ar**C-H); 1587 (**Ar**C=C); 1095 (C-O).

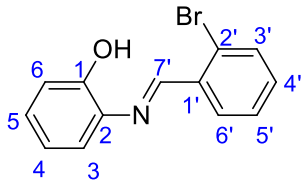
4.6.18. Synthesis of (*E*)-2-((4-chlorobenzylidene)amino)-4-methylphenol (8r)



4.6.18.1. METHOD A

Stirring time = 2 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a gold solid (2.32 g, 94%). R_f (40% EtOAc/hexane) 0.76. MP = 88 – 90 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.64 (1H, s, H7'), 7.84 (2H, d, *J* = 8.3 Hz, H3', H5'), 7.46 (2H, d, *J* = 8.3 Hz, H2', H6'), 7.10 (1H, s, H3), 7.02 (1H, d, *J* = 8.2 Hz, H5), 6.91 (1H, d, *J* = 8.2 Hz, H6), 2.32 (3H, s, CH_3). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 155.15 (C-7'), 150.16 (C-1), 137.58 (C-1'), 134.76 (C-4'), 134.44 (C-4), 129.84 (C-2), 129.82 (C-3', C-5'), 129.41 (C-2', C-6'), 129.18 (C-5), 116.25 (C-6), 114.82 (C-3), 20.79 (CH_3). **HRMS** *m/z*: calculated for $C_{14}H_{12}ClNO$: 245.70, found: [M+H]: 246.07. **IR (ν_{max}/cm^{-1})**: 3302 (**Ar**C-H); 1588, 1587 (**Ar**C=C); 1099 (C-O); 718 (C-Cl).

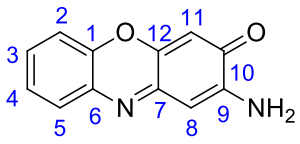
4.6.19. Synthesis of (*E*)-2-((2-bromobenzylidene)amino)phenol (8s)



4.6.19.1. METHOD A

Stirring time = 2 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a grey solid (2.58 g, 94%). R_f (40% EtOAc/hexane) 0.71. MP = 96 – 102 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 9.10 (1H, s, H7'), 8.21 (1H, d, *J* = 7.1 Hz, H3'), 7.64 (1H, d, *J* = 7.5 Hz, H4'), 7.52 – 7.17 (5H, m, H3, H5, H5', H6', NH), 7.05 (1H, d, *J* = 7.6 Hz, H6), 6.95 (1H, t, *J* = 6.8 Hz, H4). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 155.83 (C-7'), 152.54 (C-1'), 135.29 (C-1), 134.23 (C-3'), 133.41 (C-2), 132.54 (C-6'), 129.49 (C-4'), 128.81 (C-5'), 127.71 (C-2'), 126.23 (C-5), 120.20 (C-4), 116.26 (C-6), 115.21 (C-3). **HRMS** *m/z*: calculated for $C_{13}H_{10}BrNO$: 276.13, found: [M+H]: 277.00. **IR (ν_{max}/cm^{-1})**: 3083 (**Ar**C-H); 1580, 1558 (**Ar**C=C); 1098 (C-O); 702 (C-Br).

4.6.20. Synthesis of 2-amino-3H-phenoxazin-3-one (8t)



4.6.20.1. METHOD K

Stirring time = 24 h. Purification by flash chromatography (silica/EtOAc-hexane, 1:4) to afford a marron solid (1.14 g, 54 %). R_f (40% EtOAc/hexane) 0.44. MP = 248 – 250 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 7.76 (1H, d, *J* = 7.9 Hz, H5), 7.44 (1H, t, *J* = 7.4 Hz, H3), 7.41 – 7.31 (2H, m, H2, H4), 6.48 (1H, s, H11), 6.42 (1H, s, H8), 5.11 (2H, s, NH2). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 180.34 (C-10), 149.43 (C-9), 148.74 (C-7), 145.72 (C-12), 142.80 (C-1), 133.99 (C-6), 129.59 (C-5), 128.82 (C-3), 125.29 (C-4), 116.05 (C-2), 104.16 (C-8), 100.89 (C-11). **HRMS** *m/z*: calculated for $C_{12}H_8N_2O_2$: 212.20, found: [M+H]: 213.06. **IR (ν_{max}/cm^{-1})**: 3407 (N-H); 3057 (**Ar**C-H); 1805 (C=O); 1554 (**Ar**C=C); 1113 (C-O).

4.7. Synthesis of benzimidazoles

The following methods were used for the synthesis of benzimidazoles

Method L

A mixture of *o*-phenylenediamine (10.0 mmol, 1 equiv) and benzaldehyde derivative (20.0 mmol, 2 equiv) in acetonitrile (10.0 mL) and acetate buffer (10.0 mL, pH 4.5) was stirred at room temperature for 5 minutes. Suberase® (2.0 mL) was added to the mixture and the contents were stirred until reaction completes (monitored by TLC). The product precipitates from the solution as the reaction proceeds and after completion; the product was extracted with ethyl acetate (30.0 mL) and water (3 x 20.0 mL) and concentrated on a rotary evaporator. The product was washed several times with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material.

Method M

The same as Method L, except that methanol (2.0 mL) was used instead of acetonitrile.

Method N

The same as Method L, except that ethanol (4.0 mL) was used instead of acetonitrile.

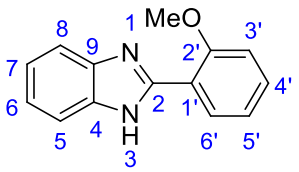
Method O

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

Method P

A repetition of **Method L** using Denilite® II Base (0.085 g) and Novoprime Base 268 (0.105 g) laccases instead of Suberase® (2.0 mL).

4.7.1. Synthesis of 2-(2-methoxyphenyl)-1H-benzimidazole (11a)



4.7.1.1. METHOD L

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (1.38 g, 62%). R_f (40% EtOAc/hexane) 0.38.

4.7.1.2. METHOD M

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (1.32 g, 59%). R_f (40% EtOAc/hexane) 0.38.

4.7.1.3. METHOD O

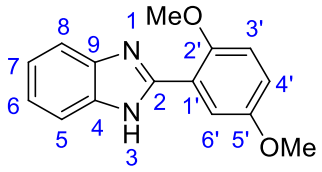
Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (1.61 g, 72%). R_f (40% EtOAc/hexane) 0.38.

4.7.1.4. METHOD P

Novoprime Base 268 (0.105 g), Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (1.55 g, 69%). R_f (40% EtOAc/hexane) 0.38. MP = 178 – 180 °C. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 12.11 (1H, s, NH), 8.33 (1H, d, J = 7.6 Hz, H6'), 7.63 (2H, dd, J = 16.6, 7.5 Hz, H5, H8), 7.48 (1H, t, J = 7.8 Hz, H4'), 7.24 (1H, d, J = 8.3 Hz, H7), 7.19 (2H, t, J = 6.5 Hz, H3', H5'), 7.12 (1H, t, J = 7.5 Hz, H6), 4.03 (3H, s, OMe). **^{13}C NMR (126 MHz, DMSO-d_6)**: δ 157.25 (C-2), 149.43 (C-2'), 143.20 (C-9), 135.19 (C-4), 131.70 (C-6'), 130.23 (C-4'), 122.51 (C-5'), 121.96 (C-1'), 121.34 (C-3'), 118.89 (C-7),

118.63 (C-6), 112.58 (C-8), 112.38 (C-5), 56.25 (OMe). **IR (v_{max}/cm^{-1})**: 3043 (**Ar**C-H); 1619 (C=N); 1584, 1537 (**Ar**C=C); 1306 (C-O).

4.7.2. Synthesis of 2-(2,5-dimethoxyphenyl)-1H-benzimidazole (11c)



4.7.2.1. Method M

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (0.253 g, 10%). R_f (40% EtOAc/hexane) 0.38.

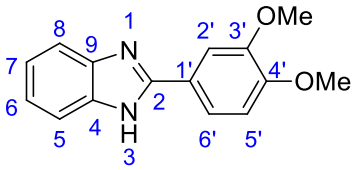
4.7.2.2. Method N

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (0.677 g, 25%). R_f (40% EtOAc/hexane) 0.38.

4.7.2.3. Method P

Novoprime Base 268 (0.105 g), stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a white solid (1.93 g, 76%). R_f (40% EtOAc/hexane) 0.38. MP = 224 – 226 °C. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 12.09 (1H, s, NH), 7.87 (1H, d, *J* = 2.8 Hz, H3'), 7.63 (2H, dd, *J* = 19.0, 7.2 Hz, H5, H8), 7.19 (3H, m, H6, H7, H6'), 7.05 (1H, dd, *J* = 9.0, 2.9 Hz, H4'), 3.98 (3H, s, OMe), 3.81 (3H, s, OMe). **^{13}C NMR (126 MHz, DMSO-d_6)**: δ 153.69 (C-2), 151.56 (C-5'), 149.21 (C-2'), 143.10 (C-9), 135.23 (C-4), 122.61 (C-7), 122.04 (C-6), 118.92 (C-1'), 117.60 (C-4'), 114.10 (C-5, C-8), 113.96 (C-2'), 112.48 (C-6'), 56.65 (OMe-5'), 56.03 (OMe-2'). **IR (v_{max}/cm^{-1})**: 2937 (**Ar**C-H); 1619 (C=N); 1523, 1492 (**Ar**C=C); 1301 (C-O).

4.7.3. Synthesis of 2-(3,4-dimethoxyphenyl)-1H-benzimidazole (11d)



4.7.3.1. METHOD L

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (1.48 g, 59%). R_f (60% EtOAc/hexane) 0.43. MP = 230 – 232 °C. **^{1}H NMR (300 MHz, Chloroform-d)**: δ 8.28 – 7.99 (1H, m, H8), 7.74 – 7.43 (3H, m, H5, H2', H6'), 7.22 (1H, d, J = 8.2 Hz, H7), 7.14 (1H, dd, J = 8.3, 3.5 Hz, H6), 7.08 – 6.89 (1H, m, H5'), 5.75 (1H, s, NH), 4.85 – 3.50 (6H, m, 2 x OMe). **^{13}C NMR (126 MHz, DMSO-d_6)**: δ 153.70 (C-2), 150.56 (C-3'), 149.39 (C-4'), 143.09 (C-9), 136.51 (C-4), 129.86 (C-1'), 122.97 (C-7), 122.85 (C-6), 122.51 (C-6'), 118.95 (C-8), 118.53 (C-5), 112.32 (C-2'), 111.38 (C-5'), 56.10 (2 x OMe): **IR (ν_{max}/cm^{-1})**: 2940 (**Ar**C-H); 1606 (C=N); 1588, 1501, (**Ar**C=C); 1320 (C-O).

4.7.3.2. METHOD M

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (0.864 g, 34%). R_f (60% EtOAc/hexane) 0.42.

4.7.3.3. METHOD O

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (1.71 g, 67%). R_f (60% EtOAc/hexane) 0.42.

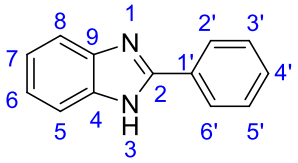
4.7.3.4. METHOD P

Denilite® II Base (0.085 g), stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a yellow oil (1.45 g, 57%). R_f (60% EtOAc/hexane) 0.42.

4.7.3.5. METHOD P

Novoprime Base 268 (0.105 g), stirring time = 8 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (1.97 g, 78%). R_f (60% EtOAc/hexane) 0.42.

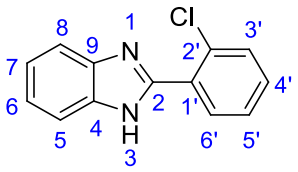
4.7.4. Synthesis of 2-phenyl-1H-benzimidazole (11e)



4.7.4.1. METHOD P

Novoprime Base 268 (0.105 g), Stirring time = 2 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (1.77 g, 92%). R_f (40% EtOAc/hexane) 0.42. MP = 290 – 293 °C. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 12.89 (1H, s, NH), 8.19 (2H, d, *J* = 7.5 Hz, H2', H6'), 7.68 (1H, d, *J* = 7.6 Hz, H8), 7.53 (4H, m, H5, H7, H3', H5'), 7.21 (2H, dt, *J* = 15.6, 6.6 Hz, H6, H4'). **^{13}C NMR (126 MHz, DMSO-d_6)**: δ 151.69 (C-2), 144.29 (C-9, C-4), 135.47 (C-1'), 130.65 (C-4'), 130.29 (C-3', C-5'), 129.40 (C-2'), 126.90 (C-6'), 122.99 (C-7), 122.12 (C-6), 119.34 (C-8), 111.78 (C-5). **IR (ν_{max}/cm^{-1})**: 3047 (**Ar**C-H); 2921 (N-H); 1622 (C=N); 1559, 1542, (**Ar**C=C).

4.7.5. Synthesis of 2-(2-chlorophenyl)-1H-benzimidazole (11f)

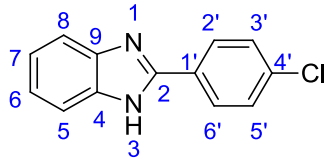


4.7.5.1. METHOD P

Novoprime Base 268 (0.105 g), stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (2.14 g, 94%). R_f (40% EtOAc/hexane) 0.58. MP = 231 – 234 °C. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 12.71

(1H, s, NH), 7.92 (1H, d, *J* = 7.1 Hz, H6'), 7.78 – 7.46 (5H, m, H5, H8, H3', H4', H5'), 7.25 (2H, d, *J* = 6.8 Hz, H6, H7). **^{13}C NMR (126 MHz, DMSO-d_6)**: δ 149.56 (C-2), 143.69 (C-1'), 135.13 (C-9), 132.54 (C-4), 132.11 (C-2'), 131.65 (C-4'), 130.81 (C-3'), 130.46 (C-6'), 127.88 (C-5'), 123.18 (C-7), 122.17 (C-6), 119.56 (C-8), 112.17 (C-5). **IR (ν_{max}/cm^{-1})**: 3045 (**Ar**C-H); 1622 (C=N); 1569, 1539, (**Ar**C=C); 700 (C-Cl).

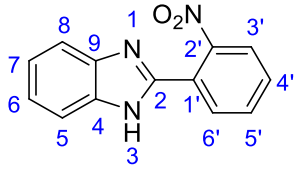
4.7.6. Synthesis of 2-(4-chlorophenyl)-1H-benzimidazole (11g)



4.7.6.1. METHOD P

Novoprime Base 268 (0.105 g), Stirring time = 4 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (2.02 g, 88%). R_f (40% EtOAc/hexane) 0.42. MP = 286 – 289 °C. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 12.98 (1H, s, NH), 8.20 (2H, d, *J* = 8.4 Hz, H2', H6'), 7.64 (4H, m, H5, H8, H3', H5'), 7.22 (2H, d, *J* = 6.7 Hz, H6, H7). **^{13}C NMR (126 MHz, DMSO-d_6)**: δ 149.56 (C-2), 143.69 (C-9), 135.13 (C-4), 132.54 (C-4'), 132.11 (C-1'), 131.65 (C-3'), 130.81 (C-5'), 130.46 (C-2'), 127.88 (C-6'), 123.18 (C-7), 122.17 (C-6), 119.56 (C-8), 112.17 (C-5). **IR (ν_{max}/cm^{-1})**: 3052 (**Ar**C-H); 1622 (C=N); 1588, 1540, (**Ar**C=C); 727 (C-Cl).

4.7.7. Synthesis of 2-(2-nitrophenyl)-1H-benzimidazole (11h)

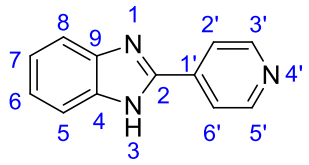


4.7.7.1. METHOD P

Novoprime Base 268 (0.105 g), Stirring time = 8 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford an orange-brown solid

(1.33 g, 56%). R_f (40% EtOAc/hexane) 0.22. MP = 191 – 193 °C. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 12.21 (1H, s, NH), 7.21 (1H, d, *J* = 8.0 Hz, H6'), 7.15 (1H, d, *J* = 7.7 Hz, H3'), 7.04 (1H, t, *J* = 7.6 Hz, H5'), 6.94 (1H, t, *J* = 7.7 Hz, H4'), 6.79 (2H, s, H5, H8), 6.43 (2H, s, H6, H7). **^{13}C NMR (126 MHz, DMSO-d_6)**: δ 149.44 (C-2), 147.77 (C-2'), 133.11 (C-4, C-9), 131.39 (C-5', C-6'), 131.36 (C-1', C-4'), 124.76 (C-6, C-7, C-3'), 124.71 (C-5, C-8). **IR (ν_{max}/cm^{-1})**: 3064 (**Ar**C-H); 1625 (C=N); 1573, 1524, (**Ar**C=C).

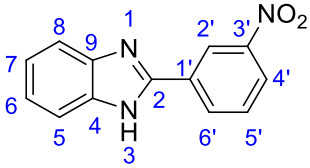
4.7.8. Synthesis of 2-(pyridin-4-yl)-1H-benzimidazole (11i)



4.7.8.1. METHOD P

Novoprime Base 268 (0.105 g), Stirring time = 2 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (1.69 g, 60%). R_f (40% EtOAc/hexane) 0.13. MP = 211 – 214 °C. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 13.23 (1H, s, NH), 8.76 (2H, d, *J* = 4.9 Hz, H3', H5'), 8.10 (2H, d, *J* = 4.8 Hz, H2', H6'), 7.66 (2H, d, *J* = 68.5 Hz, H5, H8), 7.50 – 7.02 (2H, m, H6, H7). **^{13}C NMR (126 MHz, DMSO-d_6)**: δ 150.97 (C-2), 149.24 (C-3', C-5'), 144.13 (C-1'), 137.60 (C-9), 135.49 (C-4), 124.03 (C-6, C-7), 122.73 (C-2'), 120.79 (C-6'), 119.93 (C-8), 112.27 (C-5). **IR (ν_{max}/cm^{-1})**: 3051 (**Ar**C-H); 2877(N-H); 1665 (C=N); 1562, 1537, (**Ar**C=C)

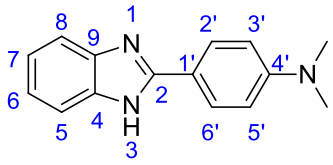
4.7.9. Synthesis of 2-(3-nitrophenyl)-1H-benzimidazole (11j)



4.7.9.1. METHOD P

Novoprime Base 268 (0.105 g), Stirring time = 4 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (2.26 g, 95%). R_f (40% EtOAc/hexane) 0.43. MP = 202 – 205 °C. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 13.27 (1H, s, NH), 9.02 (1H, s, H2'), 8.62 (1H, d, *J* = 7.7 Hz, H6'), 8.33 (1H, d, *J* = 8.2 Hz, H4'), 7.86 (1H, t, *J* = 8.0 Hz, H5'), 7.66 (2H, d, *J* = 50.7 Hz, H5, H8), 7.26 (2H, d, *J* = 6.7 Hz, H6, H7). **^{13}C NMR (126 MHz, DMSO-d_6)**: δ 147.43 (C-2), 146.75 (C-3'), 130.84 (C-4, C-9), 130.12 (C-6'), 129.04 (C-1', C-5'), 122.55 (C-4'), 119.20 (C-6, C-7, C-2'), 116.36 (C-5, C-8). **IR (ν_{max}/cm^{-1})**: 3098 (**Ar**C-H); 1623 (C=N); 1588, 1517 (**Ar**C=C).

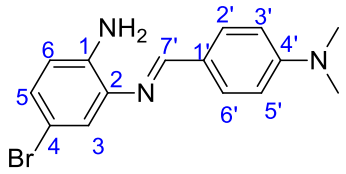
4.7.10. Synthesis of 4-(1H-benzimidazol-2-yl)-N,N-dimethylaniline (11k)



4.7.10.1. METHOD P

Novoprime Base 268 (0.105 g), Stirring time = 8 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford an orange solid (1.52 g, 64%). R_f (40% EtOAc/hexane) 0.17. MP = 201 – 202 °C. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 8.14 (1H, s, NH), 7.99 (2H, d, *J* = 8.7 Hz, H2', H6'), 7.48 (2H, d, *J* = 9.2 Hz, H5, H8), 7.14 (2H, d, *J* = 9.2 Hz, H6, H7), 6.83 (2H, d, *J* = 8.7 Hz, H3', H5'), 3.00 (6H, s, 2 x N-CH_3). **^{13}C NMR (126 MHz, DMSO-d_6)**: δ 163.47 (C-2), 152.72 (C-4'), 151.74 (C-9, C-4), 128.02 (C-2', C-6'), 121.80 (C-6, C-7), 117.83 (C-5, C-8, C-1'), 112.32 (C-3', C-5'). **IR (ν_{max}/cm^{-1})**: 2887 (**Ar**C-H); 2773 (N-H); 1637 (C=N); 1593, 1504 (**Ar**C=C).

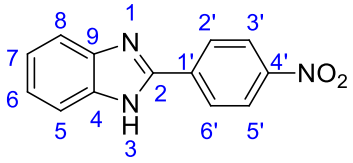
4.7.11. Synthesis of (*E*)-5-bromo-N^1-(4-(dimethylamino)benzylidene)benzene-1,2-diamine (11l)



4.7.11.1. METHOD P

Novoprime Base 268 (0.105 g), Stirring time = 12 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (3.12 g, 98%). R_f (40% EtOAc/hexane) 0.98. MP = 151 – 153 °C. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 8.43 (1H, s, H7'), 7.78 (2H, d, *J* = 8.5 Hz, H2', H6'), 6.95 (1H, d, *J* = 8.3 Hz, H6), 6.85 (1H, s, H3), 6.77 (2H, d, *J* = 8.5 Hz, H3', H5'), 6.65 (1H, d, *J* = 8.3 Hz, H5), 5.31 (2H, s, NH), 3.00 (6H, s, N-CH_3). **^{13}C NMR (126 MHz, DMSO-d6)**: δ 157.60 (C-7'), 152.76 (C-4'), 145.55 (C-1, C-2), 136.21 (C-1'), 130.76 (C-2', C-6'), 124.66 (C-5), 119.01 (C-6), 118.80 (C-3), 116.48 (C-3', C-5'), 111.92 (C-4). **IR (ν_{max}/cm^{-1})**: 2889 (**Ar**C-H); 1589 (N-H); 1547, 1522 (**Ar**C=C).

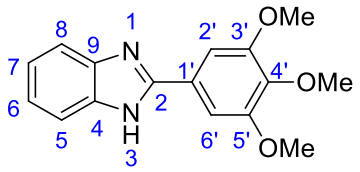
4.7.12. Synthesis of 2-(4-nitrophenyl)-1H-benzimidazole (11m)



4.7.12.1. METHOD P

Novoprime Base 268 (0.105 g), Stirring time = 2 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (2.27 g, 95%). R_f (40% EtOAc/hexane) 0.52. MP = 320 °C. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 13.28 (1H, s, NH), 8.42 (4H, s, H2', H3', H5', H6'), 7.74 (1H, d, *J* = 7.6 Hz, H8), 7.59 (1H, d, *J* = 7.6 Hz, H5), 7.28 (2H, m, H6, H7). **^{13}C NMR (126 MHz, DMSO-d_6)**: δ 149.48 (C-2), 148.30 (C-4'), 144.32 (C-1'), 136.53 (C-9), 135.71 (C-4), 127.88 (C-2', C-6'), 124.79 (C-3', C-5'), 124.07 (C-7), 122.80 (C-6), 119.94 (C-8), 112.28 (C-5). **IR (ν_{max}/cm^{-1})**: 2748 (**Ar**C-H); 1604 (C=N); 1513, 1433 (**Ar**C=C).

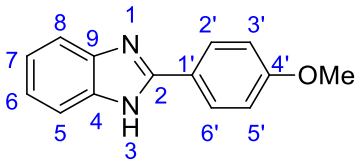
4.7.13. Synthesis of 2-(3,4,5-trimethoxyphenyl)-1H-benzoimidazole (11n)



4.7.13.1. METHOD P

Novoprime Base 268 (0.105 g), stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a white solid (2.53 g, 89%). R_f (40% EtOAc/hexane) 0.19. MP = 258-262 °C. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 12.82 (1H, s, NH), 7.66 (1H, d, *J* = 7.8 Hz, H8), 7.55-7.53 (3H, m, H5, H6, H7), 7.20 (2H, p, *J* = 7.0 Hz, H2', H6'), 3.91 (6H, s, 2 x OMe), 3.74 (3H, s, OMe). **^{13}C NMR (126 MHz, DMSO-d_6)**: δ 153.69 (C-2), 151.68 (C-3', C-5'), 144.22 (C-9), 139.38 (C-4), 135.44 (C-4'), 125.95 (C-1'), 122.89 (C-7), 122.09 (C-6), 119.17 (C-5), 111.61 (C-8), 104.30 (C-2', C-6'), 60.62 (OMe), 56.53 (2 x OMe). **IR (ν_{max}/cm^{-1})**: 2952 (**Ar**C-H); 1589 (C=N); 1498, 1482 (**Ar**C=C); 1311 (C-O).

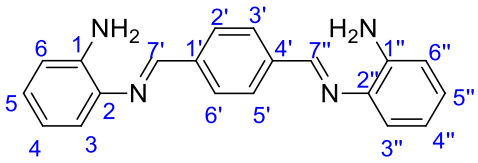
4.7.14. Synthesis of 2-(4-methoxyphenyl)-1H-benzimidazole (11o)



4.7.14.1. METHOD P

Novoprime Base 268 (0.105 g), Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a light brown solid (1.53 g, 68%). R_f (40% EtOAc/hexane) 0.31. MP = 226 – 228 °C. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 12.73 (1H, s, NH), 8.12 (2H, d, *J* = 8.6 Hz, H2', H6'), 7.56 (2H, d, *J* = 52.8 Hz, H5, H8), 7.20 – 7.14 (2H, m, H6, H7), 7.11 (2H, d, *J* = 8.6 Hz, H3', H5'), 3.84 (3H, s, OMe). **^{13}C NMR (126 MHz, DMSO-d_6)**: δ 161.07 (C-2), 151.82 (C-4'), 144.37 (C-9), 135.44 (C-4), 128.48 (C-2', C-6'), 123.19 (C-7), 122.49 (C-6), 121.98 (C-8), 121.91 (C-5), 118.96, 114.84 (C-3', C-5'), 111.48 (C-1'), 55.80 (OMe). **IR (ν_{max}/cm^{-1})**: 3044 (**Ar**C-H); 1621 (C=N); 1583, 1500 (**Ar**C=C); 1317 (C-O).

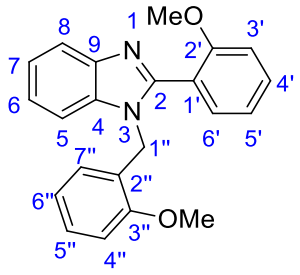
4.7.15. Synthesis of (*N*¹,*N*¹'*E*,*N*¹,*N*¹'*E*)-*N*¹,*N*¹'-(1,4-phenylenebis(methanylylidene))bis(benzene-1,2-diamine) (11p)



4.7.15.1. METHOD P

o-phenylenediamine (2 equiv), Novoprime Base 268 (0.105 g), Stirring time = 12 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford an orange solid (3.08 g, 98%). R_f (40% EtOAc/hexane) 0.63. MP = 298 – 300 °C. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 8.73 (2H, s, H7', H7"), 8.10 (4H, s, H2', H3', H5', H6'), 7.18 (2H, d, *J* = 7.8 Hz, H6, H6"), 6.99 (2H, t, *J* = 7.6 Hz, H5, H5"), 6.74 (2H, d, *J* = 7.9 Hz, H3, H3"), 6.58 (2H, t, *J* = 7.5 Hz, H4, H4"), 5.25 (4H, s, NH). **^{13}C NMR (126 MHz, DMSO-d_6)**: δ 155.94 (C-7', C-7"), 144.70 (C-1', C-4'), 135.41 (C-1, C-2, C-1", C-2"), 129.25 (C-2', C-3', C-5', C-6'), 128.39 (C-5, C-5"), 117.41 (C-4, C-4"), 116.62 (C-6, C-6"), 115.28 (C-3, C-3"). **IR (ν_{max}/cm^{-1})**: 3085 (**Ar**C-H); 2883 (N-H); 1592, 1488 (**Ar**C=C).

4.7.16. Synthesis of 1-(2-methoxybenzyl)-2-(2-methoxyphenyl)-1H-benzimidazole (12a)



4.7.16.1. METHOD M

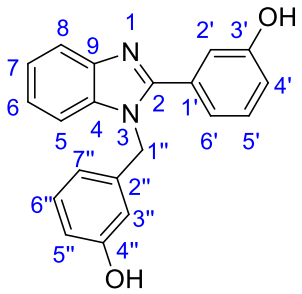
Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford an orange solid (0.689 g, 20%). R_f (40% EtOAc/hexane) 0.34. MP = 148 – 152 °C. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 7.67 (1H, d, *J* = 7.7 Hz, H6'), 7.52 (1H, t, *J* = 7.8 Hz, H5'), 7.42 (1H, d, *J* = 7.4 Hz, H7"), 7.36 (1H, d, *J* = 7.1 Hz, H3'),

7.27 – 7.12 (4H, m, H6, H7, H8, H4'), 7.07 (1H, t, *J* = 7.4 Hz, H6"), 6.92 (1H, d, *J* = 8.2 Hz, H5), 6.75 (1H, t, *J* = 7.4 Hz, H5"), 6.58 (1H, d, *J* = 7.4 Hz, H4"), 5.21 (2H, s, H1"), 3.67 (6H, s, OMe). **^{13}C NMR (126 MHz, DMSO-*d*$_6$)**: δ 157.53 (C-3"), 156.83 (C-2'), 152.31 (C-2), 143.35 (C-4), 135.68 (C-9), 132.39 (C-6'), 132.02 (C-4'), 129.17 (C-7"), 127.94 (C-5"), 124.64 (C-2"), 122.66 (C-7), 122.03 (C-6), 120.98 (C-5'), 120.57 (C-6"), 120.00 (C-1'), 119.54 (C-8), 111.96 (C-5), 111.33 (C-3'), 111.16 (C-4"), 55.75 (OMe), 55.73 (OMe), 43.25 (C-1"). **IR (ν_{max}/cm^{-1})**: 3063 (**Ar**C-H); 1603 (C=N); 1582, 1518; (**Ar**C=C); 1327 (C-O).

4.7.16.2. METHOD N

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (0.447 g, 13%). R_f (40% EtOAc/hexane) 0.34.

4.7.17. Synthesis of 3-(1-(3-hydroxybenzyl)-1H-benzoimidazol-2-yl)phenol (12b)



4.17.1. METHOD L

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 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. **^{1}H NMR (500 MHz, DMSO-*d*$_6$)**: δ 9.77 (1H, d, *J* = 30.8 Hz, H6'), 9.43 (1H, s, H2'), 7.73 (1H, d, *J* = 7.7 Hz, H8), 7.39 (1H, d, *J* = 7.5 Hz, H5), 7.33 (1H, q, *J* = 7.7 Hz, H5'), 7.23 (3H, td, *J* = 17.7, 16.0, 8.7 Hz, H6, H7, H6"), 7.17 – 7.06 (2H, m, OH), 6.95 (1H, d, *J* = 8.1 Hz, H7"), 6.65 (1H, d, *J* = 8.0 Hz, H5"), 6.50 (1H, d, *J* = 7.5 Hz, H4'), 6.40 (1H, s, H3"), 5.49 (2H, s, H1"). **^{13}C NMR (126 MHz, DMSO-*d*$_6$)**: δ 156.09 (C-3'), 155.91 (C-4"), 151.69 (C-2), 140.98 (C-4), 136.71 (C-9, C-2"), 134.25 (C-1'), 129.58 (C-5'), 128.19 (C-6"), 120.96 (C-7), 120.52 (C-6), 117.87 (C-6'),

117.55 (C-7”), 115.28 (C-8), 114.99 (C-5), 114.38 (C-4’), 112.82 (C-3”), 111.07 (C-2’), 109.45 (C-5”), 45.81 (C-1”).

4.7.17.2. METHOD N

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (3.06 g, 97%). R_f (40% EtOAc/hexane) 0.23

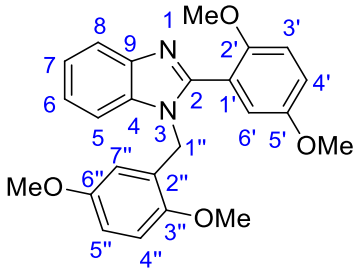
4.7.17.3. METHOD O

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (3.10 g, 98%). R_f (40% EtOAc/hexane) 0.23.

4.7.17.4. METHOD P

Novoprime Base 268 (0.105 g), stirring time = 4 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (2.72 g, 86%). R_f (40% EtOAc/hexane) 0.23.

4.7.18. Synthesis of 1-(2,5-dimethoxybenzyl)-2-(2,5-dimethoxyphenyl)-1H-benzimidazole (12c)



4.7.18.1. METHOD L

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford an oil (2.25 g, 56%). R_f (40% EtOAc/hexane) 0.36. **^{1}H**

NMR (500 MHz, DMSO-d_6): δ 7.73 – 7.57 (1H, m, H6'), 7.48 – 7.32 (1H, m, H8), 7.28 – 7.14 (2H, m, H3', H4'), 7.12 (2H, d, *J* = 9.3 Hz, H4", H5"), 7.02 – 6.85 (1H, m, H7), 6.85 (1H, d, *J* = 8.9 Hz, H5), 6.75 (1H, dd, *J* = 8.9, 2.7 Hz, H6), 6.15 (1H, s, H7"), 5.19 (2H, s, H1"), 3.74 – 3.45 (12H, m, 4 x OMe). **^{13}C NMR (126 MHz, DMSO-d_6)**: δ 153.42 (C-2), 153.32 (C-5'), 151.90 (C-6"), 151.58 (C-3"), 151.01 (C-2'), 143.22 (C-4), 135.63 (C-9), 125.80 (C-2"), 122.76 (C-7), 122.10 (C-6), 120.61 (C-8), 119.56 (C-5), 117.48 (C-1'), 117.10 (C-4'), 114.80 (C-7"), 113.23 (C-3'), 113.00 (C-6'), 112.19 (C-5"), 111.37 (C-4"), 56.22 (OMe-5'), 56.04 (OMe-6"), 55.68 (OMe-3"), 55.37 (OMe-2'), 43.26 (C-1").

4.7.18.2. METHOD M

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford an oil (3.64 g, 90%). R_f (40% EtOAc/hexane) 0.36.

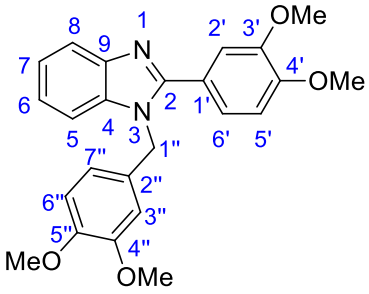
4.7.18.3. METHOD N

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford an oil (2.82 g, 70%). R_f (40% EtOAc/hexane) 0.36.

4.7.18.4. METHOD O

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford an oil (3.79 g, 94%). R_f (40% EtOAc/hexane) 0.36.

4.7.19. Synthesis of 1-(3,4-dimethoxybenzyl)-2-(3,4-dimethoxyphenyl)-1H-benzimidazole (12d)



4.7.19.1. METHOD L

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a yellow oil (1.45 g, 36%). R_f (60% EtOAc/hexane) 0.21. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 7.88 (1H, s, H2'), 7.71 (1H, d, *J* = 7.9 Hz, H6'), 7.53 (1H, d, *J* = 7.8 Hz, H8), 7.34 – 7.16 (3H, m, H5, H6, H7), 6.91 – 6.79 (2H, m, H5', H6"), 6.74 (1H, s, H3"), 6.62 (1H, d, *J* = 16.3 Hz, H7"), 5.90 (2H, m, H1"), 3.88 – 3.57 (12H, m, 4 x OMe). **^{13}C NMR (126 MHz, DMSO-d_6)**: δ 153.71 (C-2), 150.59 (C-3'), 149.41 (C-4'), 149.36 (C-4"), 149.18 (C-5"), 148.60 (C-4), 143.10 (C-9), 129.87 (C-1'), 122.99 (C-7), 122.85 (C-6), 122.16 (C-2"), 119.76 (C-6'), 119.45 (C-7"), 118.95 (C-8), 118.56 (C-5), 112.36 (C-2'), 112.49 (C-6'), 111.37 (C-5'), 110.78 (C-3"), 56.11 (4 x OMe), 47.74 (C-1"). **IR (ν_{max}/cm^{-1})**: 2970 (**Ar**C-H); 1651 (C=N); 1454 (**Ar**C=C); 1228 (C-O).

4.7.19.2. METHOD M

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a yellow oil (2.66 g, 66%). R_f (60% EtOAc/hexane) 0.21.

4.7.19.3. METHOD O

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a white solid (0.927 g, 23%). R_f (60% EtOAc/hexane) 0.21.

4.7.19.4. METHOD P

Denilite® II Base (0.085 g), stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a yellow oil (1.21 g, 30%). R_f (60% EtOAc/hexane) 0.21.

4.8. Synthesis of benzothiazoles

The following methods were used to synthesize benzothiazoles

Method Q

A mixture of 2-aminothiophenol (1.63 g, 15.0 mmol) and benzaldehyde derivative (1.06 g, 10.0 mmol) in acetonitrile (10.0 mL) and acetate buffer (10.0 mL, pH 4.0) was stirred at room for 5 minutes. Suberase® (2.0 mL) was then added into the mixture and the contents were stirred for 1 h. When the reaction completes, the product precipitates from the solution and was extracted with ethyl acetate (30.0 mL) and water (3 x 20.0 mL) and concentrated on a rotary evaporator. The product was washed several times with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material.

Method R

The same as Method Q, except that methanol (2.0 mL) was used instead of acetonitrile.

Method S

The same as Method Q, except that ethanol (4.0 mL) was used instead of acetonitrile.

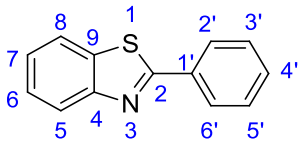
Method T

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

Method U

The same as Method Q, except that DCM (10.0 mL) was used instead of acetonitrile.

4.8.1. Synthesis of 2-phenylbenzothiazole (15a)



4.8.1.1. METHOD Q

Stirring time = 2 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a bright yellow solid (1.79 g, 85%). R_f (40% EtOAc/hexane) 0.93. MP = 96 – 98 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.15 – 8.04 (3H, m, H8, H2', H6'), 7.91 (1H, d, *J* = 8.0 Hz, H5), 7.50 (4H, m, H6, H7, H3', H5'), 7.39 (1H, t, *J* = 7.6 Hz, H4'). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 154.17 (C-2), 135.08 (C-4), 133.65 (C-1'), 130.97 (C-9), 129.03 (C-2', C-6'), 127.57 (C-5'), 126.32 (C-3'), 125.19 (C-4'), 123.26 (C-6, C-7), 121.62 (C-5, C-8). **IR (ν_{max}/cm^{-1})**: 3075 (**Ar**C-H); 1588, 1558, (**Ar**C=C); 1644 (C=N); 727 (C-S).

4.8.1.2. METHOD R

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a white solid (1.23 g, 58%). R_f (40% EtOAc/hexane) 0.93.

4.8.1.3. METHOD S

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a white solid (1.16 g, 55%). R_f (40% EtOAc/hexane) 0.93.

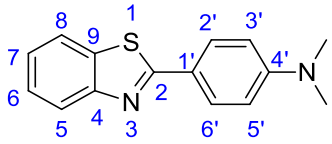
4.8.1.4. METHOD T

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a white solid (1.66 g, 78%). R_f (40% EtOAc/hexane) 0.93.

4.8.1.5. METHOD U

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a white solid (1.37 g, 65%). R_f (40% EtOAc/hexane) 0.93.

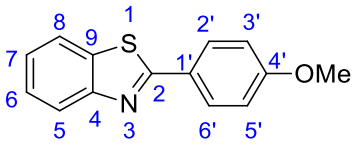
4.8.2. Synthesis of 4-(benzothiazol-2-yl)-N,N-dimethylaniline (15b)



4.8.2.1. METHOD Q

Stirring time = 2 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a cream solid (1.43 g, 56%). R_f (40% EtOAc/hexane) 0.81. MP = 172 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.03 – 7.91 (2H, m, H2', H6'), 7.84 (1H, d, *J* = 7.9 Hz, H5), 7.44 (1H, t, *J* = 7.4 Hz, H6), 7.30 (1H, t, *J* = 7.6 Hz, H7), 6.82 – 6.54 (3H, m, H8, H3', H5'), 3.01 (6H, d, *J* = 53.2 Hz, N-CH_3). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 168.79 (C-2), 154.43 (C-4'), 152.20 (C-4), 134.56 (C-9), 128.87 (C-6'), 127.71 (C-2'), 125.97 (C-6), 125.29 (C-7), 124.18 (C-1'), 122.29(C-8), 121.34 (C-5), 120.46 (C-5'), 112.29(C-3'), 40.48 (N-CH_3), 40.18 (N-CH_3). **IR (ν_{max}/cm^{-1})**: 3053 (**Ar**C-H); 2904 (C-N); 2815 (N-**C-H**), 1556, 1526, (**Ar**C=C); 1604 (C=N); 720 (C-S).

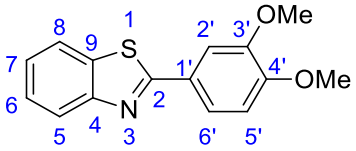
4.8.3. Synthesis of 2-(4-methoxyphenyl)benzothiazole (15c)



4.8.3.1. METHOD Q

Stirring time = 8 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford white solid (1.64 g, 68%). R_f (40% EtOAc/hexane) 0.89. MP = 120 – 122 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.04 (3H, d, *J* = 8.7 Hz, H5, H2', H6'), 7.88 (1H, d, *J* = 8.0 Hz, H8), 7.47 (1H, t, *J* = 7.6 Hz, H6), 7.35 (1H, t, *J* = 7.6 Hz, H7), 7.00 (2H, d, *J* = 8.7 Hz, H3', H5'), 3.88 (3H, s, OMe). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 167.85 (C-2), 161.94 (C-4'), 154.24 (C-4), 134.87 (C-1'), 129.12 (C-9), 126.47 (C-6'), 126.19 (C-2'), 124.79 (C-6), 122.83 (C-7), 121.50 (C-5, C-8), 114.38 (C-3', C-5'), 55.46 (OMe). **IR (ν_{max}/cm^{-1})**: 3060 (**Ar**C-H); 1574, 1557, (**Ar**C=C); 1603 (C=N); 729 (C-S), 1310 (C-O).

4.8.4. Synthesis of 2-(3,4-dimethoxyphenyl)benzothiazole (15d)

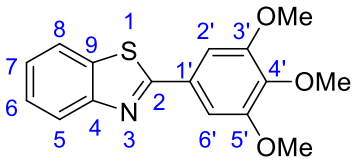


4.8.4.1 METHOD Q

Stirring time = 2 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a cream solid (1.98 g, 73%). R_f (40% EtOAc/hexane) 0.67. MP = 132 – 134 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.04 (1H, d, *J* = 8.2 Hz, H5), 7.88 (1H, d, *J* = 7.9 Hz, H8), 7.71 (1H, s, H2), 7.60 (1H, d, *J* = 9.5 Hz, H6'), 7.47 (1H, t, *J* = 7.7 Hz, H6), 7.36 (1H, t, *J* = 7.6 Hz, H7), 6.95 (1H, d, *J* = 8.4 Hz, H5'), 3.99 (6H, d, *J* = 33.3 Hz, OMe). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 167.93 (C-2), 154.17 (C-4), 151.60 (C-3'), 149.37 (C-4'), 134.92 (C-9), 126.71 (C-1'), 126.23 (C-6), 124.88 (C-7), 122.85 (C-5), 121.51 (C-8), 121.16 (C-6'), 111.05 (C-2'),

109.84 (C-5'), 56.15 (OMe-3'), 56.06 (OMe-4'). **IR (v_{max}/cm^{-1})**: 2964 (**Ar**C-H); 1520, 1479, (**Ar**C=C); 1598 (C=N); 732 (C-S), 1312 (C-O).

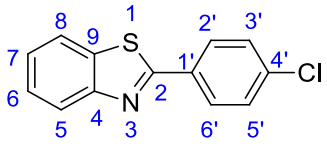
4.8.5. Synthesis of 2-(3,4,5-trimethoxyphenyl)benzothiazole (15e)



4.8.5.1. METHOD Q

Stirring time = 2 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a yellow solid (1.96 g, 65%). R_f (40% EtOAc/hexane) 0.74. MP = 148 – 151 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.06 (1H, d, *J* = 8.2 Hz, H5), 7.89 (1H, d, *J* = 8.0 Hz, H8), 7.49 (1H, t, *J* = 7.7 Hz, H6), 7.38 (1H, t, *J* = 7.6 Hz, H7), 7.34 (2H, s, H2', H6'), 3.96 (9H, d, *J* = 30.7 Hz, OMe). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 167.80 (C-2), 154.10 (C-4), 153.61 (C-3', C-5'), 140.71 (C-4'), 135.06 (C-9), 129.09 (C-1'), 126.36 (C-6), 125.13 (C-7), 123.10 (C-8), 121.56 (C-5), 104.84 (C-2', C-6'), 61.02 (2 x OMe), 56.39 (OMe). **IR (v_{max}/cm^{-1})**: 2941 (**Ar**C-H); 1581, 1518, (**Ar**C=C); 1698 (C=N); 1331 (C-O); 709 (C-S).

4.8.6. Synthesis of 2-(2-chlorophenyl)benzothiazole (15f)

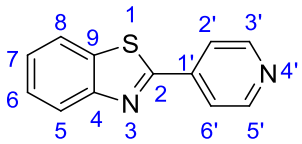


4.8.6.1. METHOD Q

Stirring time = 18 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a cream solid (1.35 g, 55%). R_f (40% EtOAc/hexane) 0.93. MP = 110 – 112 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)** δ 8.07 (1H, d, *J* = 8.2 Hz, H5), 8.03 (2H, d, *J* = 8.4 Hz, H2', H6'), 7.90 (1H, d, *J* = 8.0 Hz, H8), 7.48 (3H, m, H6, H3', H5'), 7.40 (1H, t, *J* = 7.6 Hz, H7).**^{13}C NMR (126 MHz, Chloroform-*d*)** δ 166.60 (C-2), 154.08 (C-5), 137.03 (C-1'), 135.06

(C-4'), 132.13 (C-9), 129.27 (C-6'), 128.71 (C-2'), 126.48 (C-3', C-5'), 125.41 (C-6), 123.31 (C-7), 121.65 (C-5, C-8). **IR (v_{max}/cm^{-1})**: 3055 (**Ar**C-H); 1646 (C=N); 1588, 1569, (**Ar**C=C); 730 (C-Cl), 709 (C-S).

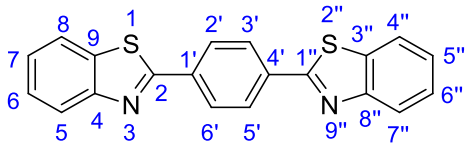
4.8.7. Synthesis of 2-(pyridin-4-yl)benzothiazole (15g)



4.8.7.1. METHOD Q

Stirring time = 2 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a bright yellow solid (1.86 g, 88%). R_f (40% EtOAc/hexane) 0.37. MP = 126 – 130 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.78 (2H, d, *J* = 5.7 Hz, H3', H5'), 8.13 (1H, d, *J* = 8.2 Hz, H5), 7.99 – 7.87 (3H, m, H8, H2', H6'), 7.55 (1H, t, *J* = 7.7 Hz, H6), 7.46 (1H, t, *J* = 7.6 Hz, H7). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 165.10 (C-4), 153.99 (C-2), 150.77 (C-3', C-5'), 140.49 (C-1'), 135.23 (C-9), 126.82 (C-6), 126.20 (C-7), 123.94 (C-8), 121.87 (C-5), 121.20 (C-2', C-6'). **IR (v_{max}/cm^{-1})**: 3035 (**Ar**C-H); 1698 (C=N); 1552, 1503, (**Ar**C=C); 704 (C-S).

4.8.8. Synthesis of 1,4-bis(benzothiazol-2-yl)benzene (15h)

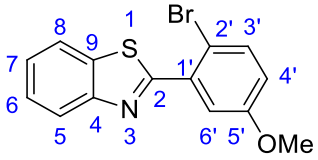


4.8.8.1. METHOD Q

2-aminothiophenol (2 equiv), Stirring time = 2 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a yellow solid (2.98 g, 87%). R_f (40% EtOAc/hexane) 0.81. MP = 261 – 264 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.23 (4H, s, H2', H3', H5', H6'), 8.12 (2H, d, *J* = 8.1 Hz, H5, H7"), 7.94 (2H, d, *J* = 8.0 Hz, H8, H4"), 7.53 (2H, t, *J* = 7.6 Hz, H6, H6"), 7.42 (2H, t, *J* = 7.5 Hz, H7, H5"). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 166.81(C-2, C-1"), 154.19 (C-4, C-8"), 135.70 (C-1', C-4"), 135.21 (C-9, C-3"),

128.09 (C-2', C-3', C-5', C-6'), 126.56 (C-6, C-6"), 125.56 (C-7, C-5"), 123.48 (C-8, C-4"), 121.70 (C-5, C-7"). **IR (v_{max}/cm^{-1})**: 3057 (**Ar**C-H); 1697 (C=N); 1558, 1522, (**Ar**C=C); 724 (C-S).

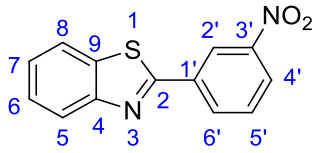
4.8.9. Synthesis of 2-(2-bromo-5-methoxyphenyl)benzothiazole (15i)



4.8.9.1. METHOD Q

Stirring time = 8 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a yellow solid (2.42 g, 76%). R_f (40% EtOAc/hexane) 0.93. MP = 123 – 125 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.68 (1H, d, *J* = 2.3 Hz, H5), 8.10 (1H, d, *J* = 8.2 Hz, H8), 7.93 (1H d, *J* = 7.9 Hz, H3'), 7.57 – 7.46 (2H, m, H6, H6'), 7.39 (1H, t, *J* = 7.5 Hz, H7), 6.94 (1H, d, *J* = 8.8 Hz, H4'), 4.04 (3H, s, OMe). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 161.43 (C-2), 156.22 (C-5'), 152.00 (C-4), 136.20 (C-1'), 134.12 (C-9), 131.88 (C-3'), 126.11 (C-6), 124.93 (C-7), 124.05 (C-8), 122.99 (C-5), 121.24 (C-4'), 113.81 (C-6'), 113.46 (C-2'), 56.03 (OMe). **IR (v_{max}/cm^{-1})**: 3058 (**Ar**C-H); 1694 (C=N); 1587, 1571, (**Ar**C=C); 1315 (C-O); 751 (C-Br); 723 (C-S).

4.8.10. Synthesis of 2-(3-nitrophenyl)benzothiazole (15j)

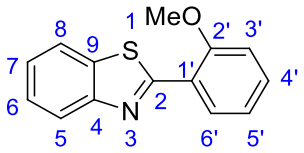


4.8.10.1. METHOD Q

Stirring time = 8 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a yellow solid (1.23 g, 48%). R_f (40% EtOAc/hexane) 0.81. MP = 185 – 187 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.94 (1H, s, H2'), 8.43 (1H, d, *J* = 7.8 Hz, H6'), 8.34 (1H, d, *J* = 8.2 Hz, H4'), 8.12 (1H, d, *J* = 8.2 Hz, H5), 7.95 (1H, d, *J* = 8.0 Hz, H8), 7.69 (1H, t, *J* = 8.0 Hz, H5'), 7.55 (1H, t, *J* = 7.7 Hz, H6), 7.46 (1H, t, *J* = 7.6 Hz, H7). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 164.89 (C-2), 153.97 (C-4), 148.79 (C-3'), 135.32 (C-6'), 135.20 (C-1'), 132.99

(C-9), 130.10 (C-5'), 126.84 (C-6), 126.03 (C-7), 125.18 (C-4'), 123.77 (C-2'), 122.35 (C-8), 121.83 (C-5). **IR (v_{max}/cm^{-1})**: 3085 (**Ar**C-H); 1612 (C=N); 1579, 1547, (**Ar**C=C); 1362 (N-O); 715 (C-S).

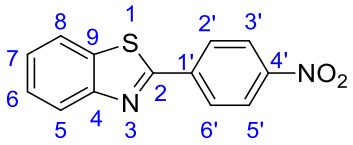
4.8.11. Synthesis of 2-(2-methoxyphenyl)benzothiazole (15k)



4.8.11.1. METHOD Q

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a brown solid (1.78 g, 74%). R_f (40% EtOAc/hexane) 0.89. MP =121 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.54 (1H, d, *J* = 7.8 Hz, H5), 8.10 (1H, d, *J* = 8.2 Hz, H8), 7.93 (1H, d, *J* = 7.9 Hz, H6'), 7.56 – 7.42 (2H, m, H6, H7), 7.37 (1H, t, *J* = 7.5 Hz, H5'), 7.14 (1H, t, *J* = 7.6 Hz, H4'), 7.06 (1H, d, *J* = 8.3 Hz, H3'), 4.05 (3H, s, OMe). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 153.49 (C-2), 150.51 (C-2'), 148.80 (C-4), 137.60 (C-9), 134.06 (C-4'), 133.86 (C-6'), 130.78 (C-6), 129.93 (C-7), 129.60 (C-1'), 125.66 (C-8), 123.29 (C-5), 116.28 (C-5'), 115.26 (C-3'). **IR (v_{max}/cm^{-1})**: 3053 (**Ar**C-H); 1604 (C=N); 1556, 1526, (**Ar**C=C); 1315 (C-O); 720 (C-S).

4.8.12. Synthesis of 2-(4-nitrophenyl)benzothiazole (15l)



4.8.12.1 METHOD Q

Stirring time = 24 h. Purification by washing with cold acetonitrile (3 x 20.0 mL) to remove any excess starting material (confirmed by TLC) to afford a yellow solid (2.15 g, 84%). R_f (40% EtOAc/hexane) 0.92. MP = 243 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.36 (2H, d, *J* = 8.7 Hz, H3', H5'), 8.27 (2H, d, *J* = 8.7 Hz, H2', H6'), 8.13 (1H, d, *J* = 8.2 Hz, H5), 7.96 (1H, d, *J* = 8.0 Hz, H8), 7.56 (1H, t, *J* = 7.7 Hz, H6), 7.47 (1H, t, *J* = 7.6 Hz, H7). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 164.84 (C-2), 154.11 (C-4), 149.05 (C-1'), 139.19 (C-4'), 135.50 (C-9), 128.24 (C-2', C-6'), 126.93 (C-6), 126.23 (C-7),

124.32 (C-3'), 123.95 (C-5'), 121.85 (C-5, C-8). **IR (v_{max}/cm^{-1})**: 3060 (**Ar**C-H); 1603 (C=N); 1574, 1557, (**Ar**C=C); 1310 (N-O); 729 (C-S).

4.9. Synthesis of diaminobenzoquinones

Method U

The primary amine (20.0 mmol, 2 equiv) was added to a mixture containing a dihydrobenzoquinone derivative (10.0 mmol, 1 equiv), acetonitrile (10.0 mL), acetate buffer (0.1 M, 10.0 mL, pH 4.0) and Denilite® II Base (0.085 g). The mixture was stirred at room temperature until the reaction completes (monitored by TLC). When the reaction completes, the product was extracted with ethyl acetate (30.0 mL) and water (3 x 20.0 mL). After extraction, the solvent was removed on the rotary evaporator to afford a solid product and purified by flash chromatography.

Method V

The same as Method U, except that methanol (2.0 mL) was used instead of acetonitrile.

Method W

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

Method X

The same as Method U, except that twice the solvent (acetonitrile, methanol, and DMF) and buffer were used.

Method Y

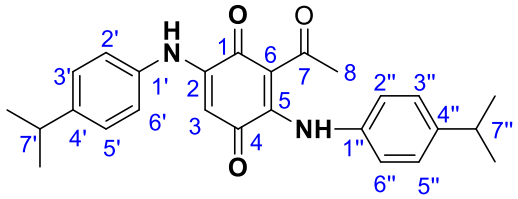
A repetition of method X using Novoprime Base 268 (0.105 g) and Suberase® (2.00 mL)

Method Z

The primary amine (20.0 mmol, 2 equiv) was added to a mixture containing, DMF (20.0 mL), acetate buffer (0.1 M, 20.0 mL, pH 4.0) and Novoprime Base 268 (0.105 g). The dihydroxy-benzoquinone derivative (10.0 mmol, 1 equiv) was dissolved in DMF and added dropwise into the mixture over time. The mixture was stirred at room temperature until the reaction completes (monitored by TLC). More laccase was added after 4, 12, and 18 h of reaction. When the reaction completes, the product was

extracted with ethyl acetate (30.0 mL) and water (3 x 20.0 mL). After extraction, the solvent was removed on the rotary evaporator to afford a solid product and purified by flash chromatography.

4.9.1. Synthesis of 3-acetyl-2,5-bis((4-isopropylphenyl)amino)cyclohexa-2,5-diene-1,4-dione (18)



4.9.1.1. Method U

Stirring time = 24 h. Purification by flash chromatography (silica/EtOAc-hexane, 1:4) to afford an orange brown solid (0.706 g, 17%).

4.9.1.2. Method V

Stirring time = 24 h. Purification by flash chromatography (silica/EtOAc-hexane, 1:4) to afford an orange brown solid (0.498 g, 12%).

4.9.1.3. Method W

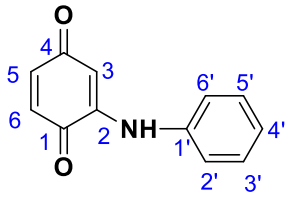
Stirring time = 24 h. Purification by flash chromatography (silica/EtOAc-hexane, 1:4) to afford an orange brown solid (1.03 g, 25%).

4.9.1.4. Method Z

Stirring time = 24 h. Purification by flash chromatography (silica/EtOAc-hexane, 1:4) to afford an orange brown solid (2.89 g, 72%). R_f (40% EtOAc/hexane) 0.72. MP = 136 – 140 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.39 (2H, s, NH), 7.29 (4H, d, *J* = 8.2 Hz, C-3', C-5', C-3", C-5"), 7.18 (4H, d, *J* = 8.2 Hz, H2', H6', H2", H6"), 6.10 (1H, s, H3), 2.93 (2H, h, *J* = 7.9, 7.4 Hz, H7', H7"), 2.65 (3H, s, CH_3-8), 1.23 (12H, dd, *J* = 30.6, 6.9 Hz, 4 x CH_3). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 206.91

(C-7), 200.03 (C-4), 177.21 (C-1, C-5), 147.84 (C-1', C-1"), 134.21 (C-4', C4', C-2), 127.75 (C-3', C-5', C-3", C-5"), 123.30 (C-2', C-6', C-2", C-6"), 115.23 (C-6), 96.94 (C-3), 33.79 (C-7', C-7"), 32.52 (C-8), 23.90 (4 x CH_3). **IR (v_{max}/cm^{-1})**: 3221 (N-H); 2967 (**Ar**C-H); 1738 (C=O); 1587, 1551, (**Ar**C=C); 1328 (C-N); 1206 (C-O).

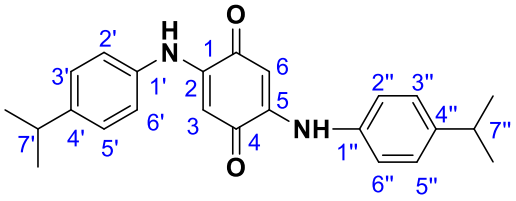
4.9.2. Synthesis of 2-(phenylamino)cyclohexa-2,5-diene-1,4-dione (19)



4.9.2.1. METHOD Z

Primary amine (10.0 mmol, 1 equiv). Stirring time = 24 h. washing with ethyl acetate (1 x 20.0 mL) to afford a dark brown solid (2.01 g, 100%). R_f (40% EtOAc/hexane). MP = 138 – 140 °C. **^{1}H NMR (300 MHz, Chloroform-*d*)**: δ 10.38 (1H, s, H3), 8.56 – 8.25 (1H, m, NH), 7.69 (2H, s, H5, H6), 7.53 – 7.46 (1H, m, H4'), 7.46 – 7.35 (2H, m, H3', H5'), 7.35 – 7.27 (2H, m, H2', H6'). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 206.95 (C-1), 178.09 (C-4), 151.53 (C-2), 150.21 (C-1'), 135.80 (C-5, C-6), 128.26 (C-2', C-5'), 125.52 (C-2', C-4', C-6'), 92.76 (C-3). **IR (v_{max}/cm^{-1})**: 3270 (N-H); 2926 (**Ar**C-H); 1633 (C=O); 1589, 1560, (**Ar**C=C); 1247 (C-N).

4.9.3. Synthesis of 2,5-bis((4-isopropylphenyl)amino)cyclohexa-2,5-diene-1,4-dione (20)

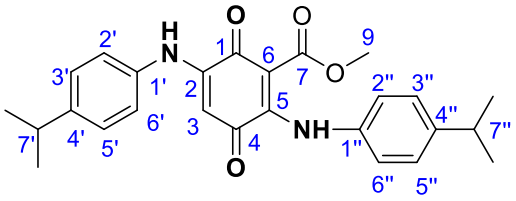


4.9.3.1. METHOD Z

Stirring time = 24 h. Purification by flash chromatography (silica/EtOAc-hexane, 1:4) to afford a dark brown solid (2.09 g, 56%). R_f (40% EtOAc/hexane) 0.82. MP = 138 – 142 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.09 (2H, s, NH), 7.19 (4H, d, *J* = 8.1 Hz, H2', H6', H2", H6"), 7.02 (1H, d, *J* = 8.1

Hz, H3"), 6.71 (2H, s, H5', H5"), 6.64 (1H, d, *J* = 8.1 Hz, H3'), 6.02 (2H, s, H3, H6), 2.93 (2H, hept, *J* = 7.0 Hz, H7', H7"), 1.26 (12H, d, *J* = 6.9 Hz,4 x CH_3). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 180.11 (C-1, C-4), 147.12 (C-2, C-5), 147.08 (C-1', C-1"), 134.73 (C-4', C-4"), 127.60 (C-3', C-5', C-3", C-5"), 122.81 (C-2', C-6', C-2", C-6"), 116.18 (C-3, C-6), 115.25, 33.75 (C-7', C-7"), 23.92 (4 x CH_3). **IR (ν_{max}/cm^{-1})**: 3236 (N-H); 2960 (**Ar**C-H); 1634 (C=O); 1560, 1512, (**Ar**C=C); 1293 (C-N); 1185 (C-O).

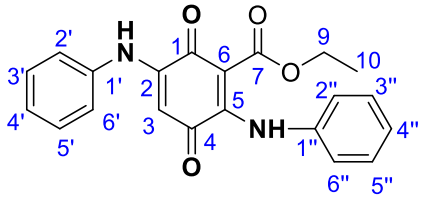
4.9.4. Synthesis of methyl 2,5-bis((4-isopropylphenyl)amino)-3,6-dioxocyclohexa-1,4-dienecarboxylate (21)



4.9.4.1. METHOD Z

Purification by flash chromatography (silica/EtOAc-hexane, 1:4) to afford a dark brown solid (1.21 g, 28%). R_f (40% EtOAc/hexane) 0.69. MP = 190 – 192 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.63 (1H, s, NH), 8.18 (1H, s, NH), 7.30 – 7.25 (4H, m, H3', H5', H3", H5"), 7.18 (2H, d, *J* = 8.2 Hz, H2', H6'), 7.07 (2H, d, *J* = 8.2 Hz, H2", H6"), 6.23 (1H, s, H3), 3.11 (3H, s, CH_3-9), 2.93 (2H, dq, *J* = 13.4, 6.8 Hz, H7', H7"), 1.25 (12H, dd, *J* = 8.3, 7.0 Hz, 4 x CH_3). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 206.90 (C-4), 178.48 (C-5), 177.01 (C-1), 148.27 (C-7), 147.58 (C-2), 147.07 (C-1', C-1") 134.35 (C-4', C-4"), 127.68 (C-3', C-5', C-3", C-5"), 127.30 (C-2', C-6', C-2", C-6"), 123.80 (C-3), 123.14 (C-6), 51.30 (C-9), 33.78 (C-7', C-7"), 23.91 (4 x CH_3). **IR (ν_{max}/cm^{-1})**: 3225 (N-H); 2959 (**Ar**C-H); 1723 (C=O); 1589, 1560, (**Ar**C=C); 1312 (C-N); 1259 (C-O).

4.9.5. Synthesis of ethyl 3,6-dioxo-2,5-bis(phenylamino)cyclohexa-1,4-dienecarboxylate (22)



4.9.5.1. METHOD Z

Stirring time = 24 h. Purification by washing with ethyl acetate (1 x 20.0 mL) to afford a dark brown solid (3.61 g, 100%). R_f (40% EtOAc/hexane). 0.72. MP = 160 – 163 °C. **^{1}H NMR (500 MHz, Chloroform-*d*)**: δ 8.69 (1H, s, NH), 8.19 (1H, s, NH), 7.46-7.37 (4H, m, H3', H5', H3", H5"), 7.31 – 7.23 (4H, m, H2', H6', H2", H6"), 7.18 (2H, d, *J* = 7.8 Hz, H4', H4"), 6.11 (1H, s, H3), 3.55 (2H, q, *J* = 7.2 Hz, H9), 1.00 (3H, t, *J* = 7.2 Hz, H10). **^{13}C NMR (126 MHz, Chloroform-*d*)**: δ 178.84 (C-4), 177.21 (C-5), 164.29 (C-1), 146.70 (C-7), 137.36 (C-2), 136.79 (C-1', C-1"), 129.75 (C-3', C-5'), 129.32 (C-3", C-5"), 127.14 (C-4'), 126.49 (C-4"), 123.76 (C-2', C-6'), 123.05 (C-2", C-6"), 102.73 (C-3), 95.89 (C-6), 60.92 (C-9), 13.80 (C-10). **IR (ν_{max}/cm^{-1})**: 3242 (N-H); 1721 (C=O); 1555, 1495 (**Ar**C=C); 1339 (C-N); 1164 (C-O).

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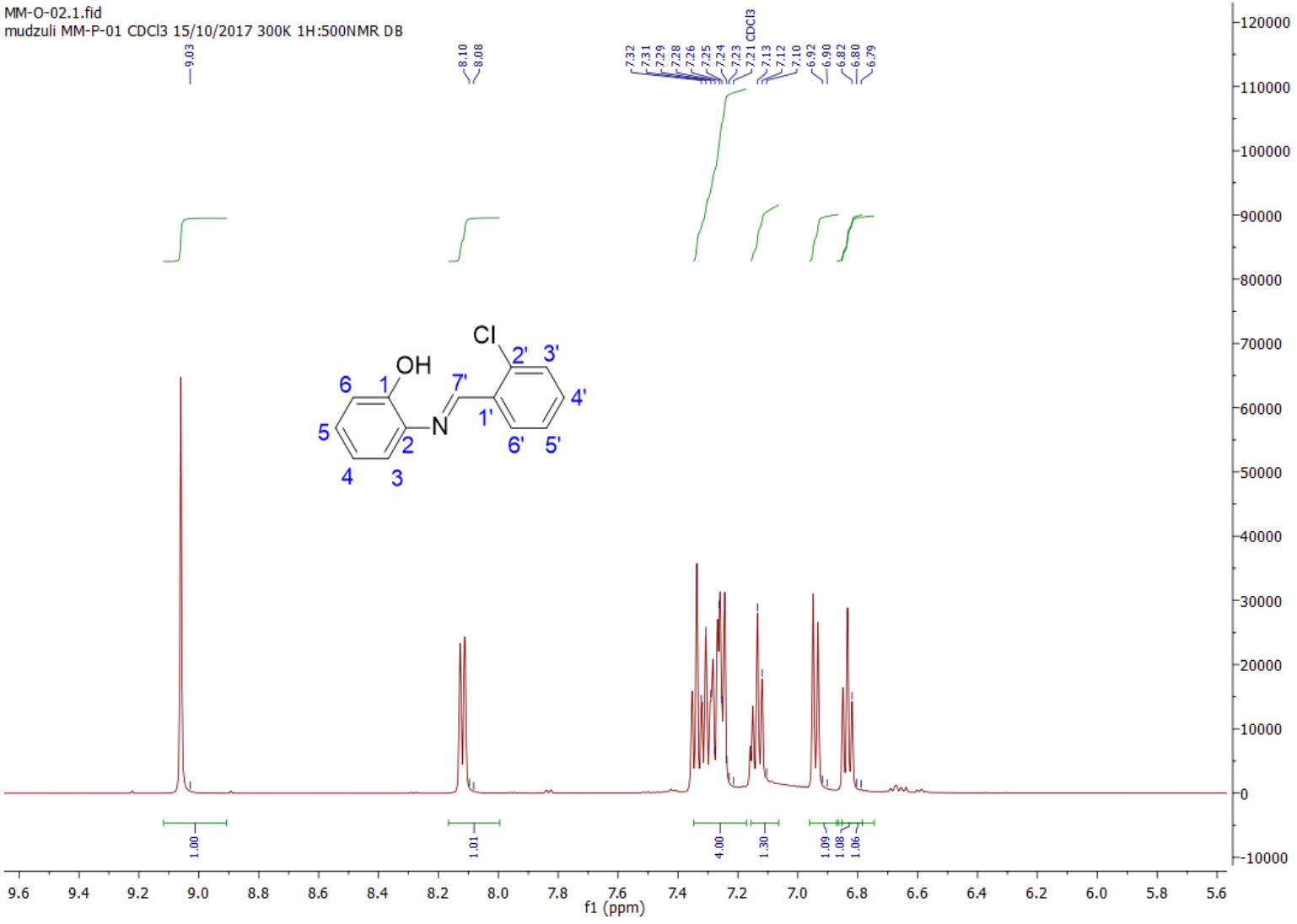
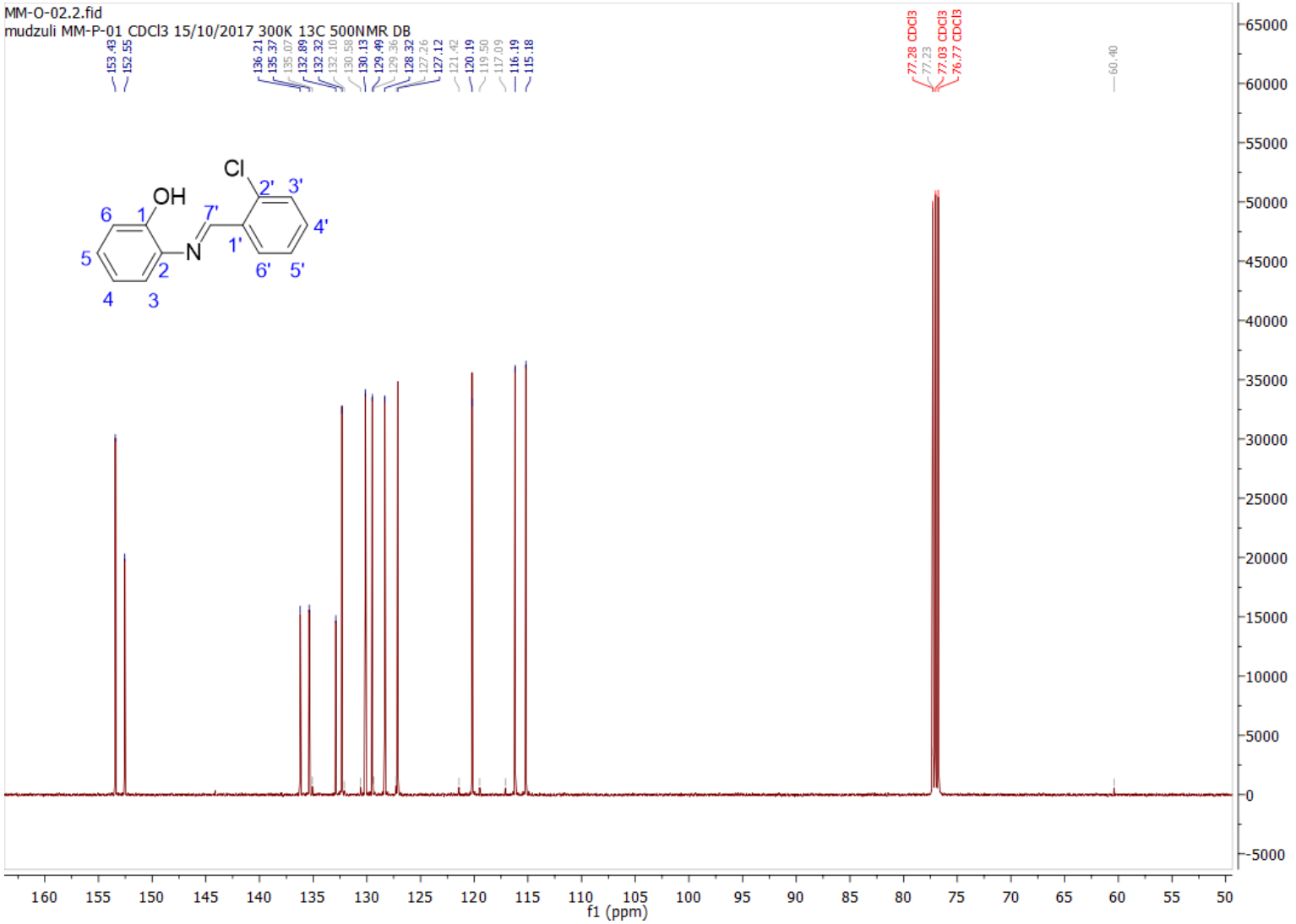
APPENDIX

Spectra of selected compound are listed below.

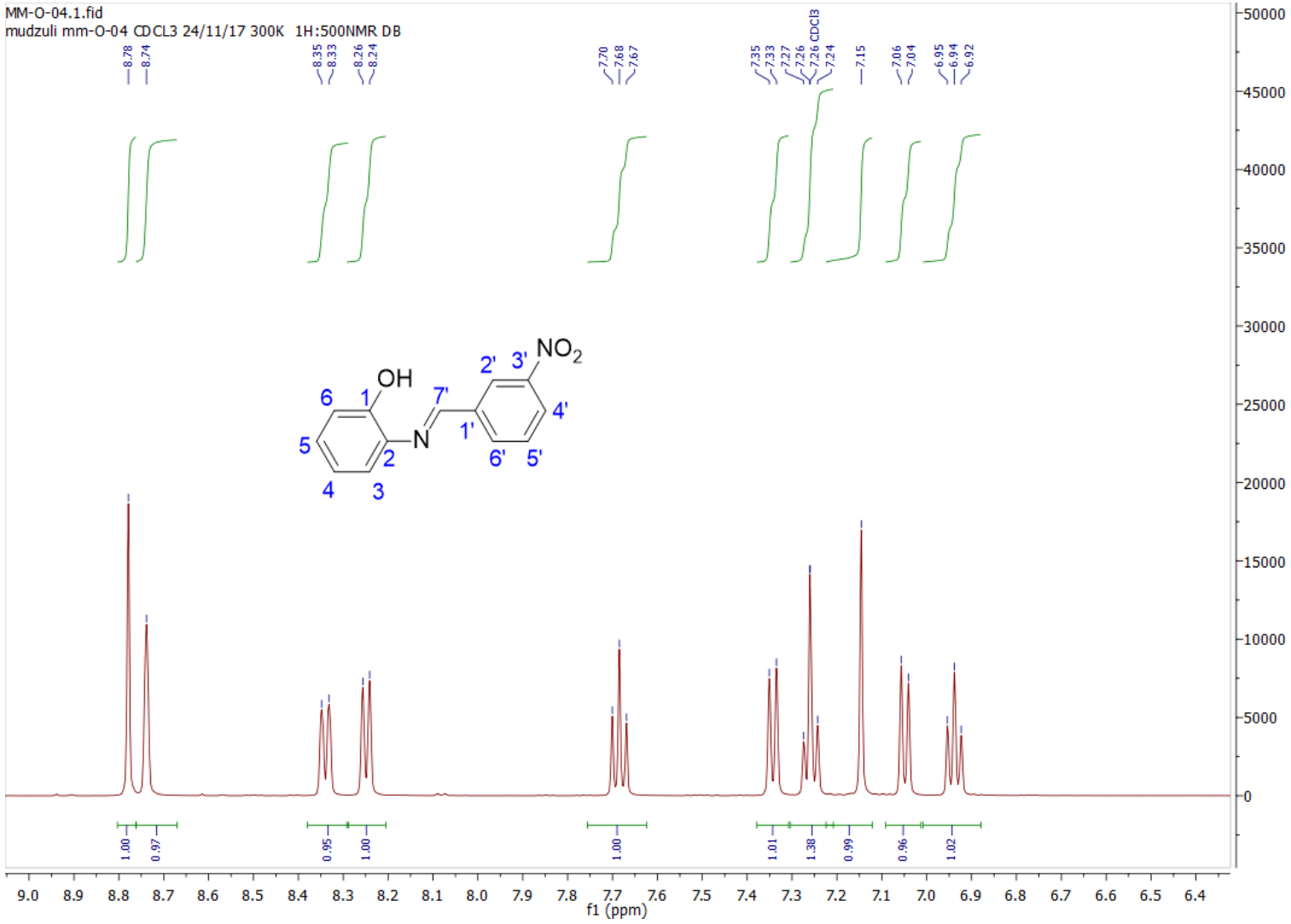
^{1}H NMR of **8a**


MM-O-02.1.fid
mudzuli MM-P-01 CDCl3 15/10/2017 300K 1H:500NMR DB
9.03
8.10
8.08
7.32
7.31
7.29
7.28
7.26
7.25
7.24
7.23
7.21 CDCl3
7.13
7.12
7.10
6.92
6.90
6.82
6.80
6.79
Cl
OH
N
1.00
1.01
4.00
1.30
1.09
1.08
1.06
f1 (ppm)

^{13}C NMR of 8a


MM-O-02.2.fid
mudzuli MM-P-01 CDCl3 15/10/2017 300K 13C 500NMR DB
153.43
152.55
136.21
135.37
135.07
132.89
132.32
132.10
130.58
130.13
129.49
129.36
128.32
127.26
127.12
121.42
120.19
119.50
117.09
116.19
115.18
77.28 CDCl3
77.23
77.03 CDCl3
76.77 CDCl3
60.40
OH
Cl
N
f1 (ppm)

^{1}H NMR of 8b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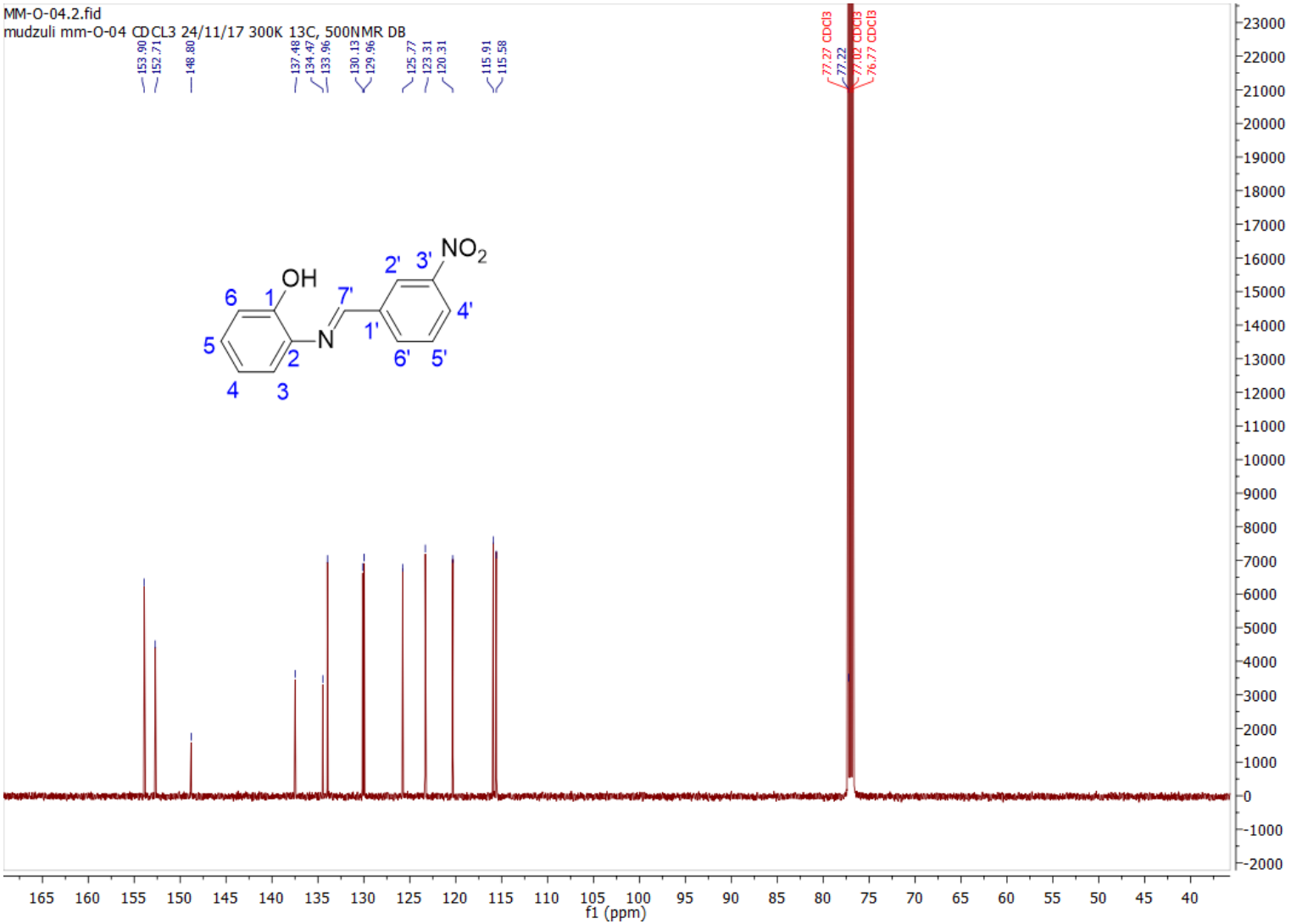
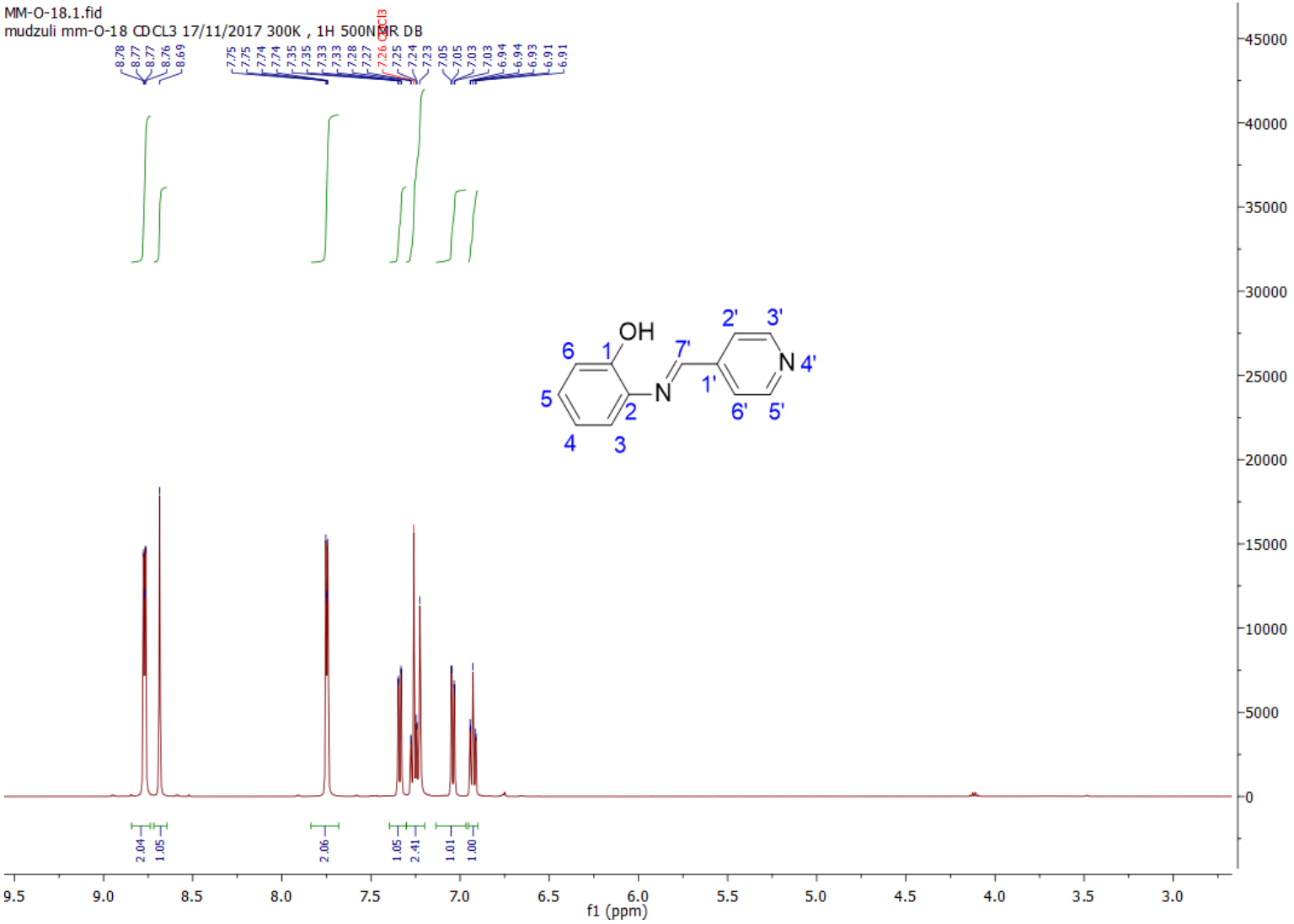
mudzuli mm-O-04 CDCL3 24/11/17 300K 1H:500NMR DB
8.78
8.74
8.35
8.33
8.26
8.24
7.70
7.68
7.67
7.35
7.33
7.27
7.26
7.26 CDCl3
7.24
7.15
7.06
7.04
6.95
6.94
6.92
OH
NO2
2'
3'
4'
1'
6'
5'
7'
1
2
3
4
5
6
N
1.00
0.97
0.95
1.00
1.00
1.01
1.38
0.99
0.96
1.02
f1 (ppm)

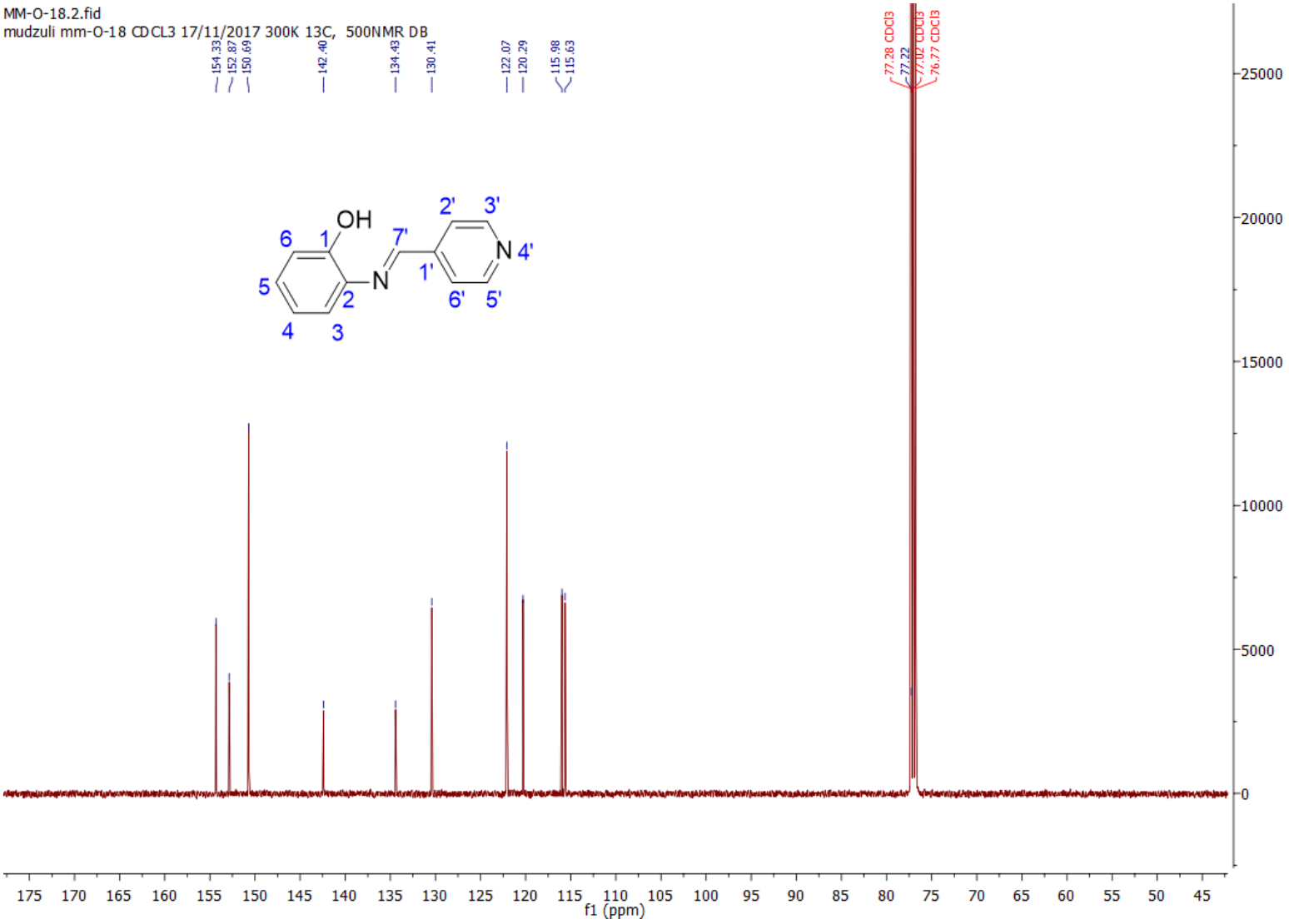
^{13}C NMR of **8b**


MM-O-04.2.fid
mudzuli mm-O-04 CDCL3 24/11/17 300K 13C, 500NMR DB
153.90
152.71
148.80
137.48
134.47
133.96
130.13
129.96
125.77
123.31
120.31
115.91
115.58
77.27 CDCl3
77.22
77.02 CDCl3
76.77 CDCl3
OH
NO2
f1 (ppm)

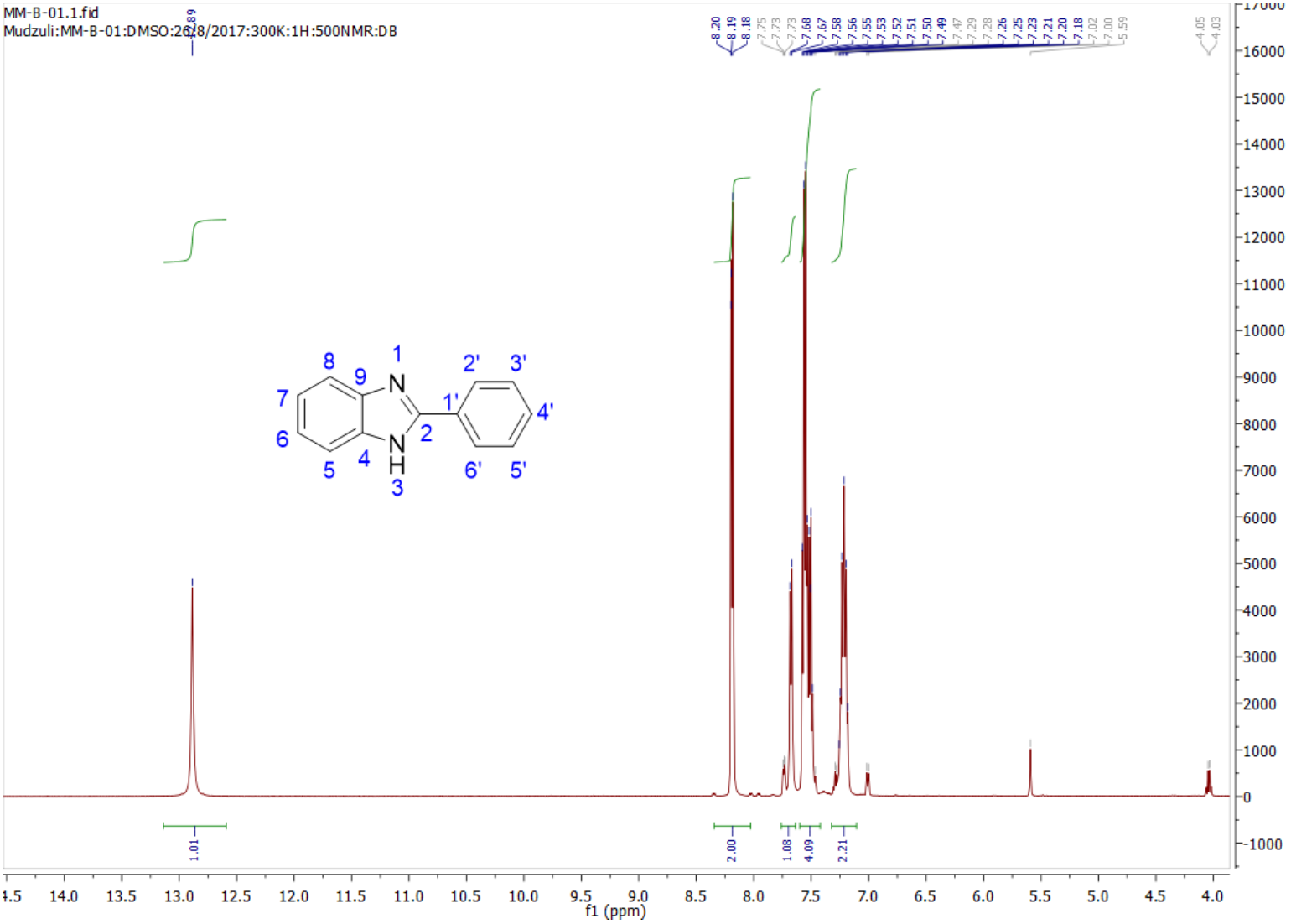
^{1}H NMR of 8o


MM-O-18.1.fid
mudzuli mm-O-18 CDCL3 17/11/2017 300K , 1H 500NMR DB
8.78
8.77
8.77
8.76
8.69
7.75
7.75
7.74
7.74
7.35
7.35
7.33
7.33
7.28
7.27
7.26
7.25
7.24
7.23
7.05
7.05
7.03
7.03
6.94
6.94
6.93
6.91
6.91
OH
1
2
3
4
5
6
N
7'
1'
2'
3'
N
4'
5'
6'
2.04
1.05
2.06
1.05
2.41
1.01
1.00
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
f1 (ppm)

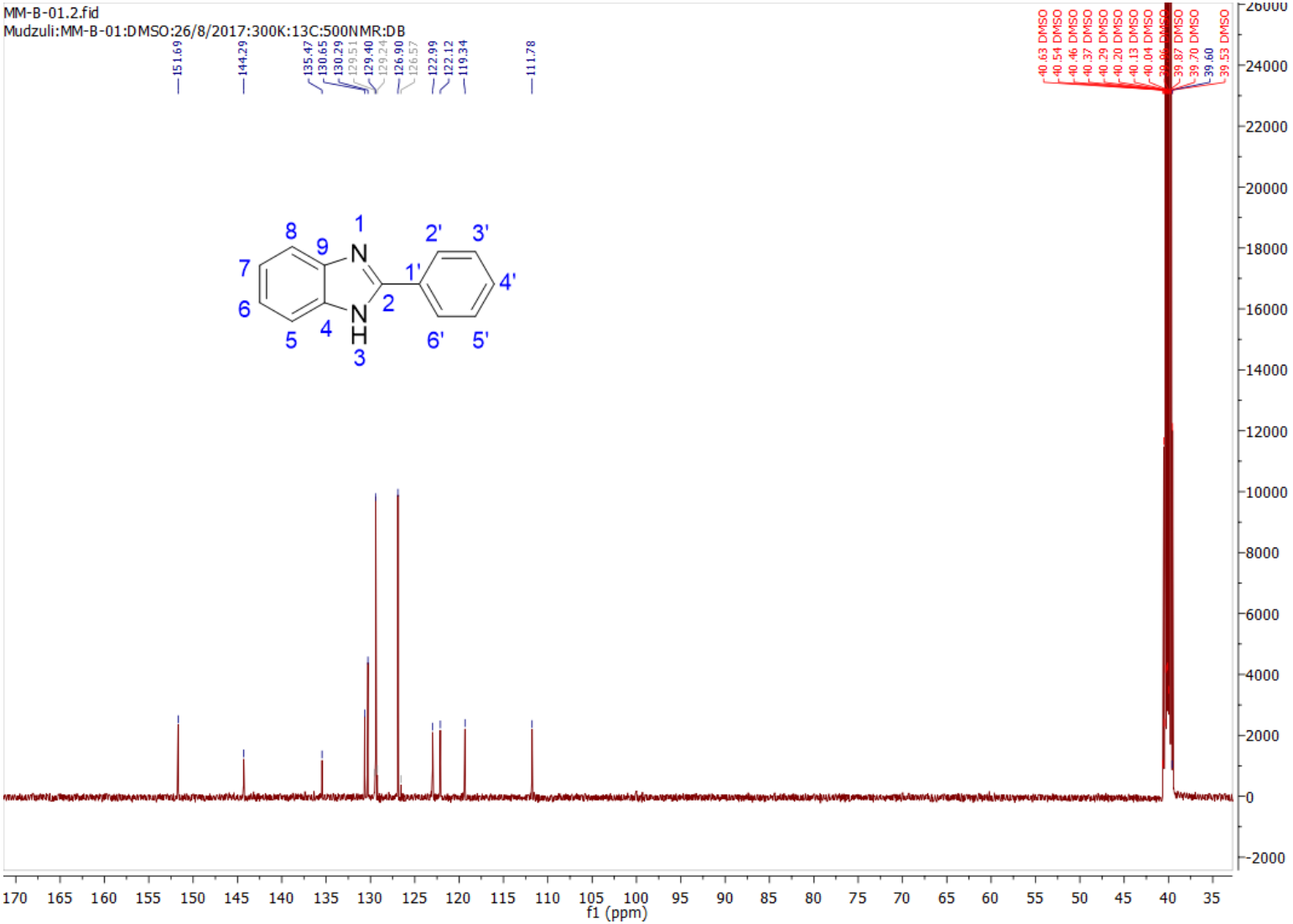
^{13}C NMR of **8o**


MM-O-18.2.fid
mudzuli mm-O-18 CDCL3 17/11/2017 300K 13C, 500NMR DB
154.33
152.87
150.69
142.40
134.43
130.41
122.07
120.29
115.98
115.63
77.28 CDCl3
77.22
77.02 CDCl3
76.77 CDCl3
OH
N
N
f1 (ppm)

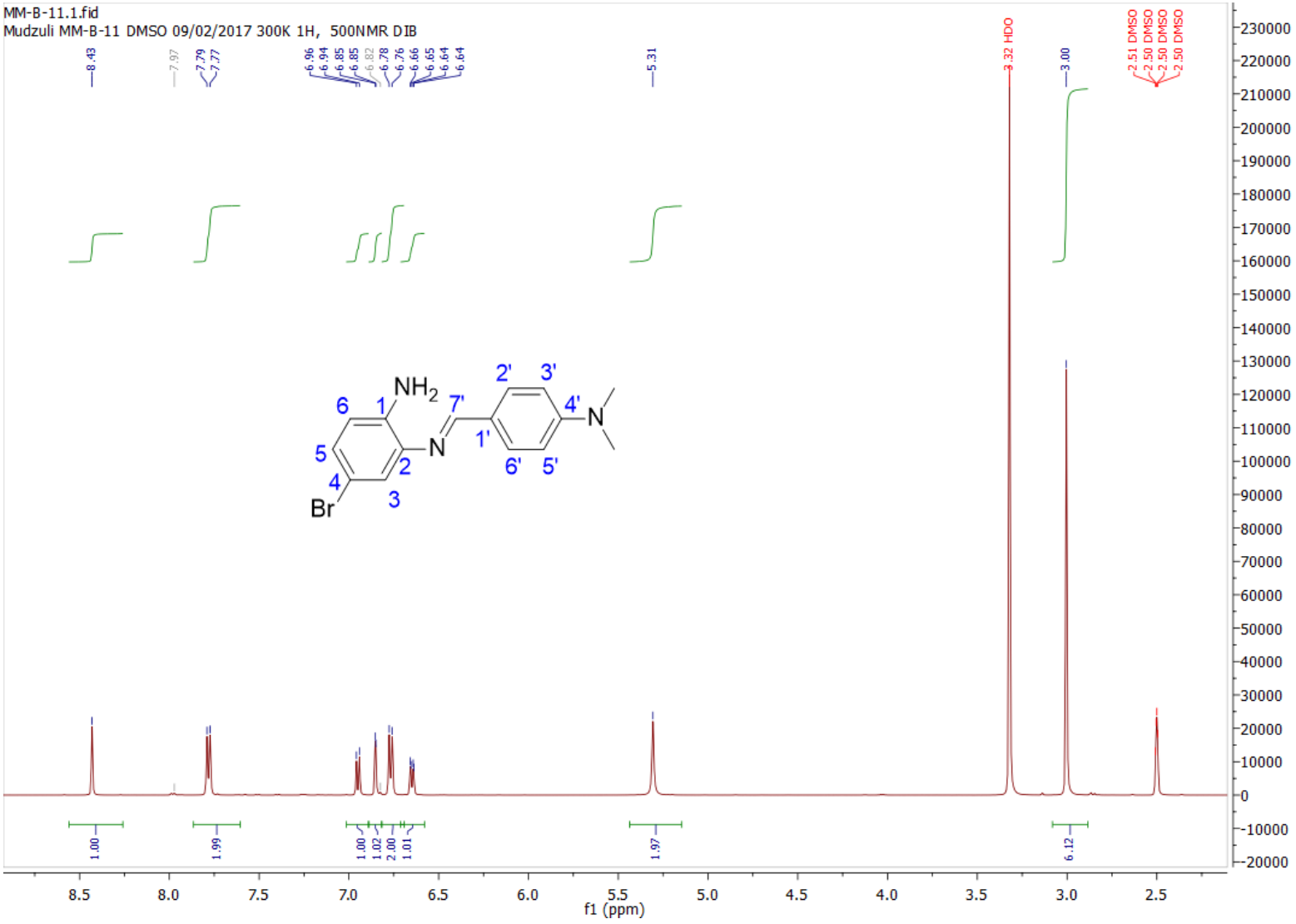
^{1}H NMR of **11a**


MM-B-01.1.fid
Mudzuli:MM-B-01:DMSO:2628/2017:300K:1H:500NMR:DB
f1 (ppm)

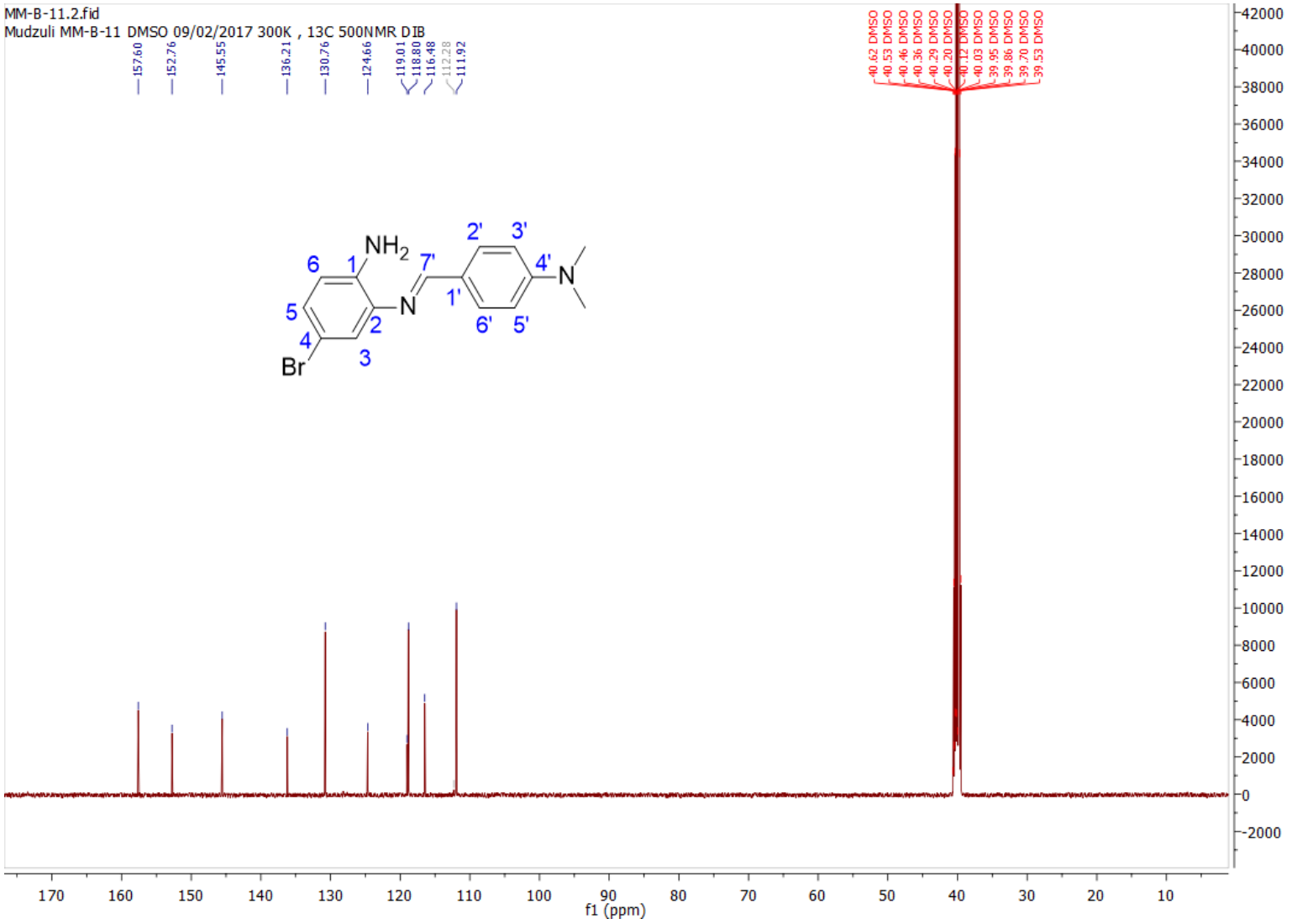
^{13}C NMR of 11a



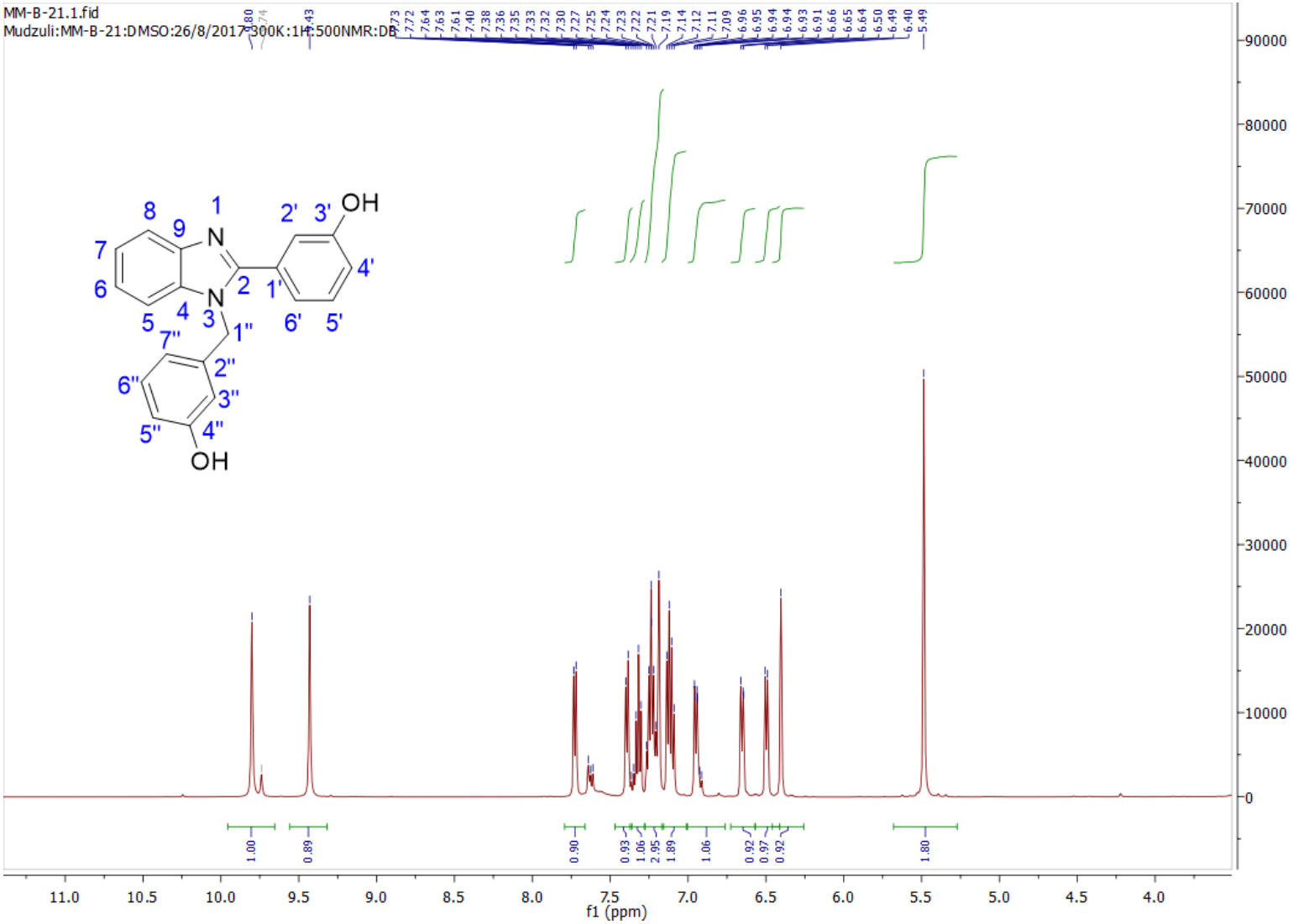
^{1}H NMR of 111


MM-B-11.1.fid
Mudzuli MM-B-11 DMSO 09/02/2017 300K 1H, 500NMR DIB
8.43
7.97
7.79
7.77
6.96
6.94
6.85
6.85
6.82
6.78
6.76
6.66
6.65
6.64
6.64
5.31
3.32 HDO
3.00
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
NH2
Br
N
1.00
1.99
1.00
1.02
2.00
1.01
1.97
6.12
f1 (ppm)

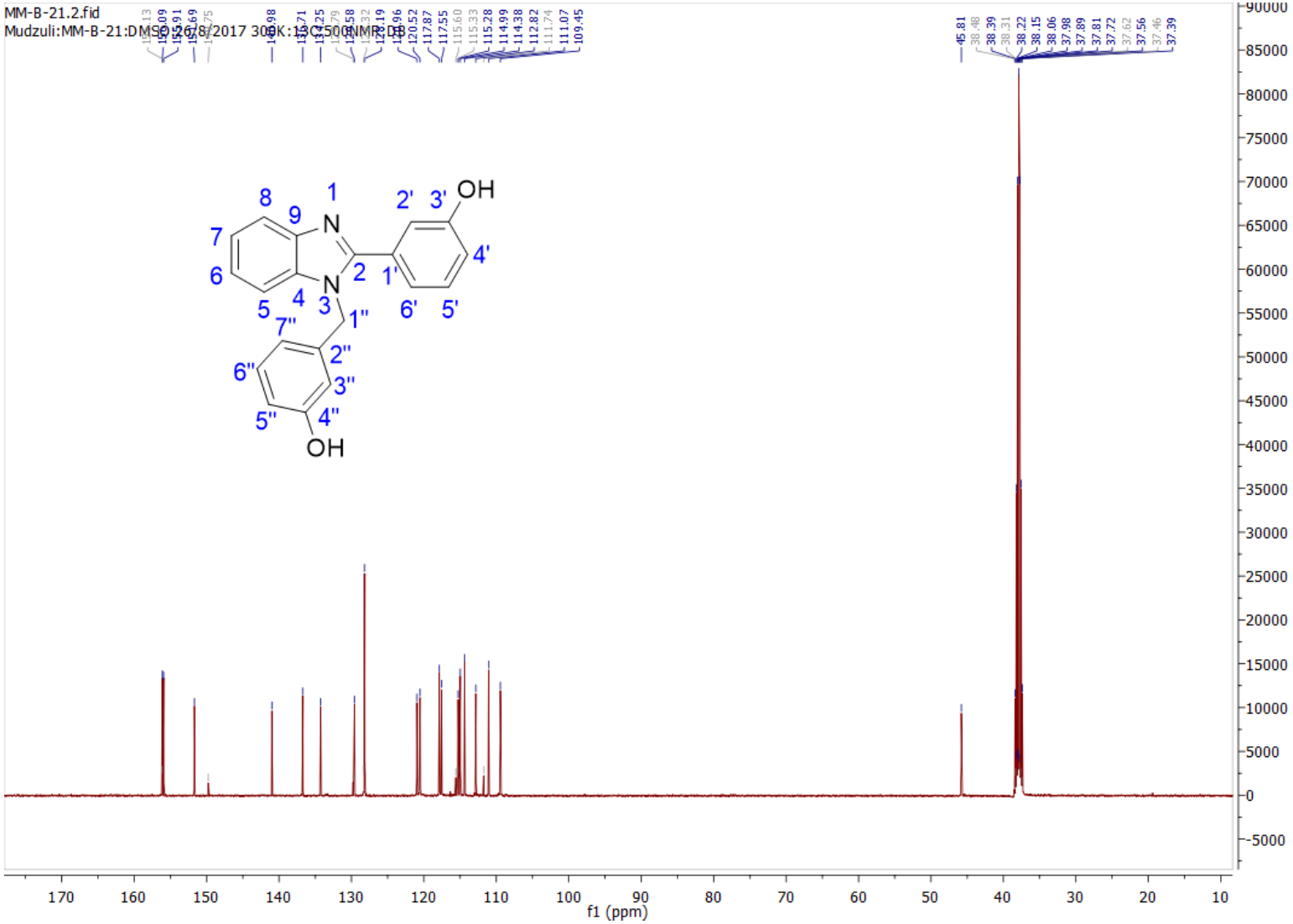
^{13}C NMR of **111**



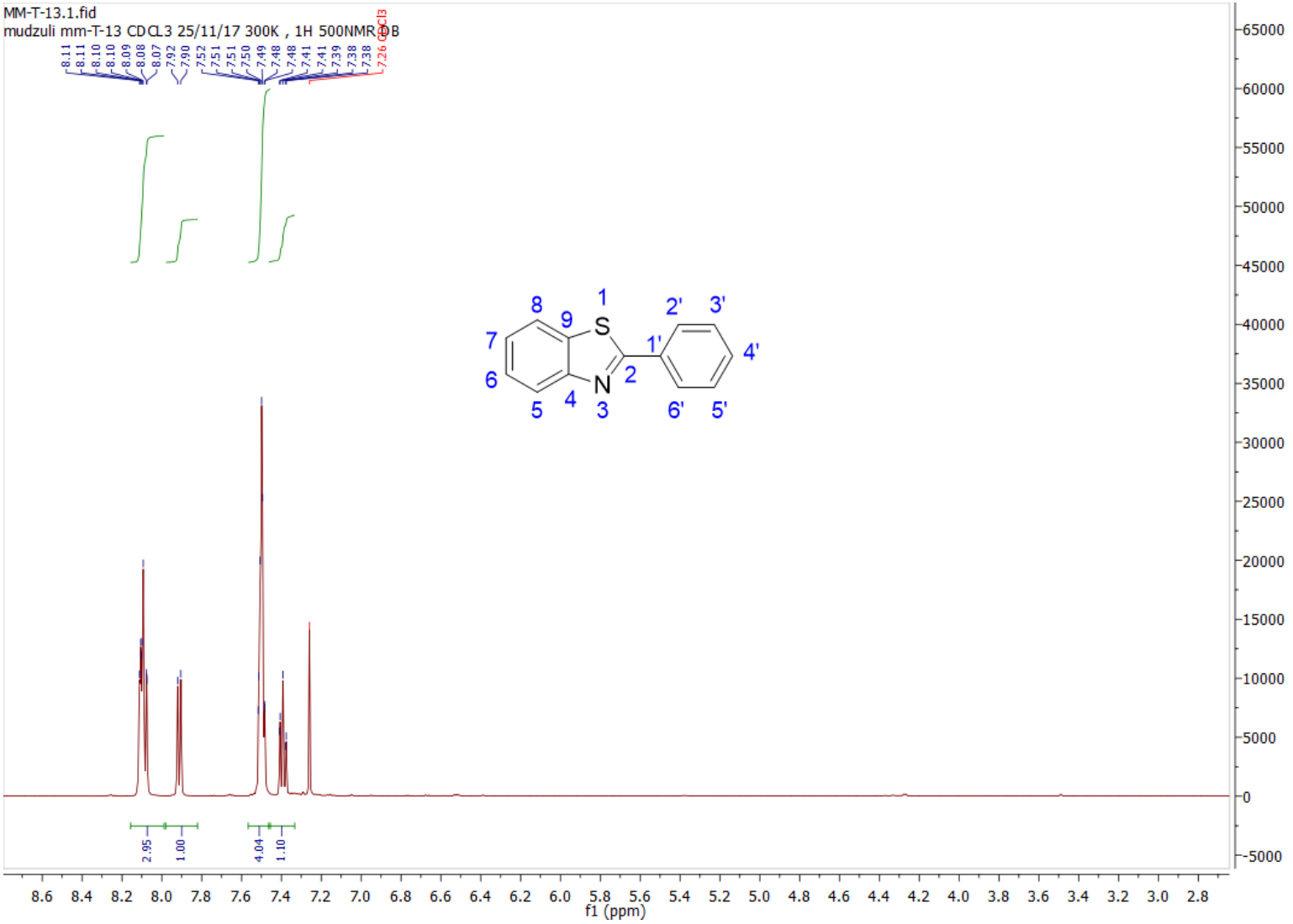
^{1}H NMR of **12b**


MM-B-21.1.fid
Mudzuli:MM-B-21:DMSO:26/8/2017:300K:1H:500NMR:DB
OH
OH
f1 (ppm)

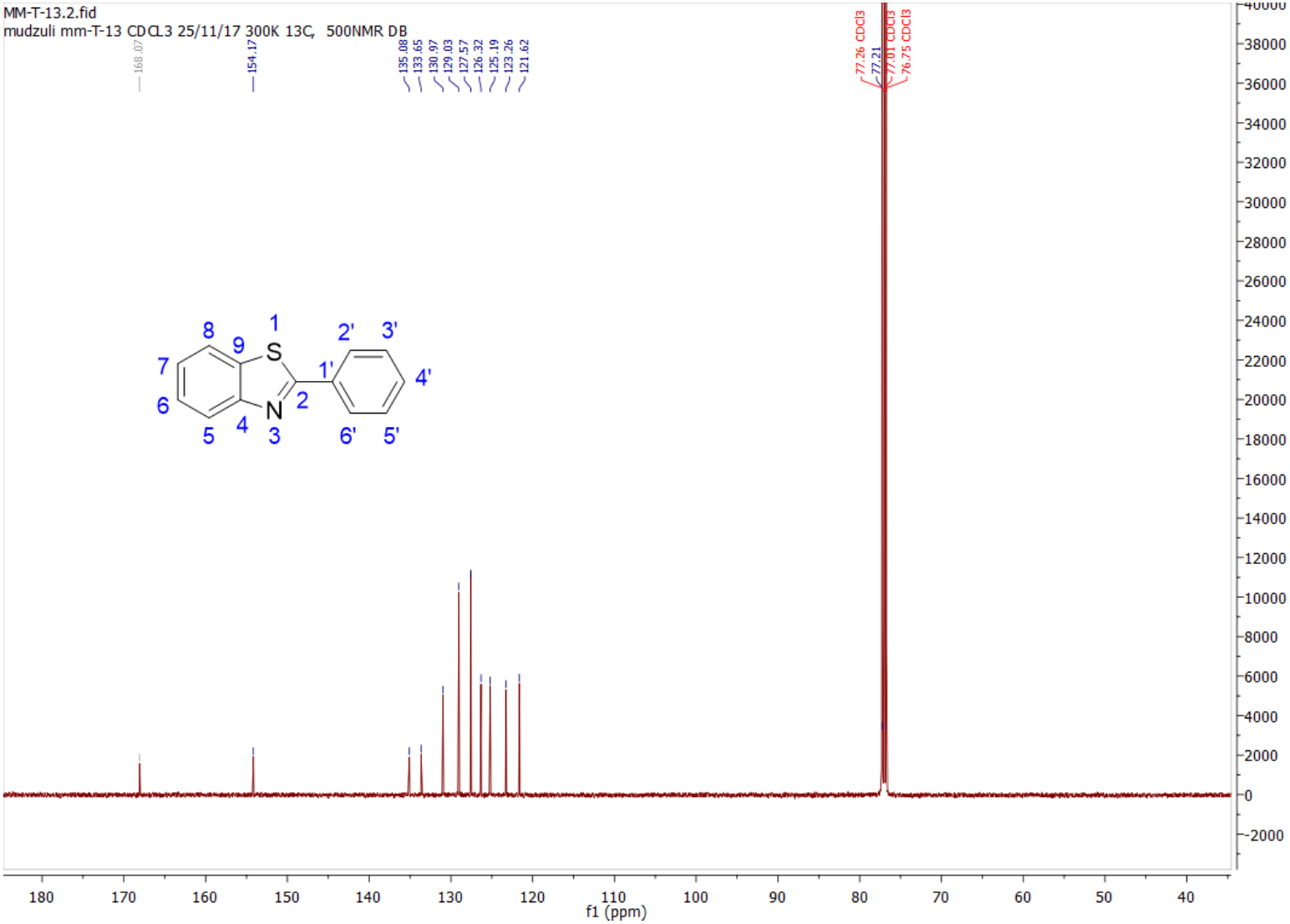
^{13}C NMR of **12b**



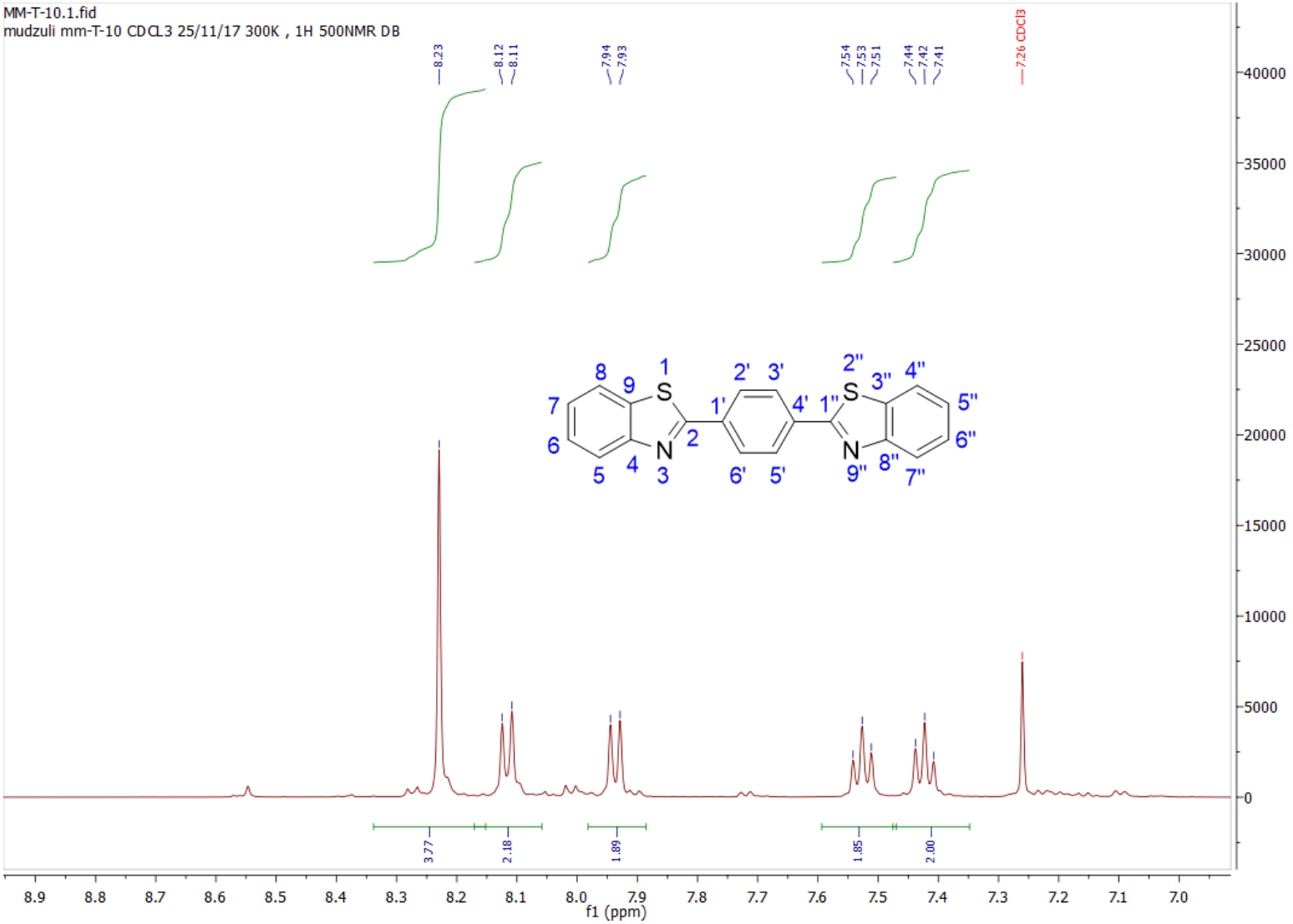
^{1}H NMR of **15a**


MM-T-13.1.fid
mudzuli mm-T-13 CDCL3 25/11/17 300K , 1H 500NMR DB
8.11
8.11
8.10
8.10
8.09
8.08
8.07
7.92
7.90
7.52
7.51
7.51
7.50
7.49
7.48
7.48
7.41
7.41
7.39
7.38
7.38
7.26
CDCl3
1
2
3
4
5
6
7
8
9
1'
2'
3'
4'
5'
6'
S
N
2.95
1.00
4.04
1.10
65000
60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000
8.6 8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8
f1 (ppm)

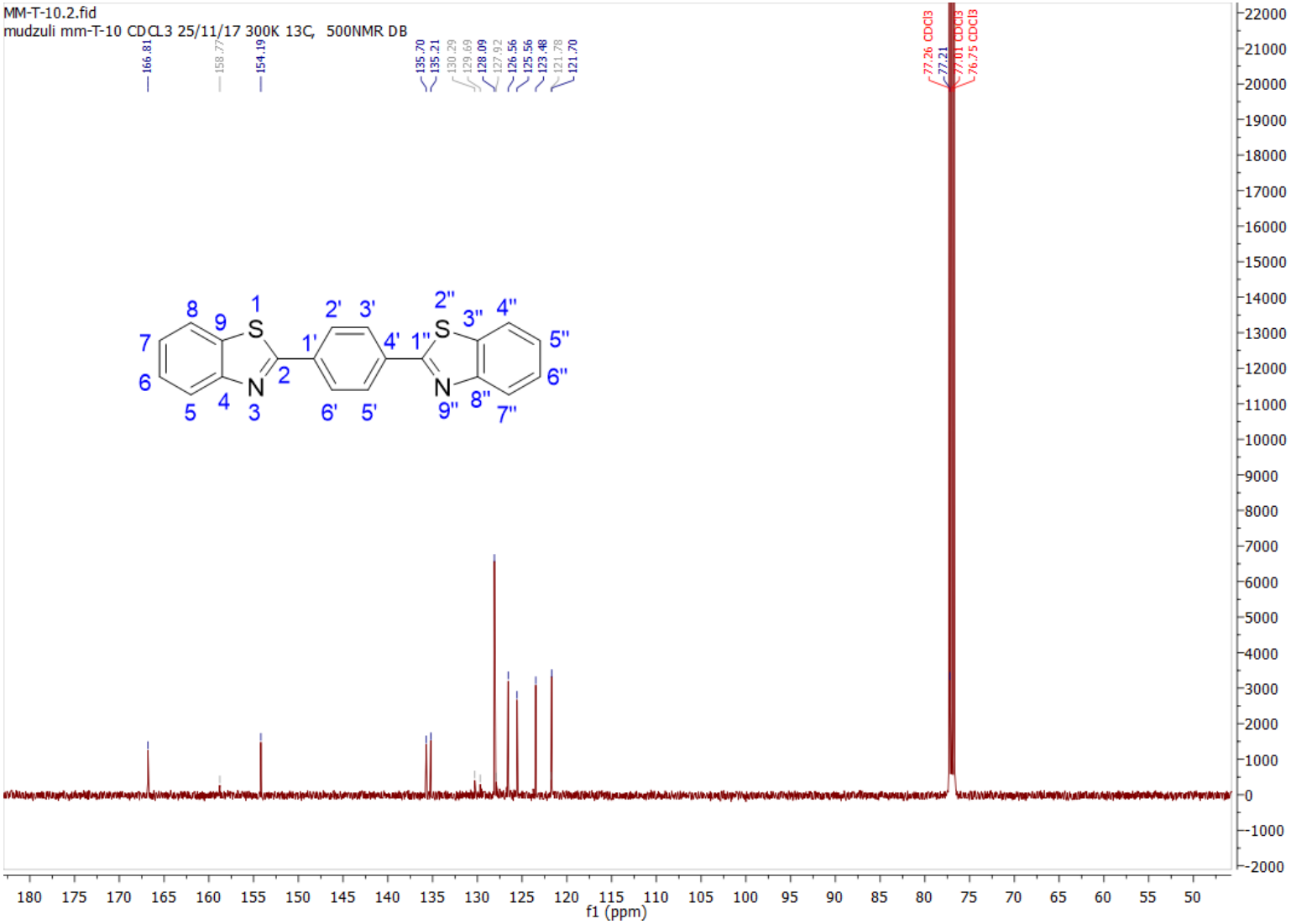
^{13}C NMR of 15a



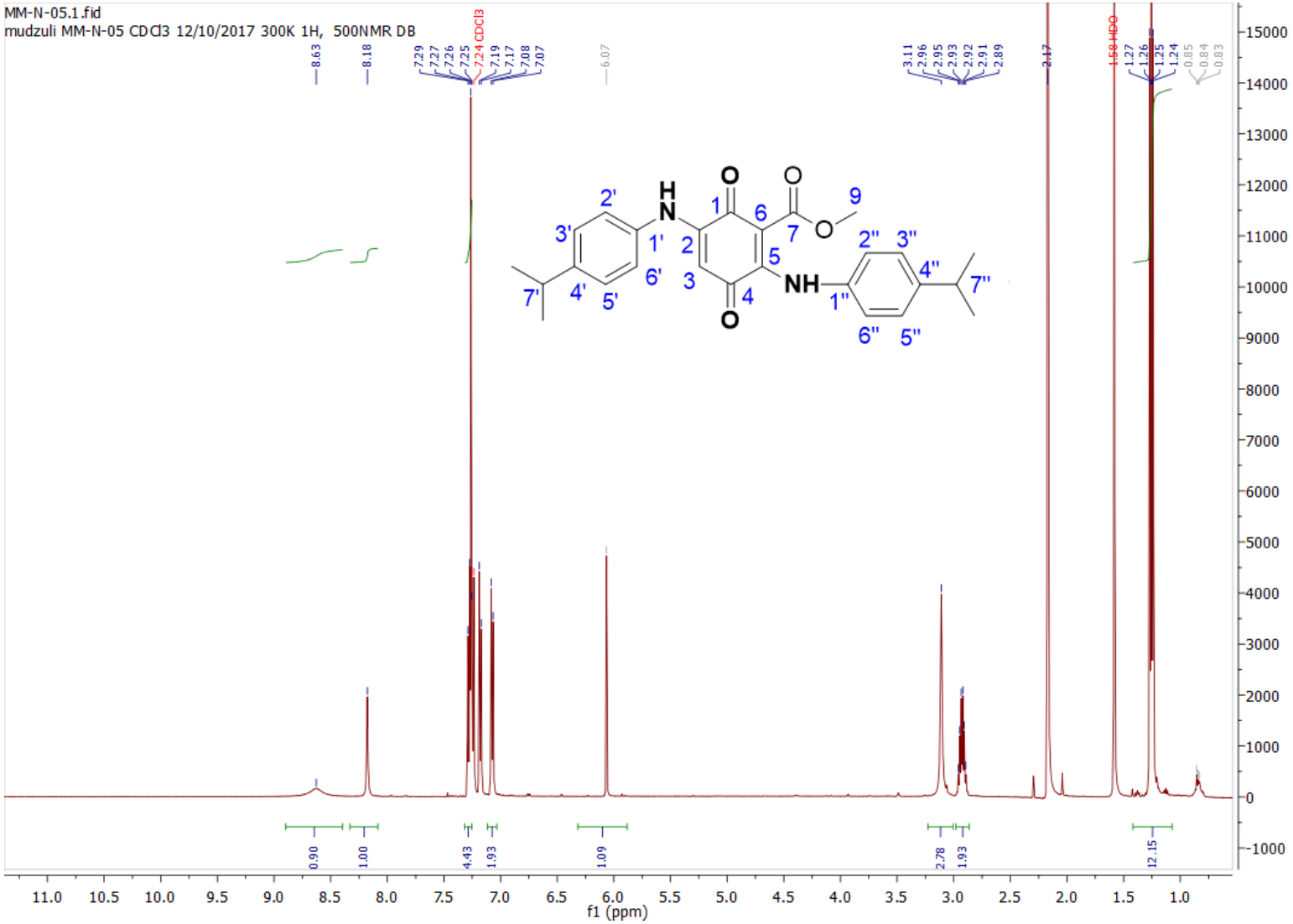
^{1}H NMR of **15h**


MM-T-10.1.fid
mudzuli mm-T-10 CDCL3 25/11/17 300K , 1H 500NMR DB
8.23
8.12
8.11
7.94
7.93
7.54
7.53
7.51
7.44
7.42
7.41
7.26 CDCl3
3.77
2.18
1.89
1.85
2.00
f1 (ppm)

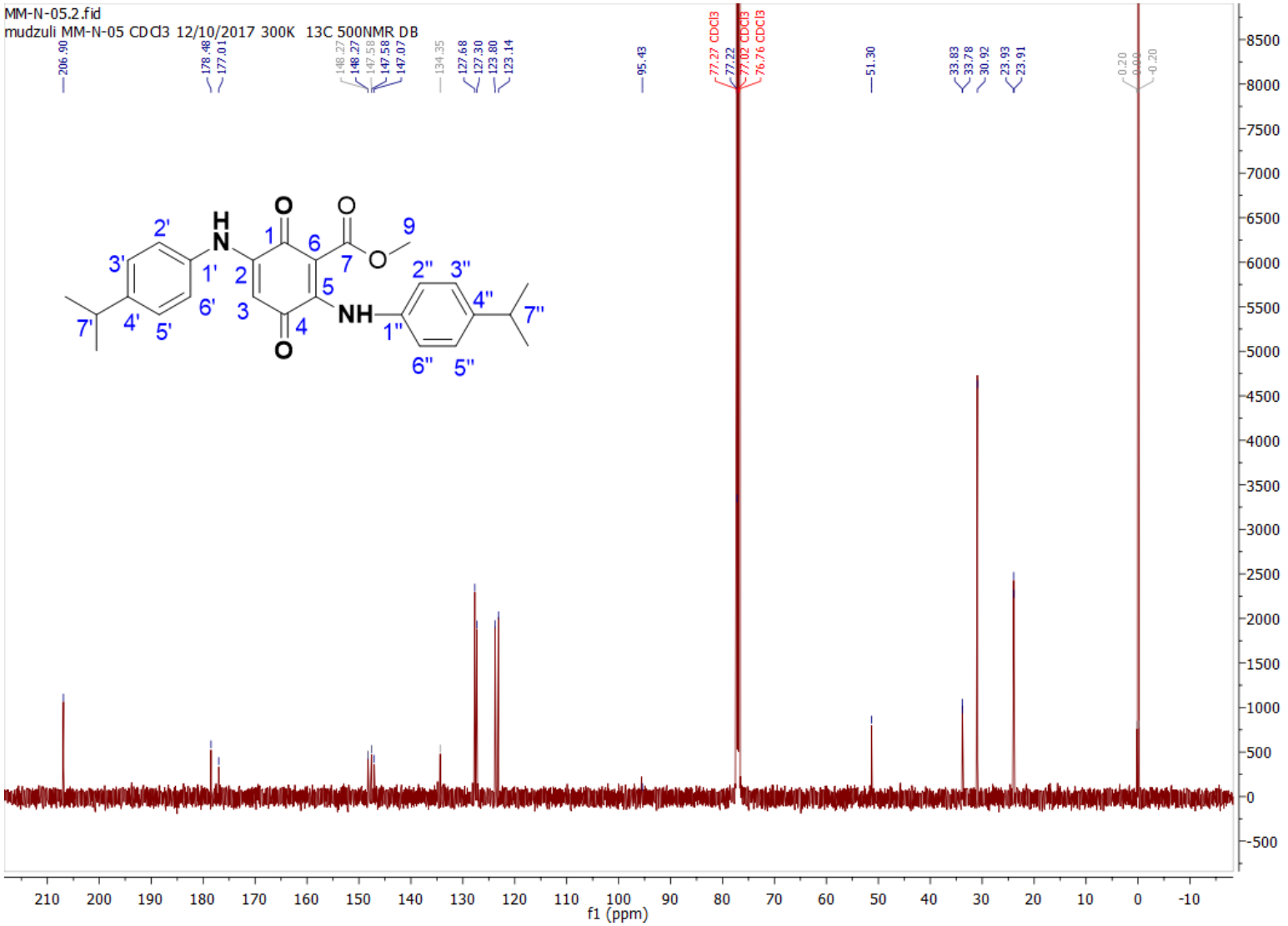
^{13}C NMR of 15h



^{1}H NMR of **21**


MM-N-05.1.fid
mudzuli MM-N-05 CDCl3 12/10/2017 300K 1H, 500NMR DB
8.63
8.18
7.29
7.27
7.26
7.25
7.24 CDCl3
7.19
7.17
7.08
7.07
6.07
3.11
2.96
2.95
2.93
2.92
2.91
2.89
2.17
1.58 HDO
1.27
1.26
1.25
1.24
0.85
0.84
0.83
0.90
1.00
4.43
1.93
1.09
2.78
1.93
12.15
f1 (ppm)

^{13}C NMR of 21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mudzuli MM-N-05 CDCl3 12/10/2017 300K 13C 500NMR DB
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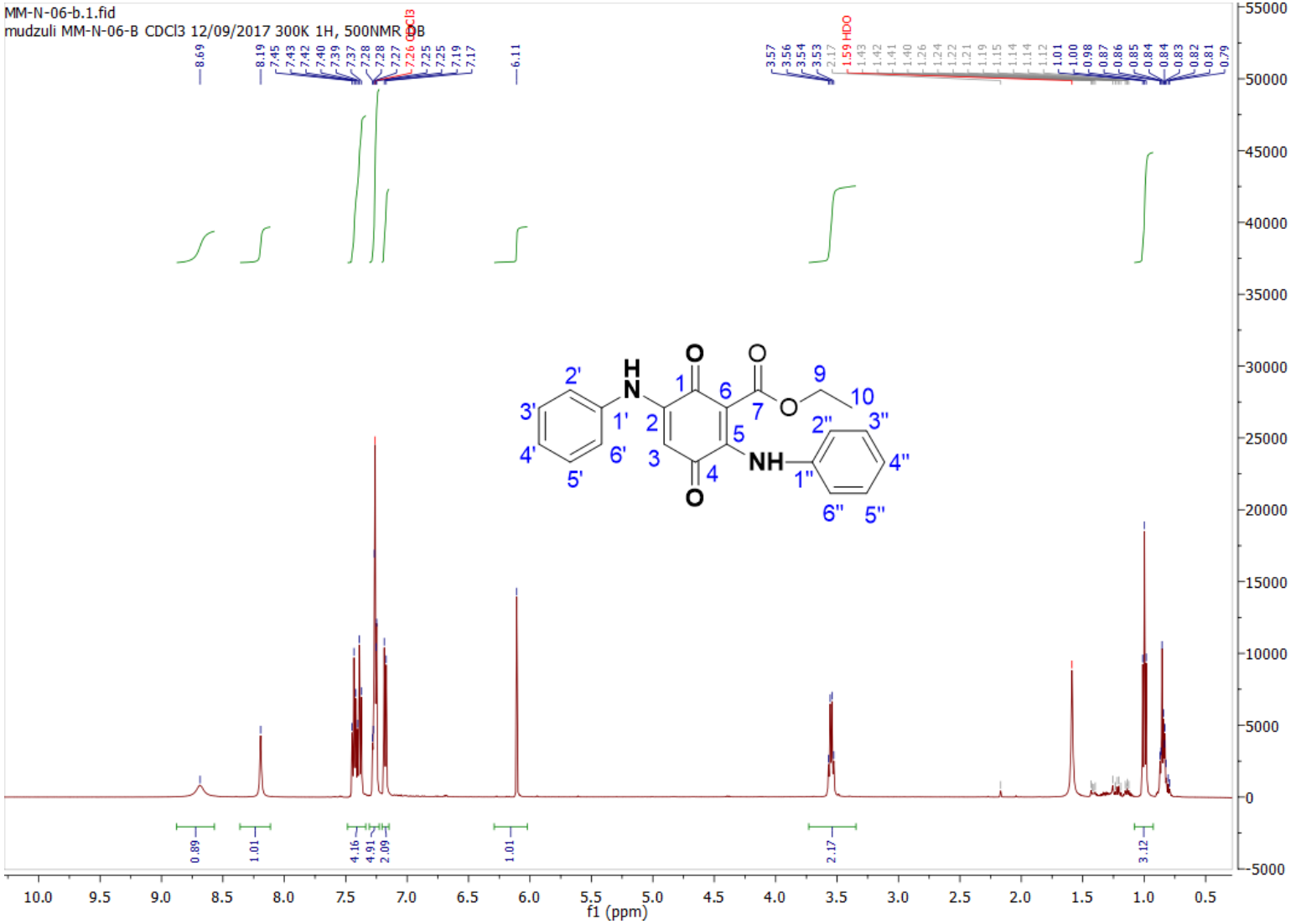
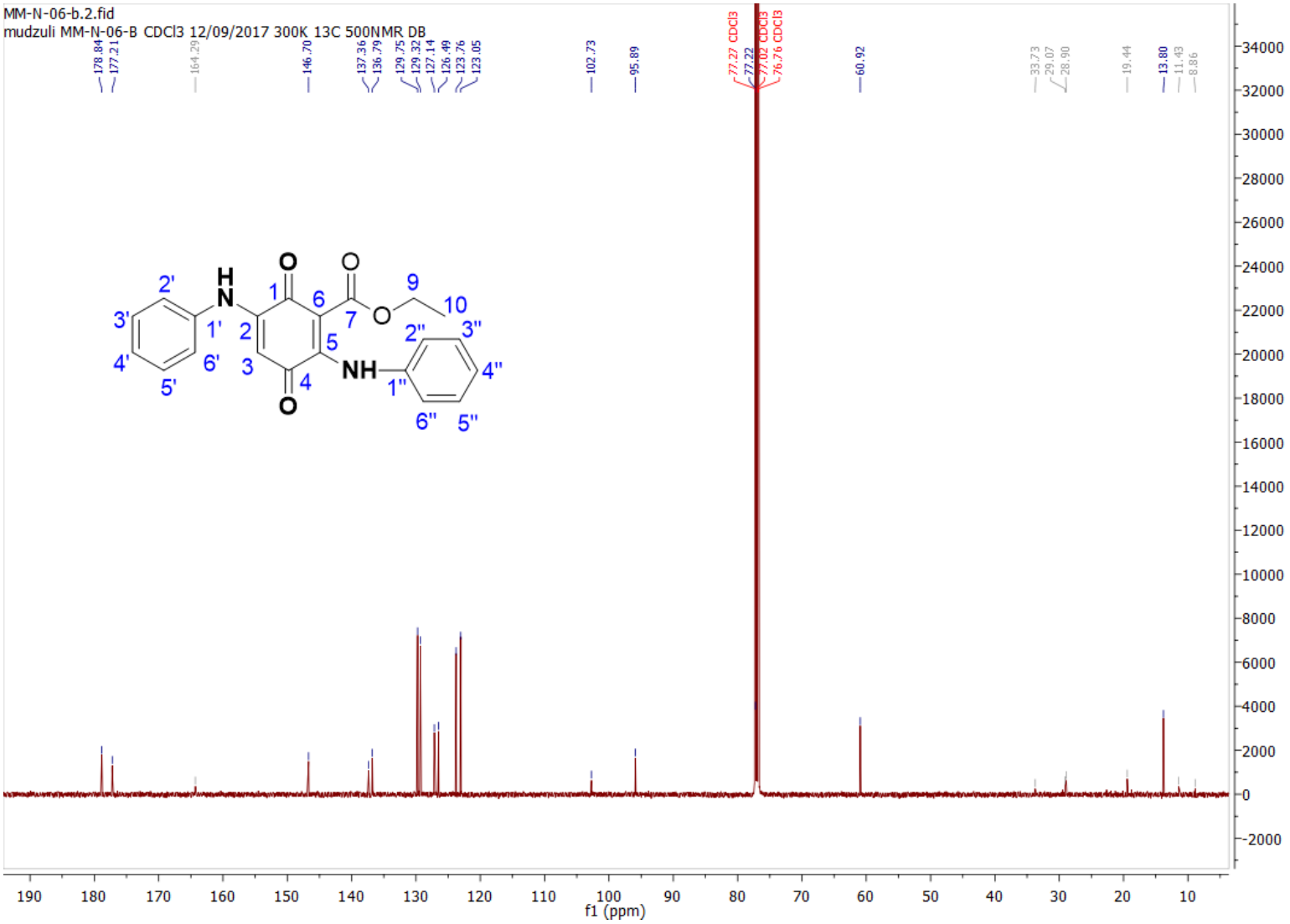
178.48
177.01
148.27
148.27
147.58
147.07
134.35
127.68
127.30
123.80
123.14
95.43
77.27 CDCl3
77.22
77.02 CDCl3
76.76 CDCl3
51.30
33.83
33.78
30.92
23.93
23.91
0.20
0.00
-0.20
f1 (ppm)

^{1}H NMR of **22**


MM-N-06-b.1.fid
mudzuli MM-N-06-B CDCl3 12/09/2017 300K 1H, 500NMR QB
8.69
8.19
7.45
7.43
7.42
7.40
7.39
7.37
7.28
7.28
7.27
7.26 CDCl3
7.25
7.25
7.19
7.17
6.11
3.57
3.56
3.54
3.53
2.17
1.59 HDO
1.43
1.42
1.41
1.40
1.26
1.24
1.22
1.21
1.19
1.15
1.14
1.14
1.12
1.01
1.00
0.98
0.87
0.86
0.85
0.84
0.84
0.83
0.82
0.81
0.79
0.89
1.01
4.16
4.91
2.09
1.01
2.17
3.12
f1 (ppm)

^{13}C NMR of **22**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mudzuli MM-N-06-B CDCl3 12/09/2017 300K 13C 500NMR DB
178.84
177.21
164.29
146.70
137.36
136.79
129.75
129.32
127.14
126.49
123.76
123.05
102.73
95.89
77.27 CDCl3
77.22
77.02 CDCl3
76.76 CDCl3
60.92
33.73
29.07
28.90
19.44
13.80
11.43
8.86
f1 (ppm)