Chalcone and curcumin hybrids of indole propargylamines as multifunctional neuroprotective agents

Lovetone Musakwa (BPharm)

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School of Pharmacy

University of the Western Cape (UWC)

Supervisor: Prof. Jacques Joubert

Co-supervisor: Prof. Sarel Malan

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For my mother, Kareni Zimunda.

Declaration

I declare that *Chalcone and curcumin hybrids of indole propargylamines as multifunctional neuroprotective agents* is my work, that it has not been submitted for any degree or examination in any other university, and that all sources I have used or quoted have been indicated and acknowledged by complete references.

Lovetone Musakwa

Signed….

Date: 06/08/2020

Abstract

Neurodegenerative disorders (NDs) are a range of chronic brain disorders that includes amongst others motor function loss. Parkinson's disease (PD) is one of the common NDs that has an insidious onset and diagnosed when dopaminergic neurons in the substantia nigra are already lost. The loss creates a deficiency of the dopamine (neurotransmitter) thereby causing neurochemical imbalance resulting in the signs and symptoms of PD. NDs overlap at multiple levels so some of the symptoms overlap as well. NDs currently have no cure yet and current drug therapies only improve the quality of life of the patients by targeting the symptoms mainly. Treatment of PD currently involves different classes of drugs and depending on the stages of the disease, some drugs can be only used as an adjunct therapy. Anti-oxidants and monoamine oxidase inhibitors (MAO-I) are part of the treatment options.

Polypharmacy in the treatment of NDs including PD is common and usually poses a high risk of non-compliance and -adherence. This study objective was to synthesize multi-targeted directed ligands (MTDLs) that could help slow down or halt the progression of PD and other NDs. A series of chalcone- and curcumin-derived hybrids of indole propargylamines were proposed to have monoamine oxidase (MAO) inhibitory activity, antioxidant and neuroprotective activity. MAO enzymes oxidatively deaminate neurotransmitters such as dopamine thereby by creating a deficiency and produces oxidative species that cause oxidative stress on the brain neurons. MAO enzymes inhibition approach protects the neurotransmitter (dopamine) from metabolism thereby reducing the deficiency or oxidative species. Seven compounds were successfully synthesized and confirmed by NMR, IR and MS.

Biological assays such as cytotoxicity, neuroprotection, MAO-A-, MAO-B inhibition and anti-oxidant activity were conducted for the synthesized compounds. Generally, all the compounds at 10 µM concentration were non-toxic to the human neuroblastoma SH-SY5Y cells except for the curcumin-derived indole hybrid (**3A**) that was toxic at 10 µM. Compound **2B** showed the best cell viability of 115.49% and seemed to encourage cell proliferation of the SH-SY5Y cells. Compound **3A** was excluded in neuroprotection studies because of the cytotoxicity results. Compounds **1A, 1B, 1C, 2A, 2B** and **2C** are effective in neuroprotection, showing results ranging from 69.81% to 80.37% with the best activity being produced by compound **2B**. All

compounds had a poor anti-oxidant activity with compound **2B** having an activity of IC_{50} = 127.90 µM compared to Trolox that had an IC_{50} of 17.12 µM.

All the compounds exhibited dual MAO inhibitory activity except for compound **3A** which had an IC_{50} of more than 100 µM. All chalcone derived hybrid compounds **(1C, 1A, 2A, 2C, 1B** and **2B)** were classified as non-selective hMAO-A/B inhibitors. Compound **1A** (hMAO-A IC_{50} = 1.10 µM and hMAO-B IC_{50} = 0.91 µM) had MAO-B activity comparable to rasagiline (MAO-B = 0.22 µM) a known MAO-B inhibitor. Compounds **1A, 1B, 2A** and **2B** showed great potential as neuroprotective agents, they are non-toxic and effective MAO inhibitors hence they could be an effective therapeutic agent in neurodegenerative disorders.

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Keywords

- Neurodegenerative disorders
- Parkinson's diseases
- Monoamine oxidase
- Oxidative stress
- Neuroprotection
- Dopamine
- Chalcone
- Curcumin
- Propargylamine
- Indole
- Multi-target directed ligands (MTDLs)

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Chapter 1

1.1. Background

Neurodegenerative disorders (NDs) are a broad group of neurological conditions comprising of heterogeneous clinical and pathological manifestations targeting various subsets of neurons (Przedborski *et al.*, 2003). Alzheimer's disease, Huntington's disease, Parkinson's disease and amyotrophic lateral sclerosis are examples of NDs (Gitler *et al.*, 2017; Durães *et al.*, 2018). Ageing is the principal contributing factor for most NDs and the number of individuals aged 60 and above is anticipated by the United Nations to increase up to 2 billion or more (Lin and Beal, 2006; UnitedNations, 2013). On the African continent, particularly Sub-Saharan, the risk of NDs is high also due to public health success which increased life expectancy (United Nations, 2017). No cure currently exist for NDs, but highly advanced imaging with positron emission tomography (PET) or computed tomography (CT) may be valuable in diagnosis, but it is limited in population-based epidemiological research (de Lau and Breteler, 2013).

Parkinson's disease (PD) is among the most prominent NDs that is characterised by dopamine meagreness in the corpus striatum of the forebrain (Chen and Tsai, 2010). Parkinson's disease treatment is based on symptomatic relief and the drugs used for treatment act on a single target whereas the disease has multiple causative factors (Kriebel-Gasparro, 2016; Nutt and Wooten, 2005; de Lau and Breteler, 2006). Oxidative stress and monoamine oxidase (MAO) activity, amongst other factors considered as significant in the development of NDs including PD (Bhat *et al.*, 2015; Robakis and Fahn, 2015; Youdim and Bakhle, 2006).

1.2. Rationale

1.2.1. Chalcone and curcumin

Many clinical studies have shown medicinal plants to have a therapeutic effect on disease control by modulating different biological activities (Rahmani *et al.*, 2018). Chalcone and curcumin are natural product-based compounds with medicinal effect and can also be synthesized (Belen'kii and Evdokimenkova, 2017; Cocconcelli *et al.*, 2008). Research on these compounds has been ongoing and has shown that chalcone and curcumin are still important compounds even though they have been in use for decades (Valavanidis and Vlachogianni, 2013).

Chalcones can be synthesized by conjugating an aromatic ketone and an aromatic aldehyde using a reaction called Claisen–Schmidt condensation under basic conditions (Andrade *et al.*, 2018). Structurally, chalcones comprise of two benzene rings linked together by a three-carbon α, β-unsaturated carbonyl complex (**Figure 1.1**). Adding groups on ring A and B can increase or decrease the activity of the chalcone and the three-carbon α, β-unsaturated carbonyl acts as a Michael acceptor (Silva *et al.*, 2013; Rangel *et al.*, 2013). Moieties such as allyl, prenyl and hydroxyl on the two rings generally increase chalcone activity (Monti *et al.*, 2008). Chalcone has a variety of biological activities as is shown below in **Figure 1.1.** In this current study, chalcone's antioxidative effect (Silva *et al.*, 2013) is of particular importance as we aim to structurally modify the chalcone compound to potentiate its activity in neurodegenerative disorders.

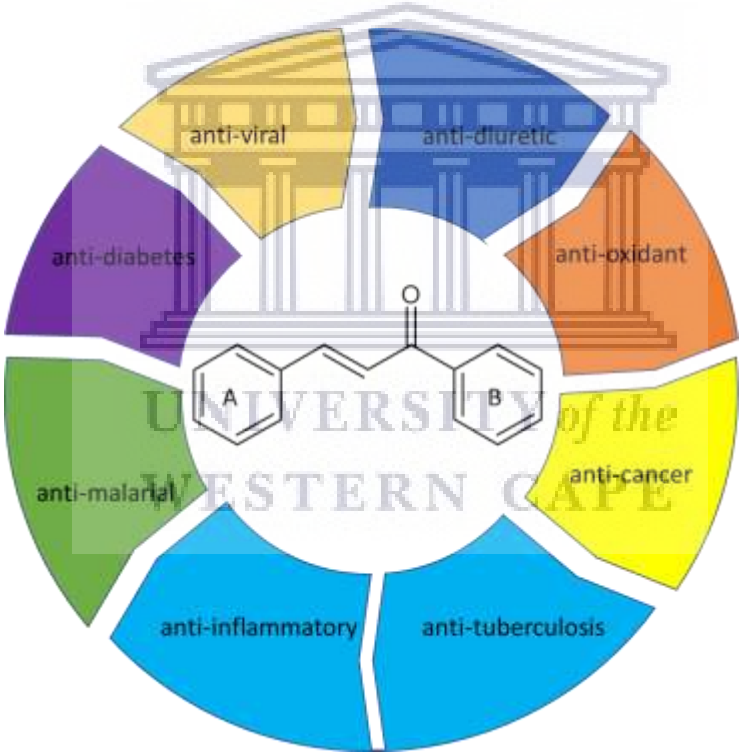


Figure 1.1: Chalcone activities (Silva *et al.*, 2013).

Curcumin which can be found in turmeric preparations is a polyphenol compound with a molecular weight of 368.37g/mol (Anand *et al.*, 2007). Several biological activities such as antioxidant, antifungal, anti-cancer and anti-inflammatory have been reported on curcumin (Rahmani *et al.*, 2018; Sharma *et al.*, 2005). Curcumin has two methoxy phenol rings that are linked by heptadienone shown in Figure 1.2 below. Generally, curcumin and its enol tautomer

coexist at equilibrium but at alkaline pH (pH above 8) enolate form predominates resulting in electron donor activity similar to phenolic antioxidants (Holder *et al.*, 1978). However, curcumin is unstable at alkaline pH and degrades to various products such as ferulic acid, vanillin and feruloyl methane (Holder *et al.*, 1978). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are thought to be involved in neurodegeneration, so the use of curcumin or its derivatives could play a pivotal role in neurodegeneration prevention since it can scavenge ROS and RNS effectively (Sharma *et al.*, 2005; Rao and Kunchandy, 1990). The curcumin-derived hybrid in series 2 was formed by conjugation of the indole and cinnamic acid resulting in an ester bond, the cinnamic acid was for antioxidant activity.

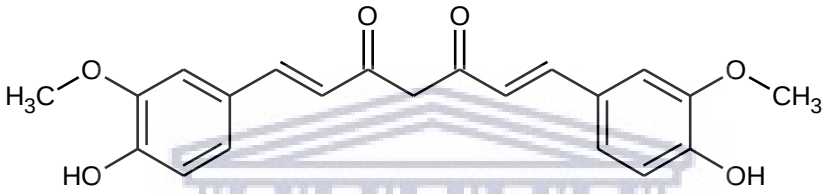


Figure 1.2: Curcumin structure.

1.2.2. Propargylamine moiety

The propargylamine moiety has been used in neurodegenerative disorders and the moiety is present in selegiline and rasagiline, which are drugs that irreversibly inhibit brain MAO (Chen *et al.*, 2007). Interest was placed on the *N*-propargyl moiety of both selegiline and rasagiline, which led to structure-activity findings that demonstrated that both drugs have a similar neuroprotective pathway due to the propargylamine moiety (Bar-Am *et al.*, 2004; Bar-Am *et al.*, 2005). This key finding illustrated the moiety's importance in both drugs' activity and neurodegenerative disorders (Bar-Am *et al.*, 2005). In addition to the above activities, the propargaylamine moiety was shown to have anti-apoptotic properties (Youdim and Weinstock, 2001).

1.2.3. Enzyme inhibition of monoamine oxidase (MAO)

MAO enzymes have been a key drug target since their discovery several decades ago and they are involved in neurodegeneration pathogenesis (Zeller, 1938; Deftereos *et al.*, 2012). MAO enzymes deactivate amine compounds including neurotransmitters such as dopamine. Disorders of the neurodegenerative type such as Parkinson's disease are significantly linked

with low dopamine levels in the forebrain (Brooks, 2008; Chen and Tsai, 2010; Kriebel-Gasparro, 2016). MAO has two isoforms and abnormal levels of MAO-B isoforms are associated with neurological conditions such as depression and neurodegeneration (Grimsby *et al.*, 1991; Garpenstrand *et al.*, 2000). Selective MAO-B inhibition is important in NDs as there is about 4-fold more MAO-B isoenzyme contrasted to MAO-A isoenzyme in the extrapyramidal area of an individual's brain (O'Carroll *et al.*, 1983). MAO-B in Alzheimer's disease is linked to the damage of the cholinergic neurones and amyloid plaque development (Cai, 2014).

1.2.4. Indole scaffold

The indole scaffold has been the core building block in medicinal chemistry research and can be used in neurodegenerative disorders. Several bioactive indole derivatives with neurodegenerative activities have been reported in the literature; more on this is discussed in chapter 2. The indole scaffold is heterocyclic, the benzene ring of the scaffold can form several bonds including cation π interaction while the pyrrole can form hydrogen bonds and this can be useful in active site-ligand interaction (Shimazaki *et al.*, 2009, 2015). The scaffold has also been reported to be neuroprotective because of its effective in scavenging free radicals (Buemi *et al.*, 2013).

1.3 Aim of study

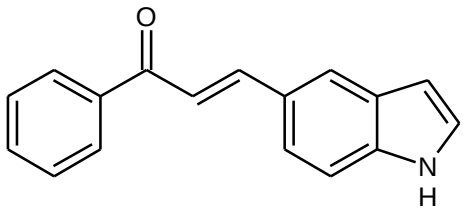
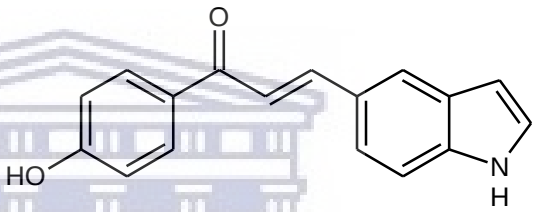
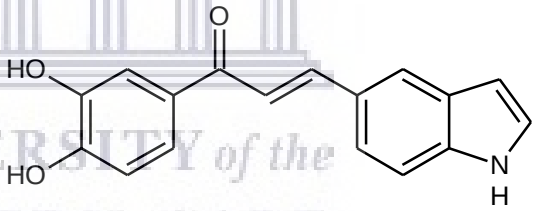
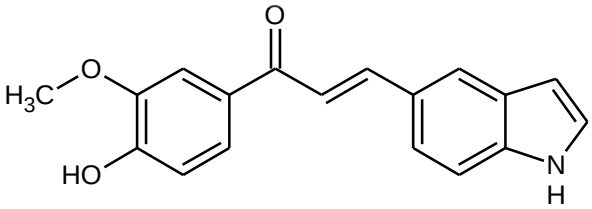
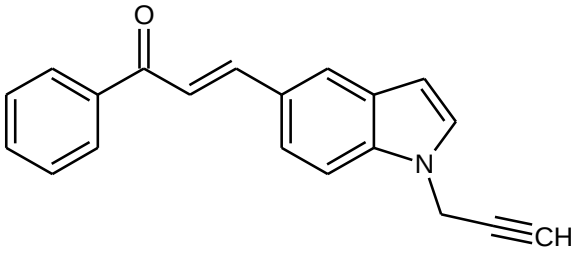
According to all the above literature information, chalcone and curcumin-derived indole propargylamine compounds that could be biologically active were proposed for synthesis. The heterocyclic indole scaffold acts as a framework to which either the chalcone or curcumin-derived moiety could be linked to the propargylamine group. The aim was to synthesize and evaluate chalcone- and curcumin-derived hybrids of indole propargylamine compounds (**Table 1.1**).

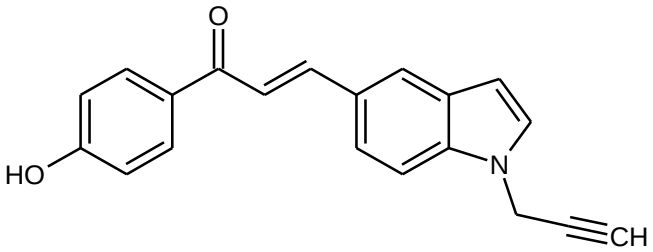
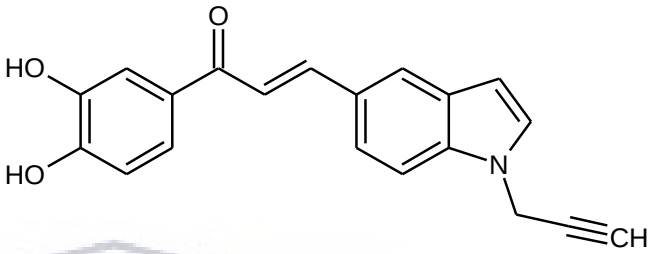
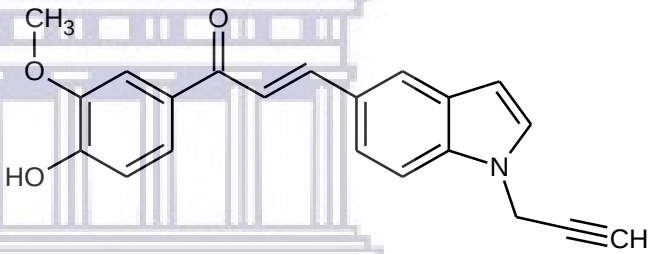
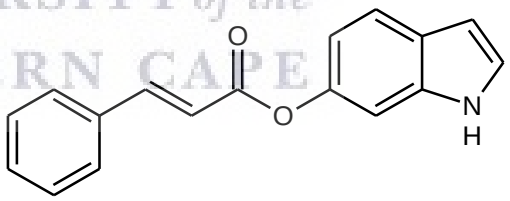
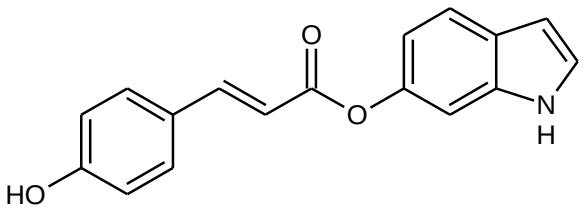
In summary, the aim(s) of the study is as follow(s):

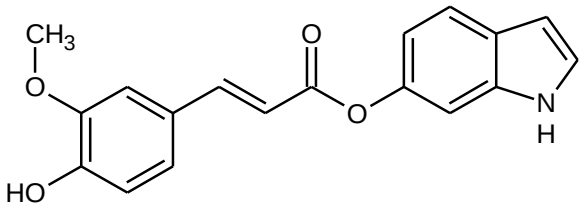
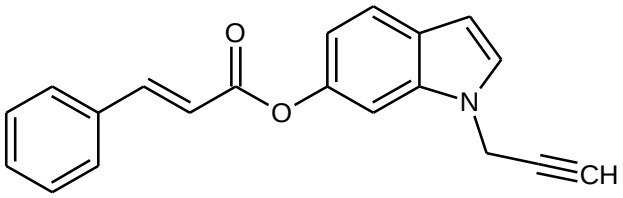
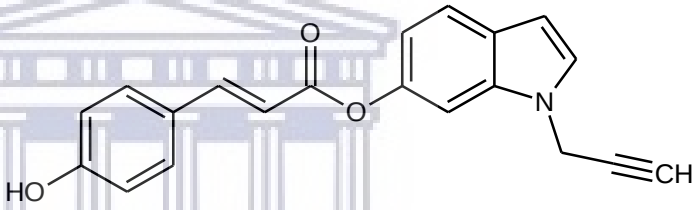
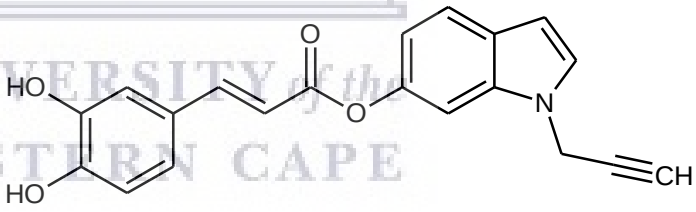
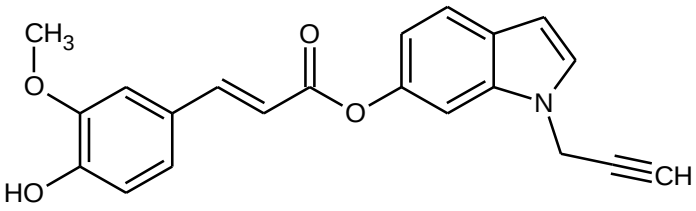
- To design compounds based on literature and *in silico* studies acting on multiple target sites involved in the development of neurodegenerative disorders.
- To synthesize the designed compounds using green chemistry where possible.

- To perform biological evaluations of the synthesized compounds.
- To analyse the biological results to assess the level of bioactivity of the compounds.

Table 1.1: Structures proposed as neuroprotective agents.

Compound	Structure
(2*E*)-3-(1*H*-indol-5-yl)-1-phenylprop-2-en-1-one	
(2*E*)-1-(4-hydroxyphenyl)-3-(1*H*-indol-5-yl)prop-2-en-1-one	
(2*E*)-1-(3,4-dihydroxyphenyl)-3-(1*H*-indol-5-yl)prop-2-en-1-one	
(2*E*)-1-(4-hydroxy-3-methoxyphenyl)-3-(1*H*-indol-5-yl)prop-2-en-1-one	
(2*E*)-1-phenyl-3-[1-(prop-2-yn-1-yl)-1*H*-indol-5-yl]prop-2-en-1-one	

(2*E*)-1-(4-hydroxyphenyl)-3-[1-(prop-2-yn-1-yl)-1*H*-indol-5-yl]prop-2-en-1-one	
(2*E*)-1-(3,4-dihydroxyphenyl)-3-[1-(prop-2-yn-1-yl)-1*H*-indol-5-yl]prop-2-en-1-one	
(2*E*)-1-(4-hydroxy-3-methoxyphenyl)-3-[1-(prop-2-yn-1-yl)-1*H*-indol-5-yl]prop-2-en-1-one	
1*H*-indol-6-yl (2*E*)-3-phenylprop-2-enoate	
1*H*-indol-6-yl (2*E*)-3-(4-hydroxyphenyl)prop-2-enoate	

1*H*-indol-6-yl (2*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoate	
1-(prop-2-yn-1-yl)-1*H*-indol-6-yl (2*E*)-3-phenylprop-2-enoate	
1-(prop-2-yn-1-yl)-1*H*-indol-6-yl (2*E*)-3-(4-hydroxyphenyl)prop-2-enoate	
1-(prop-2-yn-1-yl)-1*H*-indol-6-yl (2*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoate	
1-(prop-2-yn-1-yl)-1*H*-indol-6-yl (2*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoate	

The compounds shown in **Table 1.1** are likely to have monoamine oxidase activity and anti-oxidant activities due to the presence of bioactive pharmacophores such as propargylamine and chalcone- or curcumin-derived hybrids of indole, respectively. This could help reduce polypharmacy and possibly halt the progression of PD and other neurodegenerative diseases thereby increasing the quality of patient's life.

Chapter 2

2.1. Introduction

This chapter describes neurodegenerative disorders in general and shows how various factors such as monoamine oxidase enzymes and oxidative stress are involved in the process. A general overview of the indole heterocyclic compound will also be explored. The chapter will also substantiate the use of multi-target-directed ligands as a treatment option.

2.2. Neurodegenerative disorders

Neurodegenerative disorders are a range of brain disorders that overlap at multiple levels and involves, but is not limited to the disintegration of the neuronal network (Przedborski *et al.*, 2003). Alzheimer's disease and Huntington's disease, Parkinson's disease and amyotrophic lateral sclerosis are examples of neurodegenerative disease and their pathophysiology differ (Gitler *et al.*, 2017; Durães *et al.*, 2018). The World Health Organization (WHO) predicts that diseases affecting motor function including neurodegenerative disorders will come second to cardiovascular disease as the leading cause of mortality in 20 years (Gammon, 2014).

There is no cure for NDs but understanding their aetiology could result in effective treatment options for the NDs (Gitler *et al.*, 2017). Treatment is based on symptomatic relief and Gammon (Gammon, 2014) indicated that around the United States $20 billion is being spent on treatment and this amount is projected to grow. In light of this growing burden of disorder, it is imperative that a new drug which can reverse or slow down the progression of the disorders be developed (Tan *et al.*, 2015).

Ageing is a public health success, but also a constant risk factor in neurodegenerative disorders, especially for Parkinson's disease and Alzheimer's disease (Lekoubou *et al.*, 2014). Global population life expectancy is on the rise and in Sub-Saharan Africa, life expectancy at birth has increased by more than 20 years from 1960 to 2017 (United Nations, 2017). This overall increase in life expectancy for Sub-Saharan countries could be attributed to improved sanitation, the introduction of drugs to treat infectious diseases such as malaria, tuberculosis and HIV, thereby resulting in more people reaching the age of 60 years compared to 1960 (Lekoubou *et al.*, 2014; United Nations, 2017). The aetiology and drugs currently being used in therapy for some of the NDs are discussed below to highlight the necessity of new drugs.

2.2.1. Alzheimer's disease

Alzheimer's disease (AD) is one of the most prevalent NDs that is characterised by multifactorial features such as mitochondrial defects, misfolding of proteins, oxidative stress and protein aggregation (Goedert and Spillantini, 2006; Bajda *et al.*, 2011). Patients with AD also delineate cardinal features of the disease which include a progressive decrease in language structure, delusion, paranoia, gradual memory loss and impaired cognitive activity (Selkoe, 1991). Dementia is commonly caused by AD, dementia is the most prevalent cause of death found in AD patients' death certificates and patients usually survive for about 3 to 9 years from diagnosis (Brookmeyer *et al.*, 2002; Ganguli *et al.*, 2005).

The cholinergic theory indicates that when neurons containing the neurotransmitter acetylcholine (ACh) in the brain dysfunction, ACh levels decline as well and this contributes significantly to a decline in cognition (Greig *et al.*, 2001). ACh (which is a non-monoamine neurotransmitter) primarily activates the somatic regions of the peripheral nervous system, activating mechanisms responsible for the regulation of the motor system (Kapalka, 2010). Acetylcholinesterase(AChE) is the main enzyme implicated in ACh's catalytic hydrolysis and AChE is also proposed to be involved in the transformation of soluble Aβ into amyloid fibrils and fosters Aβ peptide aggregation (Alvarez *et al.*, 1997; Inestrosa *et al.*, 2008). Butyrylcholinesterase (BuChE), an enzyme that is related to AChE, acts as supporting enzyme to AChE making up for around 10% of cholinesterase activity inside the temporal cortex (Mushtaq *et al.*, 2014). Amyloid plaques and neurofibrillary tangles (NFTs) are an important feature of AD (Bajda *et al.*,2011; Selkoe, 2005).

MAO-B activities damage cholinergic neurons and contribute to the development of amyloid plaques (Cai, 2014). Schedin-Weiss and colleagues conducted a study that led to a finding that MAO-B controls Aβ production through γ-secretase (an enzyme that releases the neurotoxic Aβ), thus providing a framework for establishing MAO-B association with AD aetiology (Schedin-Weiss *et al.*, 2017). Chronic inflammation is also an inherent feature of AD due to the brain's microglial cells which may secrete episodes of inflammatory factors thereby affecting bystander neurons (Jia *et al.*, 2014).

Treatment of AD has been based on neurotransmitter replacement and a variety of treatment options which include monoamine oxidase inhibitors, cholinesterase inhibitors, anti-amyloid drugs, antioxidants, anti-inflammation drugs and nerve growth factors (Knopman and Morris,

1997; Nordberg and Svensson, 1998). Currently, several drugs are being used to manage the symptoms of AD and for neurotransmitter replacement such as the cholinesterase inhibitors. Examples of these drugs are donepezil, rivastigmine and galantamine (Nordberg and Svensson, 1998; Yiannopoulou and Papageorgiou, 2013).

2.2.2. Huntington's disease

Huntington's disease is a hereditary genetic disorder that causes fatal neurodegeneration in the brain cells (Perry *et al.*, 1973; Mahalingam and Levy, 2014). People with HD have an increased number of nucleobases (cytosine, adenine and guanine) repeats in the huntingtin gene located on chromosome number 4 (The Huntington's Disease Collaborative Research Group, 1993). The huntingtin gene's function may involve internal cell signalling, neuronal toxicity prevention and maintaining cyclic adenosine monophosphate response element that binds protein (Nucifora *et al.*, 2001). Mutations arise from defective genes and this could lead to protein aggregates that result from misfolded proteins which could trigger cellular dysfunction, cell death and synaptic connection loss (Walker, 2007; Bucciantini *et al.*, 2002).

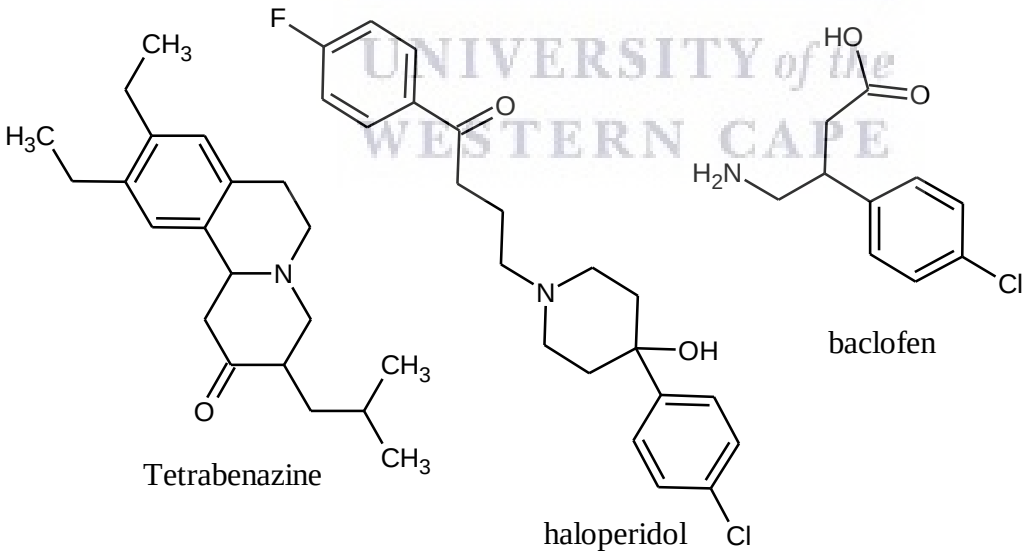


Figure 2.1: Structures of drugs used in HD for symptomatic pharmacotherapy.

Progressive brain atrophy is involved in HD and the neostriatum, that involves the caudate nucleus and putamen, is the main and earliest location of pathology (Vonsattel and DiFiglia,

1998; Vonsattel *et al.*, 1985). As the brain atrophy advances, the striatum and other brain components lose their structural and functional connectivity (Snowden, 2017). Glutamate, gamma-aminobutyric acid and dopamine are considered to be the neurotransmitters most impacted in HD and are presently the focus of treatment (Weeks *et al.*, 1996; Perry *et al.*, 1973; Ribeiro *et al.*, 2010). HD is incurable and therapy focuses on symptomatic relief of chorea, dystonia and loss of motor skills (Snowden, 2017). Tetrabenazine is one of the drugs used treat HD and studies have shown that it ameliorates chorea and involuntary movements in HD (Kenney *et al.*, 2007). Drugs such as mood-stabilizing drugs, antipsychotics, selective serotonin reuptake inhibitors (SSRI) and anti-epileptics can be of great use also in the management of HD symptoms (Duijn, 2010).

2.2.2. Parkinson's disease

Parkinson's disease (PD) is a progressive neurodegenerative disease characterised by a range of symptoms that fall under motor and non-motor symptoms (Winklhofer and Haass, 2010). Motor symptoms experienced in PD are as a result of dopamine deficiency in the corpus striatum (Chen and Tsai, 2010), which results from moderate to severe dopaminergic neuron decrease in the region of the brain called the substantia nigra pars compacta (SNpc) and generally correlates with parkinsonism-consistent clinical characteristics (De Pablo-Fernández *et al.*, 2016; Greffard *et al.*, 2006). PD diagnostic tests or markers that are reliable and easy to use are currently unavailable, thereby making primary clinical symptom presentation key in public based epidemiological diagnosis (de Lau and Breteler, 2013).

The symptoms of PD are bradykinesia, resting tremor, postural imbalance or rigidity and for a patient to be diagnosed with PD at least two of the symptoms and exclusion of possible secondary parkinsonism cause is required (de Lau and Breteler, 2013). The accuracy of PD clinical diagnosis is low and it is difficult to diagnose in early stages as well as when it progresses (Litvan *et al.*, 2003; De Rijk *et al.*, 1997). About 80-90% of clinical diagnosis cases done on patients with PD were confirmed by autopsy and this clinicopathological validation exposed the limitations in diagnosis because there are no universal neuropathological criteria for diagnosis and the patient dies years after diagnosis (Litvan *et al.*, 2003). The concise aetiology of PD is currently unknown, but researchers have proposed several theories proposed to be involved in PD progression shown in **Figure 2.2** (Chen and Tsai, 2010).

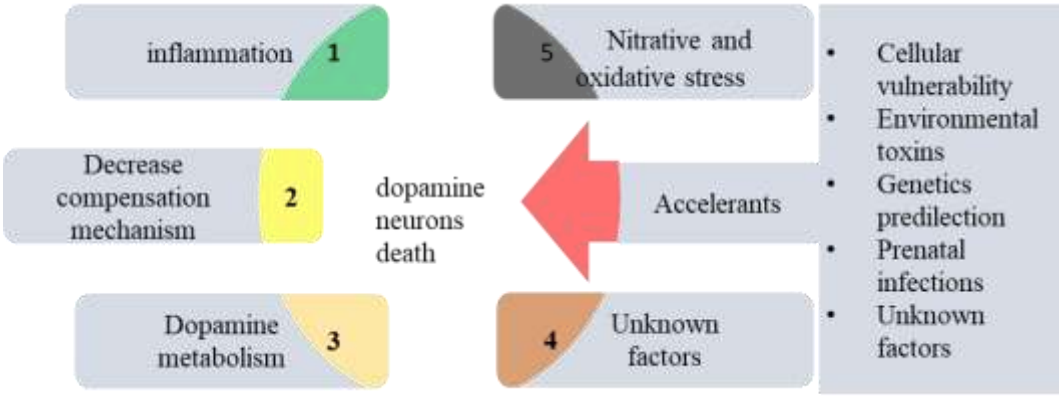


Figure 2.2: Factors that cause the death of neurons in the brain (Collier *et al.*, 2011).

PD anomalies also manifest beyond the key dopaminergic pathways due to extensive dysfunction displayed by factors such as biochemical alteration, gene expression, functional deficiencies in several core neurons lacking dopamine, autonomous and sensory pathways as well as peripheral structures along with non-neural tissue (Hindle, 2010; Halliday, 2009; Braak *et al.*, 2003; Przedborski *et al.*, 2003; Beach *et al.*, 2010; Hargreaves *et al.*, 2008; Zheng *et al.*, 2010; Gibson *et al.*, 2003).

Treatment methods become more complex as the illness advances and the immediate therapy expenses will also rise considerably in proportion to the rise in Hoehn and Yahr (Jankovic, 2008). Current treatment tends to be effective in ameliorating symptoms of PD by correcting distinctive dopaminergic deficiencies but fail to stop PD progression (Phillipson, 2014). A combination of various medical therapies like anticholinergics, monoamine oxidase inhibitors, amantadine, levodopa, and catechol-O-methyl transferase inhibitors can be prescribed on diagnosis (McInerney-Leo *et al.*, 2004).

2.3. Monoamine oxidase enzymes

Monoamine oxidases (MAOs) are a group of enzymes which oxidatively deaminate amine moieties from biogenic to xenobiotics amine groups such as dopamine and aniline, respectively (Croom, 2012). MAO enzymes were discovered eight decades ago and were initially proposed as tyramine oxidase before their activity was extended (Hare, 1928). Papers published by Bkaschko and Zeller challenged the classical view postulated in 1928 of MAO (known then as tyramine oxidase) being only able to breakdown tyramine and showed evidence that tyramine oxidase can catalyse catecholamines and histamines in addition to tyramine; this then led to the

renaming of tyramine oxidase to MAO (Blaschko *et al.*, 1937; Zeller, 1938; Slotkin, 1999). More than 20,000 research papers have been published on MAO since they gained interest in the 1950s and MAOs are still important enzymes in medical research (Edmondson *et al.*, 2007).

The process of oxidative deactivation of amines by MAO enzymes acts as a protective mechanism for example in neurotransmitter metabolism, MAO enzymes can scavenge excess neurotransmitters in the glial cells (Youdim *et al.*, 2006a). MAO enzymes require activation from a cofactor flavin adenine dinucleotide (FAD) which is bound to the enzyme by a covalent bond (Edmondson *et al.*, 1993). Multiple hypotheses have been postulated on how flavin works as it can accept more than one electron (Gaweska and Fitzpatrick, 2011). Single-electron transfer mechanism, hydrogen atom transfer mechanism, nucleophilic mechanism, and hydride transfer mechanism are some of the postulated hypothesises (Gaweska and Fitzpatrick, 2011).

2.3.1. Monoamine oxidase nomenclature

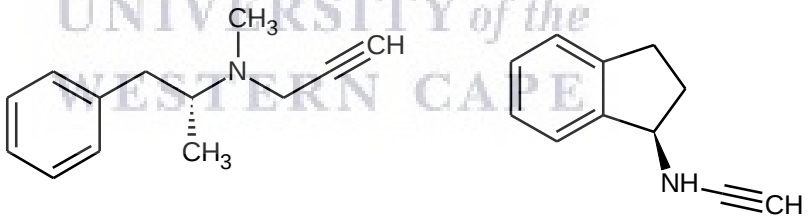
Two isoforms of the mitochondrial enzyme MAO exist which are MAO type A and MAO type B (Garpenstrand *et al.*, 2000). The two isoforms are encoded by two distinct genes and both genes have 14 introns with a minimum of 60 kilobases, 15 exons and are located on Xp11.23-Xp22.1 of the X chromosome (Garpenstrand *et al.*, 2000; Kochersperger *et al.*, 1986). The exon-intron arrangement is identical in both genes and indicates that they originated from similar ancestral gene duplication (Grimsby *et al.*, 1991). Using oligonucleotide analysis, cDNA clones encoding the two MAO isoforms inferred that MAO-A/ -B comprises of 527 and 520 amino acids, respectively (Bach *et al.*, 1988; Kuwahara *et al.*, 1990). MAO-A/ -B are 70% related and have 59700 Da and 58 800 Da molecular weights, respectively (Bach *et al.*, 1988).

The human cavity length of MAO-A/ -B varies, it is about 400 $Å^3$ and 700 $Å^3$ respectively (Hong and Li, 2019). Structural human MAO-A/B cavity contrast indicated that MAO-A has a bigger entrance cavity compared to MAO-B resulting in MAO-A preferring bulkier ligands while MAO-B prefers smaller ligands (Hong and Li, 2019). The specificity of the substrate and inhibitor of the hMAO isoforms are determined by residues Phe-A208 and Ile-A335 in MAO-A and residues Ile-199 and Tyr-326 in of MAO-B (Tsugeno and Ito, 1997). The protein molecular folding of MAO-A and -B vary and have regions which are conserved such as the C terminal (residue 491-511), the region of FAD covalent bonding (residues 350-458), substrate binding area (residues 178-221) and residues 6-23 (Wouters *et al.*, 1995; Wouters, 1998).

Using X-ray crystallography, hMAO-A and hMAO-B were found to exist as dimer crystals and monomeric units respectively (De Colibus *et al.*, 2005; Binda *et al.*, 2002). The cavities of both MAO isoforms are generally hydrophobic but MAO-B has an entrance cavity with a conserved hydrophilic region (De Colibus *et al.*, 2005; Kumar *et al.*, 2017). MAO-A/B have varying distribution in human tissues and substrate specification (Garpenstrand *et al.*, 2000). Platelets only have the isoform type B of the enzyme MAO and the activity of the enzyme in platelets have been linked to personality traits such as impulsiveness and sensation seeking (Oreland and Hallman., 1995; Shih *et al.*, 1999).

2.3.2. Rational for MAO inhibitors approach

Tyramine and dopamine are the conventional substrates for both MAO enzymes but MAO-A selectively breaks down epinephrine, 5-HT and norepinephrine whereas MAO-B prefers deaminating benzylamine and 2-phenylethylamine (Knoll and Magyar, 1972; Strolin Benedetti and Dostert, 1987). MAO-A is involved in alteration of monoaminergic activity and they have been proposed to be related to mood disorder etiopathogenesis, thereby making MAO-A inhibition a therapeutic strategy (Lipper *et al.*, 1979). Neurological conditions such as neurodegeneration, depression and schizophrenia have been connected to abnormal levels of MAO-B activity (Grimsby *et al.*, 1991).



selegiline rasagiline

Figure 2.3: Examples of MAO-B inhibitors on the market.

Selegiline and rasagiline inhibit the MAO-B enzyme from metabolising dopamine, thus making the dopamine readily available to the brain (Kriebel-Gasparro, 2016). MAO-B inhibiting drugs enables dopamine neurotransmitters to stay in the brain thereby prolonging its action and can increase the duration of action of the registered drug Sinemet® (carbidopa-levodopa combination) in early PD diagnosis (Kriebel-Gasparro, 2016; Brooks, 2008).

2.4. Oxidative stress in neurodegeneration

Oxidative stress is the state where the equilibrium between antioxidant defence and free radicals are offset in favour of the free radicals (Sies, 1991). If the equilibrium is offset, homeostasis is not maintained, resulting in increased free radicals and then ultimately pathophysiological events that cause oxidative damage (Sies, 1986). Free radicals are chemical species with an electron that is unpaired and can cause chain reactions if they react with a molecule or atom (Betteridge, 2000; Halliwell, 1994). Examples include, but are not limited to, transition metals, nitric monoxide (NO·), superoxide ($O_2^{\cdot-}$) from oxygen and the hydroxyl radical (·OH) that reacts at the site of formation thereby giving it a short lifespan in vivo (Betteridge, 2000). Free radicals are involved in biochemical activities of the cell, for example, oxygen radicals are involved in soluble guanylate cyclase activity regulation and gene transcription (Uttara *et al.*, 2009).

Reactive oxygen species (ROS) is an umbrella term used to represent oxygenated radicals such as superoxide, singlet oxygen (½ O_2), hydroxyl radical and non-free radical hydrogen peroxide or H_2O_2 (Pisoschi and Pop, 2015). The mitochondria produce significant amounts of ROS and the oxidative deamination of dopamine by MAO enzymes which produces a variety of oxidative species such as oxygen and H_2O_2 adds more oxidative stress on neurons (Sulzer, 2007; Reeve *et al.*, 2014). The effectiveness of the electron transport chain (ETC) and adenosine triphosphate (ATP) are reduced with age due to leakages (Phillipson, 2014). Oxidative stress markers increase in ageing and a decrease in the nuclear signal (erythroid-derived 2) such as (Nrf2) for antioxidation protection occurs (Suh *et al.*, 2004; Tufekci *et al.*, 2011).

Neuropathological modification can accumulate if endogenous defences fail to withstand stress, particularly in dopamine neurons, thereby making the neurons vulnerable due to oxidative stress coupled by energy deficit from dopamine metabolism, superoxide anions in excess and catechol structure (Cohen, 1994; Lamensdorf *et al.*, 2000; Marchitti *et al.*, 2007: Kristal *et al.*, 2001). This could lead to the formation of additional ROS through Fenton chemistry or even the accumulation of harmful RNS (Halliwell, 1992; Beckman, 1994; Harman, 1956). Multiple evidence showing ROS damage to brain regions that degenerated has been documented in disorders like AD and PD(Bhat *et al.*, 2015). Due to the assumed key involvement of oxidative stress in the pathogenesis of neurodegenerative diseases including PD, use of antioxidants would neutralise free radicals (de Lau and Breteler, 2013).

2.5. Indole general overview

Indole is a heterocyclic compound composed of both a pyrrole and benzene ring fused which can be synthesised or extracted from natural products. Several active pharmaceutical products containing the indole scaffold (**a**) such as; indomethacin, one of the most potent non-steroidal anti-inflammatory drugs (**b**), indalpine (**c**) a selective serotonin reuptake inhibitor (SSRI), atevirdine, a non-nucleoside reverse transcriptase inhibitor for HIV treatment (**d**) and roxindole (**e**) a selective dopamine agonist that also inhibits serotonin uptake (Darragh *et al.*, 1985; Gründer *et al.*, 1993; Romero *et al.*, 1996; Zhou *et al.*, 2010).

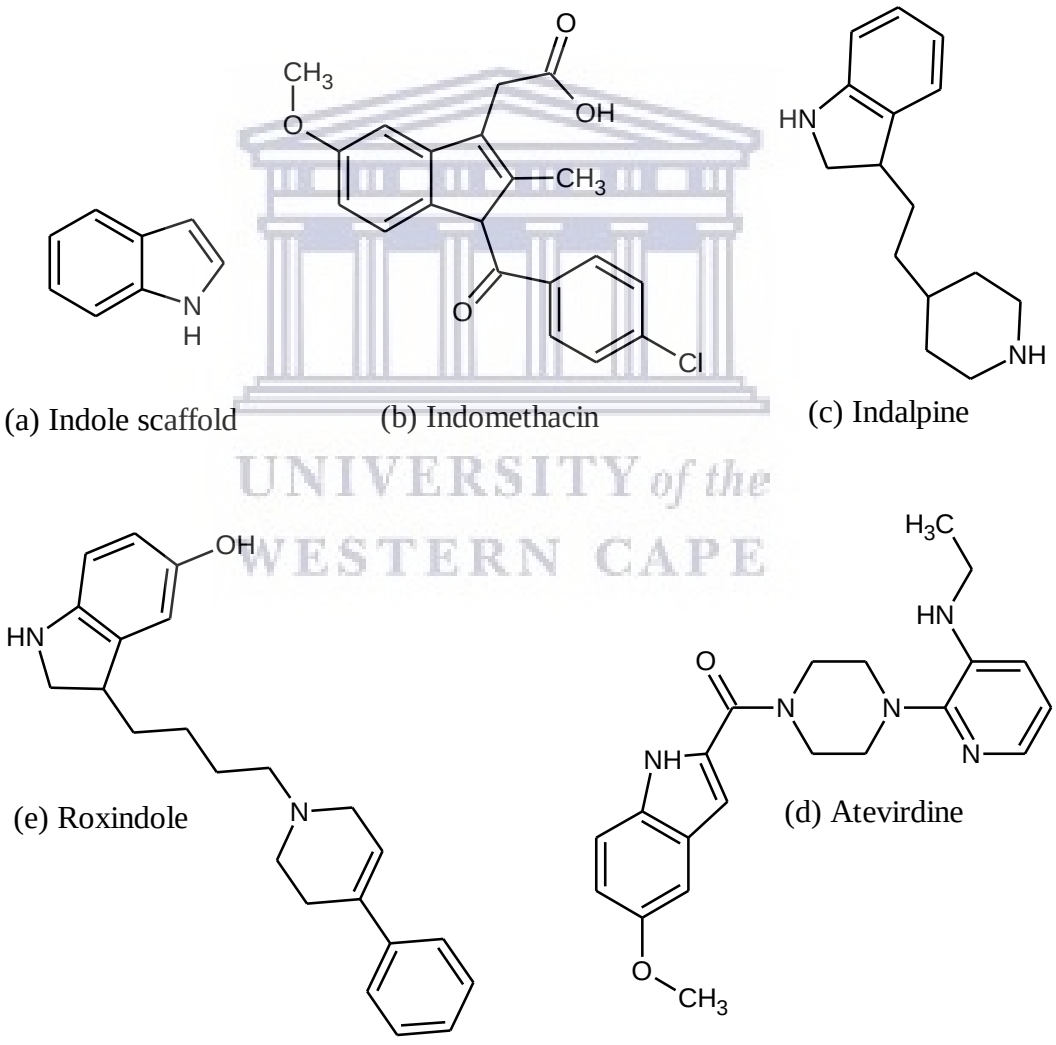


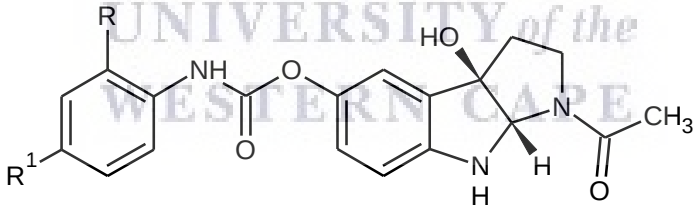
Figure 2.1: Drugs used in HD for symptomatic pharmacotherapy.

The indole scaffold is also found in tryptophan an essential amino acid, which is metabolised into eight bioactive compounds that include these common ones; nicotinamide, kynurenine,

tryptamine and the neurotransmitter serotonin can be derived from tryptophan in vivo (Friedman, 2018). A myriad of indole derivatives have been synthesised and still capture the attention of chemists as these derivatives enable them to discover compounds that have diverse pharmacological effects. In medicinal chemistry, several indole derivatives have been used as anticancer, antimicrobial, antihypertensive, antimalarial, antiviral and in neuroprotective agents. While several bioactive indole derivatives have been recorded in the research papers, the primary objective of this study was on indole derivatives in neurodegeneration.

2.5.1. Indole scaffolds in neurodegenerative disorders

The indole ring has been a core building block in research for agents that can be used to ameliorate the burden of neurodegenerative disorders. The pyrrole ring of the indole scaffold has an NH group that can form hydrogen bonds while the aromatic group of the indole forms cation π interaction and π-π stacking (Shimazaki *et al.*, 2009). The structure of the indole scaffold is comparable with that of serotonin which is a neurotransmitter connected to brain cognition and function (Hamid *et al.*, 2017). Adjustments in the serotonergic framework assume a huge role in the pathogenesis of neurological ailments and neuropsychiatric diseases (Dorszewska *et al.*, 2017).



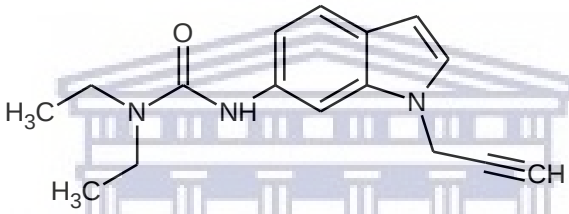
a. R =H, R' =H
b. R =OCH3, R' =H
c. R =H, R' =OCH3
d. R =H, R' =O(CH2)6 CH3

Figure 2.5: An indole based series with biological activity for Alzheimer's disease treatment (Siwicka *et al.*, 2008).

In Alzheimer's disease, BuChE and AChE enzymes are involved in the pathogenesis with AChE being a key enzyme because it breaks down the neurotransmitter acetylcholine and studies show that it promotes Aβ aggregation *in vitro* (Goyal *et al.*, 2018; Reyes *et al.*, 2004). Compounds that inhibit AChE and BuChE can be useful as a treatment option for Alzheimer's

and Siwicka *et al.* (2008) reported on some of the active indole containing compounds. The AChE inhibition assay showed that compound **a** has an activity of IC_{50} = 0.253 ± 0.033 µM which is close to the positive standards, physostigmine and phenserine. All compounds within this series had poor BuChE activity apart from compound **c** which had an IC_{50} = 2.306 ± 0.172 µM (Siwicka *et al.*, 2008).

Denya *et al.* (2018) also designed and synthesised indole compounds as agents for Alzheimer's disease. The indole series synthesised were based on ladostigil which is an MTDL that has rasagiline and rivastigmine features (Weinreb *et al.*, 2012). Compound 6 of the synthesized series showed better inhibitory activity comparable to ladostigil with IC_{50} in the single-digit µM ranges (Denya *et al.*, 2018).



compound 6

Figure: 2.6: Most active Ladostigil based compound from the series (Denya *et al.*, 2018).

2.6. Multitarget directed ligand

Numerous diseases have multifactorial origins, such as neurodegenerative disorders and cancer; hence their etiopathology involves various potential drug targets, organ system and tissues (Bansal and Silakari, 2014). Single drug therapy which has been consistently used for multifactorial diseases has proved ineffective because the drugs failed to act on multiple targets to mitigate the diverse signs (Maggiora, 2011). The polypharmacy approach in which cocktails of therapeutic agents are used for drug therapy of multifactorial disorder including neurodegenerative diseases will then be considered but it is a complex drug therapy which is linked to drug-drug interactions and poor patient compliance (Ziller *et al.*, 1991; Keith *et al.*, 2005; Reddy and Zhang, 2013).

Compounds with individual targets are of great importance in drug discovery, but current research suggests that promiscuous drugs are valuable as well (Wermuth, 2004; Mencher and Wang, 2005). Compounds with multiple targets are known as multi-target-directed ligands (MTDLs) and these compounds might have an extended duration of action or enhanced

druggable features (Morphy *et al.*, 2004; Bolognesi *et al.*, 2007; Bansal and Silakari, 2014). Public and private companies' databases have also shown an increase in drug discovery of multi-target drugs and they are perceived to be one of the significant innovations of the pharmaceutical industry (Koutsoukas *et al.*, 2011).

The most popular approach for the rational design of MTDLs was to link two correctly chosen pharmacophores with two distinct molecular activities through a spacer (Bolognesi *et al.*, 2007). MTDLs can also be designed from fusing and merging pharmacophores resulting in reduced molecular weight of the MTDL as shown in **Figure 2.7** (Morphy and Rankovic, 2006). Another method of MTDL design is analoging, where a single starting compound with multiple activities is analoged to enhance its activity (Morphy and Rankovic, 2006). Using three starting compounds usually results in an MTDL with high molecular weight which is perceived to be a pharmacokinetic disadvantage and may lead to a reduced inherent likelihood of ideal drug-likeness (Hopkins *et al.*, 2006; Morphy and Rankovic, 2006)

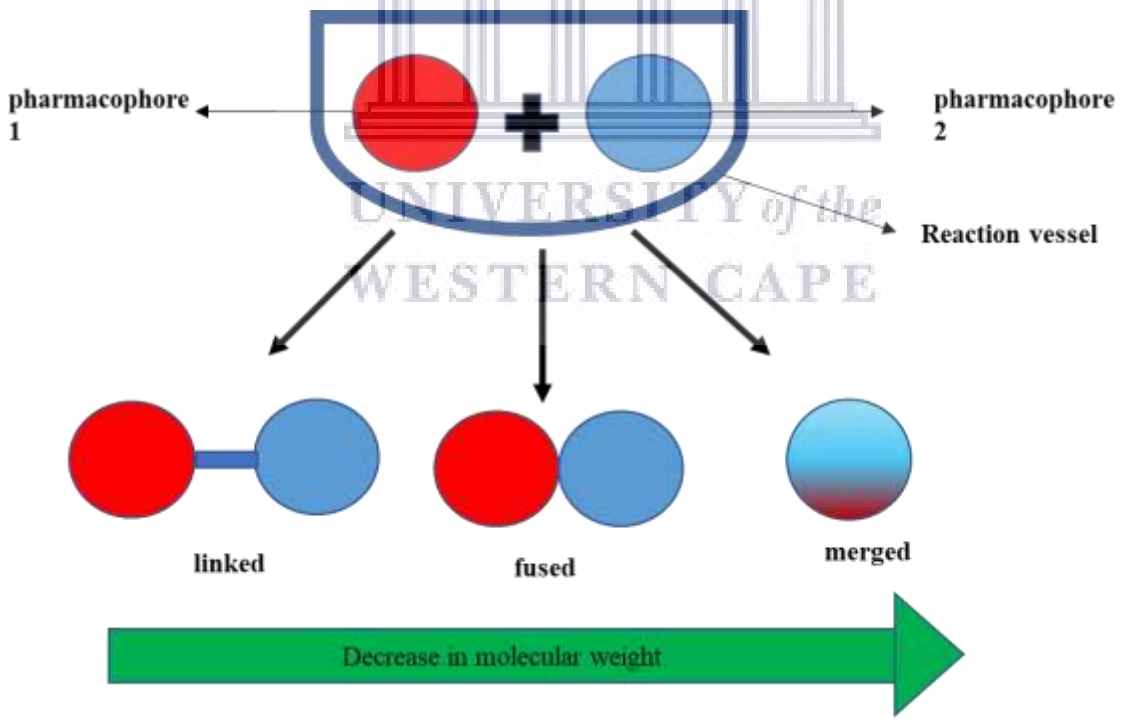


Figure 2.7: Common methods for MTDL design (Morphy and Rankovic, 2006).

2.6.1. Literature examples of designed MTDLs

Aspirin is renowned for its promiscuity and its activity spans from analgesic to being used for anti-arthritis, cancer and pre-eclampsia prevention amongst others (Espinoza-Fonseca, 2006).

For cancer treatment, Pfizer Inc developed a small molecule called SU11248 (Sunitinib) that interacts with factors involved in cancer (platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF)) and inhibits tyrosine kinase enzyme (O'Farrell *et al.*, 2003; Espinoza-Fonseca, 2006). The MTDL approach has also been used in the development of the central nervous system (CNS) drugs such as Ziprasidone for the treatment of schizophrenia (Bansal and Silakari, 2014). Ziprasidone (**Figure 2.8**) is made from hybridization of a 5-hydroxytryptamine (5-HT) ligand and a D_2 dopamine receptor agonist thereby giving Ziprasidone dual serotonergic and D_2 antagonistic activities (Bansal and Silakari, 2014).

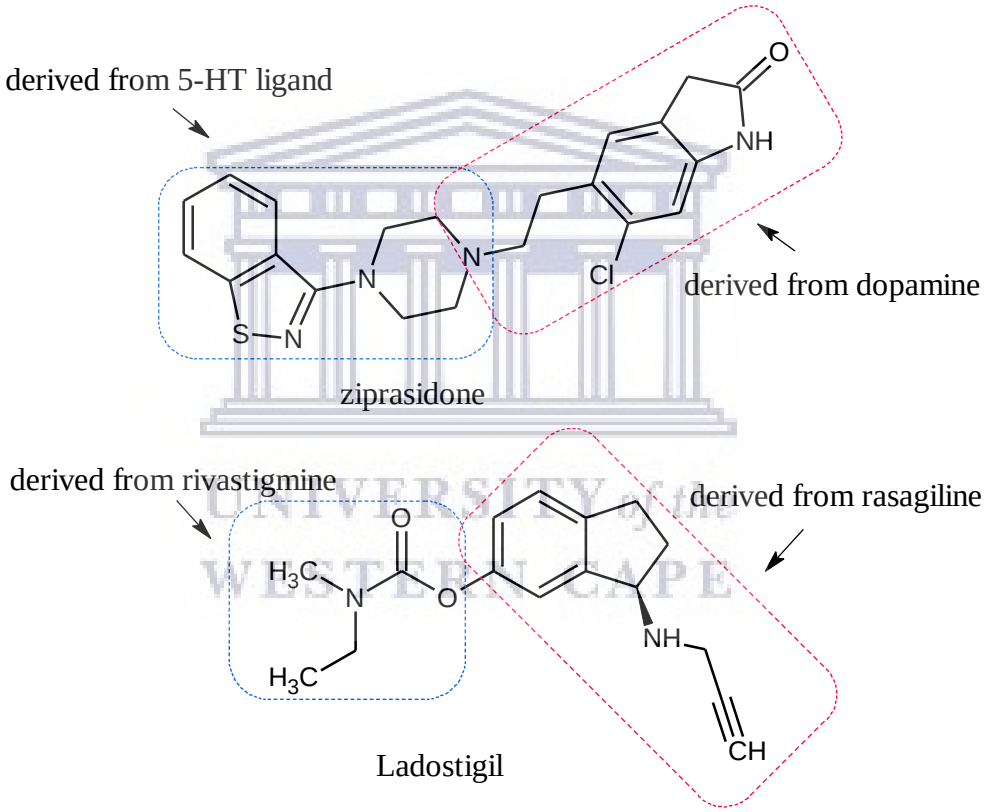


Figure 2.8: Ladostigil and Ziprasidone are examples of MTDLs.

Clozapine (not an MTDL) which is indicated for the treatment of schizophrenia exhibits less desirable D_2/5-HT_2 ratio and has a higher tendency of causing orthostatic hypotension compared to Ziprasidone (Warnez and Alessi-Severini, 2014; Bansal and Silakari, 2014). Ladostigil is another MTDL designed with the carbamate pharmacophore and an aminoindan moiety conjugated together for the treatment of neurodegenerative disease-related dementia and depression (Weinreb *et al.*, 2012; Bansal and Silakari, 2014). The carbamate pharmacophore is from a cholinesterase inhibitor rivastigmine and the aminoindan moiety from

a selective MAO-B inhibitor rasagiline (Weinreb *et al.*, 2009). Ladostigil exhibits the cholinesterase inhibitory activity of rivastigmine and neuroprotective activity that is comparable to rasagiline (Weinreb *et al.*, 2009).

2.7. Conclusion

Neurodegenerative disorders are complicated to treat because of their multifactorial pathogenesis which includes, but is not limited to, oxidative stress and MAO activity in the brain which generally causes damage to brain neurones. The indole scaffold has several biological activities and can be used in ND in conjugation with other active moieties when designing an MTDL. The MTDL approach has been used before in combating other diseases with multifactorial causes and proved to be successful, thereby causing researchers to adopt this approach in ND. The conjugation of the indole scaffold with other biologically active moieties might help in replacing the current treatment options for ND which are based on a polypharmacy approach.

Chapter 3

3. Standard experimental approaches

3.1. Reagents and chemicals

Sigma-Aldrich® (3050 Spruce Street, St Louis, USA) and Alfa Aesar® (76870 Kandel, Germany) was used to procure the reagents, except if indicated otherwise and utilized without further purification. Solvents purchased from various sources were used in reactions and chromatography.

3.1.2. Instrumentation

Nuclear magnetic resonance spectroscopy (NMR): ^{1}H and ^{13}C NMR spectra were obtained at 400 MHz using a Bruker Avance III HD Nanobay instrument equipped with a 5 mm BBO probe. The internal standard was tetramethylsilane (TMS), and the solvent used was deuterated. Chemical shifts are in parts per million, internal standard (d=0) and the deuterated solvent peak were used as a reference point. The following abbreviations are used to symbolize the multiplicity of associated signals: s- singlet; bs-broad singlet; d-doublet; dd-doublet of doublets; t-triplet and m-multiplet

Infrared spectroscopy (IR): A Perkin Elmer Spectrum 400 FT - IR / FT-NIR spectrometer was used to obtain the IR spectrum of the synthesized compounds.

Mass spectroscopy (MS): Perkin Elmer Flexar SQ 300 detector was used to obtain the MS spectra.

Melting point (MP) determination: A Stuart SMP10 melting point device was used to determine the solid compounds' melting points.

Microwave reactor: A CEM Discover™ microwave reactor was used in the synthesis of some of the compounds.

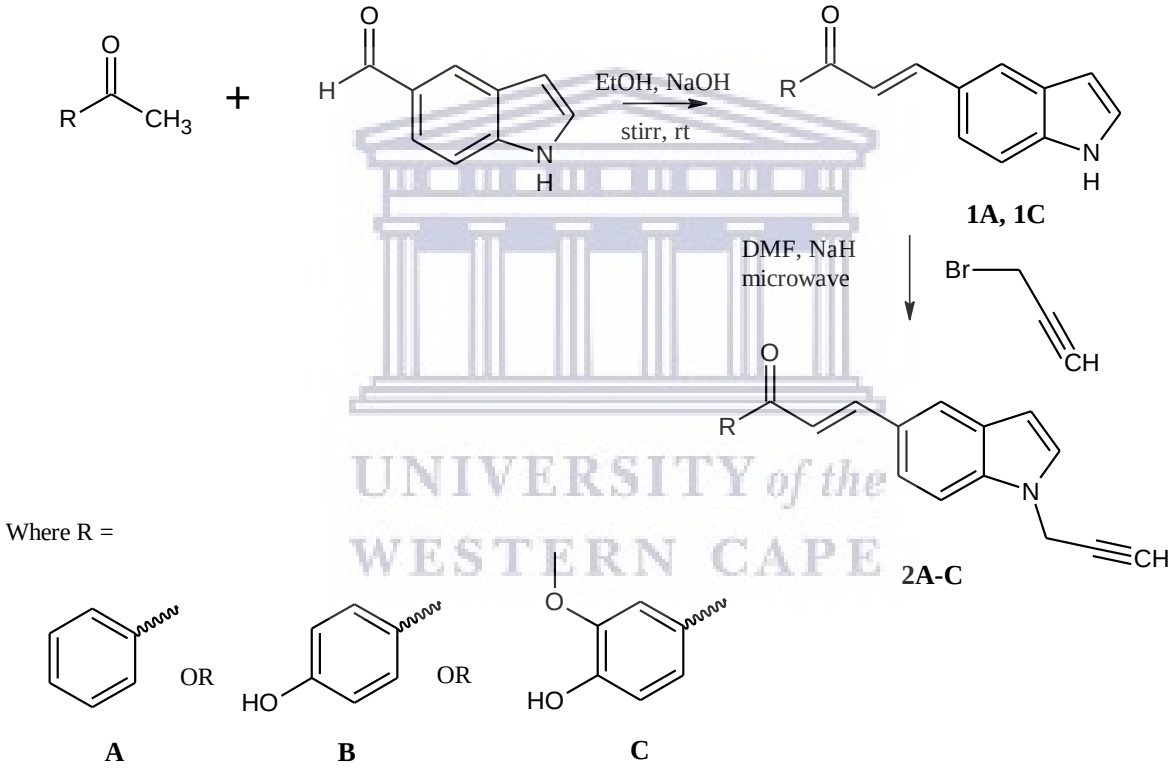
3.1.3. Chromatographic techniques

Thin-layer chromatography (TLC): Aluminium sheets with silica gel 60 F_{254} was used for TLC and visualized under UV light (254 nm and 366 nm) or iodine chamber.

Column chromatography: Standard glass columns of different sizes were used to purify reaction mixtures, and the stationary phase used for each compound was silica gel (0.063-0.200 mm/70-230 mesh ASTM, Macherey-Nagel, Duren, Germany) with varying mobile phases.

3.2. Synthesis

For the synthesis of the chalcone derived indole hybrids, the respective starting ketone was reacted with indole-5-carboxaldehyde using a Claisen–Schmidt condensation reaction in the presence of a strong base (**Scheme 3.1**). These hybrids were then reacted with propargyl bromide through an S_N2 nucleophilic reaction to give the final compounds where the propargyl moiety is conjugated to the *N*-atom of the indole (**Scheme 3.1**).



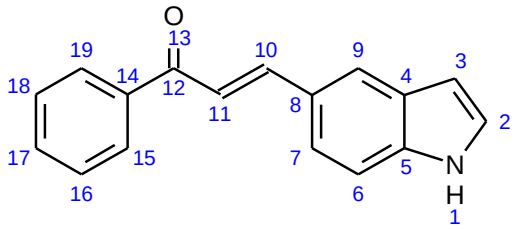
Scheme 3.1: Chalcone derived indole propagylamine compounds synthesis.

3.3. General synthesis of chalcone derived indole hybrids (1A, 1C)

To a solution of substituted ketone (1.0 mmol) and ethanol (4 ml), 1.0 mmol indole-5-carboxaldehyde was added. After that, NaOH (1 N) 60-100 µL was added. The mixture was then stirred up at RT for 2-8 hours. The residue was filtered and washed with ethanol, H_2O, and then cold ethanol. In the case of no residue, chloroform (30 ml x 3) was used to extract the mixture. The organic phase was collected, and the solvent removed *in-vacuo* and purified by

column chromatography using four parts hexane and one-part ethyl acetate (4:1). A slightly modified method was used for the synthesis of **1B** and is described under section 3.3.2. The analytical data of compounds **1A** and **1B** obtained matched with the ones found in the literature (Cocconcelli *et al.*, 2008; Cui *et al.*, 2011).

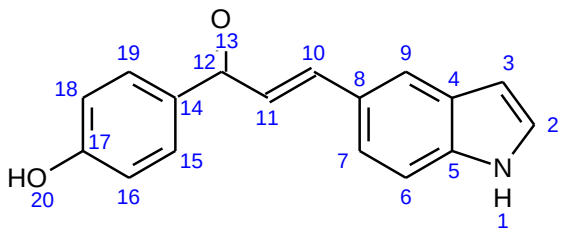
3.3.1. (2*E*)-3-(1*H*-Indol-5-yl)-1-phenylprop-2-en-1-one (1A)



Synthesis: The compound was prepared using the reaction conditions as described under section 3.3.

Physical data: $C_{17}H_{13}NO$; **mass**:112 mg; **yield**: 77.2%; **mp**: 169-171˚C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ_H (Spectrum 1): 11.38 (s, 1 H), 8.14 (d, *J* = 7.2 Hz, 2 H), 8.06 (s, 1 H), 7.87(d, *J* = 15.6 Hz, 1 H), 7.81 (d, *J* = 15.6 Hz, 2 H), 7.71-7.69 (dd, *J* = 8.4; 1.2 Hz, 2 H), 7.64 (d, *J* = 7.2 Hz, 1 H), 7.59-7.55 (t, *J* = 7.6; 2.0 Hz, 2 H), 7.46 (d, *J* = 8.4 Hz, 1 H), 7.42 (d, *J* = 2.8 Hz, 1 H), 6.52 (d, *J* = 2.8 Hz, 1 H); **^{13}C NMR** (100 MHz, DMSO-d_6) (Spectrum 2): 190.99, 147.17, 138.75, 137.30, 132.45, 128.55,128.44, 128.28, 126.96, 125.46, 123.08, 122.01, 199.35, 111.70, 103.57; **IR** (FT-IR, cm^{-1}) (spectrum 3): 3273.93, 1646.80, 1556.59, 1286.39; **MS** (EI, 70 eV) m/z (spectrum 4): 247.10 (M^+); **HR-ESI [M+H]$^+$**: calc. 248.1075, exp. 248.1073.

3.3.2. (2*E*)-1-(4-Hydroxyphenyl)-3-(1*H*-indol-5-yl)prop-2-en-1-one (1B)

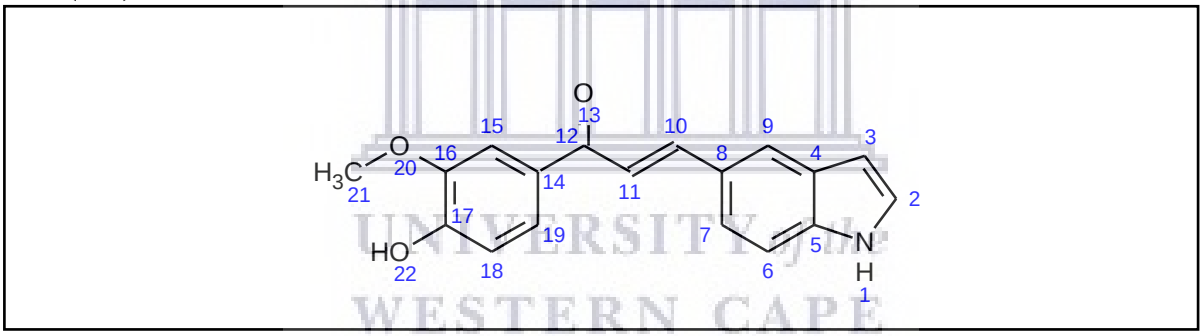


Synthesis: To a solution of 4-hydroxyacetophenone (1 mmol, 136 mg) in ethanol (4 ml), piperidine (0.12 ml) was added. Indole-5-carboxaldehyde (0.8 mmol, 116 mg) was then added

to the mixture and stirred at 95˚C for 48 hours. On completion, the reaction mixture was quenched with aqueous HCl to pH 6 and extracted with ethyl acetate. The organic layer was washed with brine, H_2O and then dried *in vacuo*. Column chromatography was used to purify the concentrate with hexane and ethyl acetate (4:1) used as eluent.

Physical data: $C_{17}H_{13}N0_2$; **mass**:14 mg; **yield**: 12%; **mp**: 189-192 ˚C; **^{1}HNMR** (400 MHz, DMSO-d_6) δ_H (Spectrum 5): 11.34 (s, 1 H), 8.06 (d, *J* = 8.4 Hz, 2 H), 8.01 (s, 1 H), 7.81-7.73 (m, 2 H), 7.66 (d, *J* = 8.8 Hz, 1 H), 7.44 (d, *J* = 8.4 Hz, 1 H), 7.41 (t, *J* = 2.8 Hz, 1 H), 6.89 (d, *J* = 8.8 Hz, 2 H), 6.51 (bs, 1 H); **^{13}C NMR** (100MHz, DMSO-d_6) (Spectrum 6): 187.06; 161.86; 145.26; 137.28; 130.95; 127.97; 126.65; 122.80; 122.69; 122.54; 121.42; 118.38; 115.30; 111.95; 102.08; **IR** (FT-IR, cm^{-1}) (spectrum 7): 3222.57, 2923.50, 1527.61, 1600.89; **MS** (EI, 70 eV) m/z (spectrum 8): 263.09 (M^+); **HR-ESI [M+H]$^+$**: calc. 264.1025, exp. 264.1019.

3.3.3. (2*E*)-1-(4-Hydroxy-3-methoxyphenyl)-3-(1*H*-indol-5-yl)prop-2-en-1-one (1C)



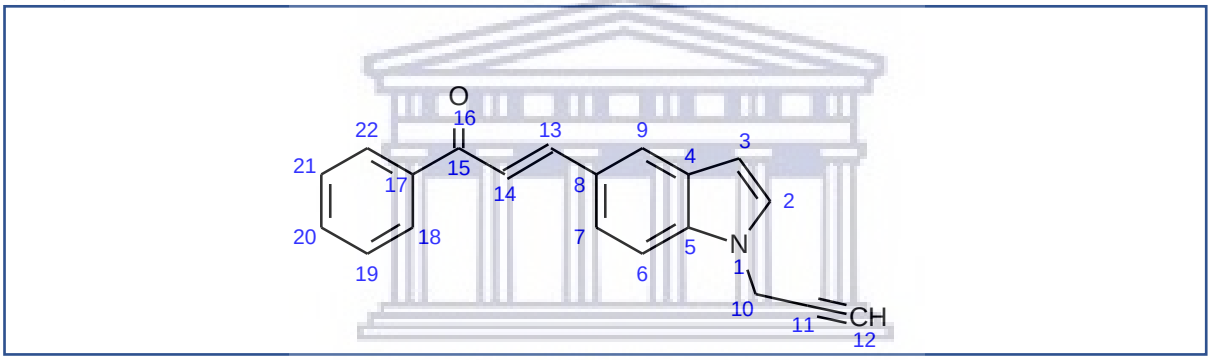
Synthesis: The compound was prepared using the reaction conditions as described under section 3.3.

Physical data: $C_{17}H_{13}NO$; **mass**:37 mg; **yield**: 25%; **mp**: 167-170˚C; **^{1}H NMR** (400 MHz, DMSO) δ_H (Spectrum 9): 11.36 (s, 1 H), 8.02 (s, 1 H), 7.78 (d, J= 4.8 Hz, 2 H), 7.74-7.73 (dd, *J*= 8.5; 2.0 Hz, 1 H), 7.66 (d, *J* = 7.6 Hz, 1 H), 7.53 (d, , *J* = 2.0 Hz, 1 H), 7.44 (d, *J* = 8.4 Hz, 1 H), 7.42-7.41 (t, *J* = 2.4 Hz, 1 H), 7.04 (d, *J* = 8.8 Hz, 1 H), 6.51 (bs, 1 H), 3.87 (s, 3 H); **^{13}C NMR** (100 MHz, DMSO) (spectrum 10): 187.87; 152.42; 147.00; 145.88; 137.77; 131.66; 128.44; 127.13; 126.47; 123.21; 121.89; 121.88; 118.79; 115.27; 112.45; 111.66; 102.58; 56.19; **IR** (FT-IR, cm^{-1}) (spectrum 11): 3305.18, 2921.66, 1543.47, 1511.83; **MS** (EI, 70 eV) m/z (spectrum 12): 293.11 (M^+); **HR-ESI [M+H]$^+$**: calc. 294.1130, exp. 294.1123.

3.4. General synthesis of chalcone derived indole propargyl hybrids (2A, 2B, 2C)

An amount of 0.40 mmol of compound **1A**, **1B** or **1C** was measured into a microwave glass-vessel and dissolved in DMF (10 ml). NaH (60%, 9.8 mg) was added to the mixture and stirred for 10 minutes at RT. A propargyl bromide solution (53 µL, 71.85 mg, 0.60 mmol) was added to the mixture and irradiated in a microwave reactor (T = 95 ˚C, P = 228 psi, µλ = 150 W). On completion, the mixture was extracted five times with ethyl acetate and H_2O. Afterwards, the organic layer was washed with brine and H_2O. The ethyl acetate solvent was then expelled *in vacuo* to give the desired compounds **2A**, **2B** or **2C**.

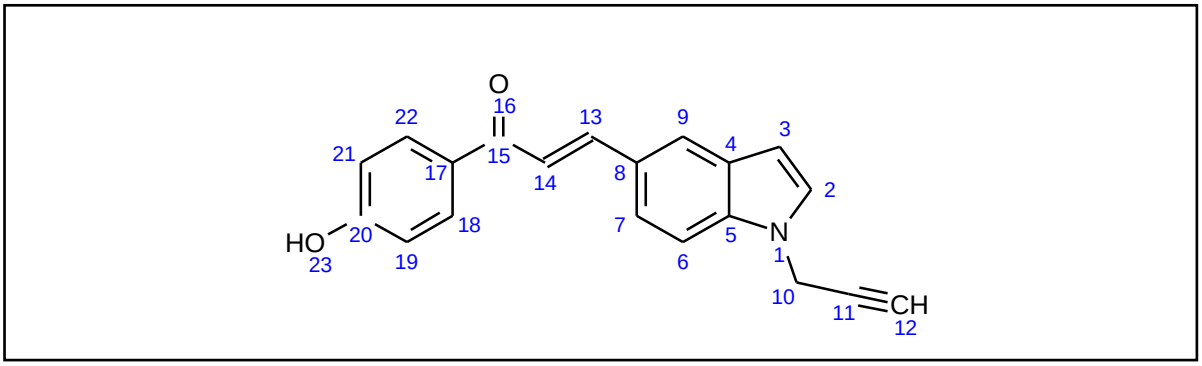
3.4.1. (2*E*)-1-Phenyl-3-[1-(prop-2-yn-1-yl)-1*H*-indol-5-yl]prop-2-en-1-one (2A)



Synthesis: The compound was prepared using the reaction conditions as described under section 3.4.

Physical data: $C_{17}H_{13}NO$; **mass**: 30 mg; **yield**: 30%; **mp**: 176-179 ˚C; **^{1}H NMR** (400 MHz, DMSO) δ_H (Spectrum 13): 8.16 (d, *J* = 7.2 Hz, 2 H), 8.08 (s, 1 H), 7.89 (m, 2 H), 7.82-7.79 (dd, *J* = 8.8; 1.2 Hz, 1 H), 7.71-7.62 (m, 2 H), 7.60-7.56 (t, *J* = 7.2 Hz, 2 H), 7.49-7.48 (d, *J* = 3.2 Hz, 1 H), 6.56 (d, *J* = 3.2 Hz, 1 H), 5.14 (d, *J* =2.4 Hz, 2H), 3.45-3.44 (t, *J* = 2.0 Hz, 1 H); **^{13}C NMR** (100 MHz, DMSO) (spectrum 14): 189.08, 146.27, 138.06, 136.97, 132.92, 129.80, 128.81, 128.44, 126.51, 123.39, 121.99, 118.97, 110.76, 102.55, 79.09, 75.80, 35.34; **IR** (FT-IR, cm^{-1}) (spectrum 15): 3232.12, 3101.82, 2924.02, 1651.83; **MS** (EI, 70 eV) m/z (spectrum 12): 285.12 (M^+); **HR-ESI [M+H]$^+$**: calc. 286.1232, exp. 286.1228.

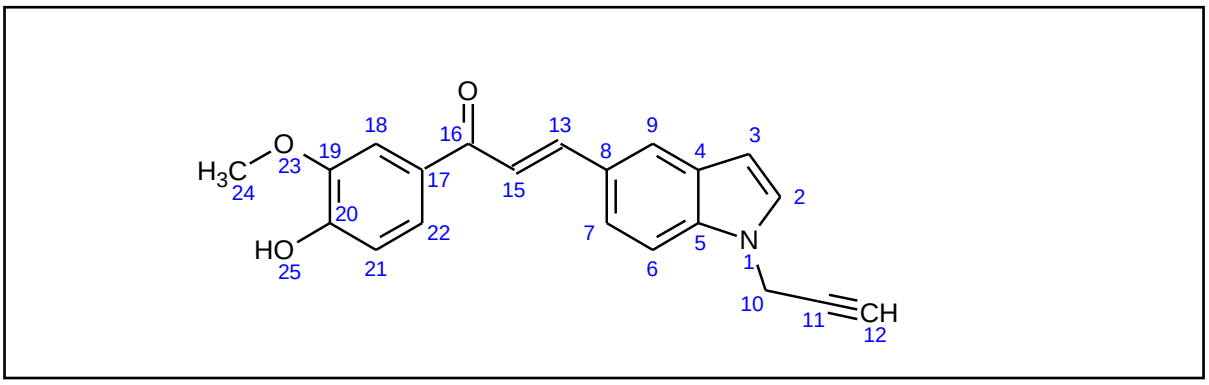
3.4.2. (2*E*)-1-(4-Hydroxyphenyl)-3-[1-(prop-2-yn-1-yl)-1*H*-indol-5-yl]prop-2-en-1-one (2B)



Synthesis: The compound was prepared using the reaction conditions as described under section 3.4.

Physical data: $C_{17}H_{13}NO$; **mass**: 40mg; **yield**: 40%; **^{1}H NMR** (400 MHz, DMSO-d_6) δ_H (Spectrum 17): 8.18 (d, *J* = 8.8 Hz, 2 H), 8.06 (s, 1 H), 7.86 (d, *J* = 6.8 Hz, 2 H), 7.79 (d, *J* = 6.8 Hz, 1 H), 7.60 (d, *J* = 8.4 Hz, 1 H), 7.48 (d, *J* = 3.2 Hz, 1 H), 7.13 (d, *J* = 8.8 Hz, 2 H), 6.56 (d, *J* = 3.2 Hz, 1 H), 5.15 (d, *J* = 2.0 Hz, 2 H), 3.66-3.65 (t, *J* = 4.4 Hz, 1 H); **^{13}C NMR** (100 MHz, DMSO-d_6) (spectrum 18): 187.32, 162.34, 160.89, 145.45, 136.84, 131.46, 130.65, 129.71, 128.63, 126.61, 123.15, 121.90, 118.87, 114.80, 102.43, 78.81, 55.74, 35.81; **IR** (FT-IR, cm^{-1}) (spectrum 19): 3398.05, 1650.72, 1451.07, 800.02; **MS** (EI, 70 eV) m/z (spectrum 20): 301.11 (M^+); **HR-ESI [M+H]$^+$**: calc. 302.1181, exp. 302.1167.

3.4.3. (2*E*)-3-(4-Hydroxy-3-methoxyphenyl)-1-[1-(prop-2-yn-1-yl)-1*H*-indol-5-yl]prop-2-en-1-one (2C)



Synthesis: The compound was prepared using the reaction conditions as described under section 3.4.

Physical data: $C_{17}H_{13}NO$; **mass**: 27 mg; **yield**: 27%; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H (Spectrum 21): 7.95 (d, *J* = 15.6 Hz, 2 H), 7.84 (s, 1 H), 7.77 (d, *J* = 8.4 Hz, 2 H), 7.61-7.54 (m, 2 H), 7.40 (d, *J* = 8.8 Hz, 1 H), 7.27 (d, *J* = 9.9 Hz, 2 H), 6.97 (d, *J* = 8.4 Hz, 1 H), 4.83 (d, *J* = 2.0 Hz, 2 H), 3.97 (s, 3 H), 2.58-2.57 (t, *J* = 2.4 Hz, 1 H); **^{13}C NMR** (100 MHz, DMSO-d_6) (spectrum 22): 190.80, 146.84, 146.01, 138.73, 137.14, 132.44, 129.28, 128.54, 127.10, 123.28, 121.97, 121.07, 120.45, 119.54, 109.96, 103.186, 73.99, 40.97, 36.05, 29.70; **IR** (FT-IR, cm^{-1}) (spectrum 23) 2852.02, 1649.46, 1597.16, 800.73; **MS** (EI, 70 eV) m/z (spectrum 24): 331.12 (M^+); **HR-ESI [M+H]$^+$**: 332.8127.

3.5. Challenges experienced

NMR structure elucidation of the organic compounds presented to be challenging. The organic compounds synthesised have multiple ring structures and most of the protons are aromatic and alkene protons. The alkene protons are of particular interest on the structures because they help confirm the conjugation of the starting materials and they are common in all compounds. The compounds form a resonance structure as shown below in **Figure 3.1**. The proton on the positively charged carbon is more de-shielded and hence appears further down-field on the proton NMR spectrum around 7.70-8.0 ppm (Hwang *et al.*, 2011; Lahsasni *et al.*, 2014; Ohkatsu and Satoh, 2008).

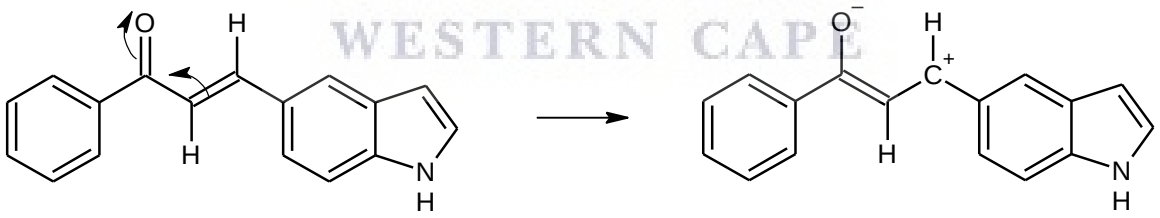


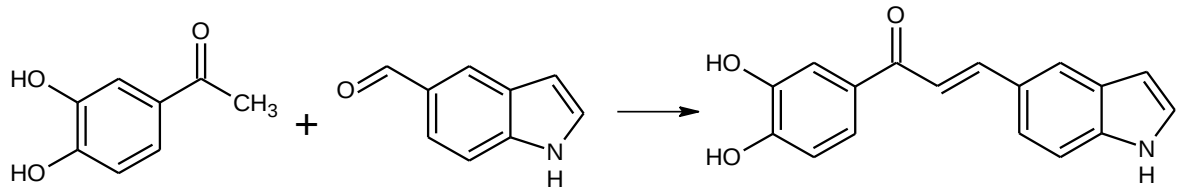
Figure 3.1: General resonance structure of the synthesised compounds.

Two series of compounds were proposed; series one being the chalcone derivatives. For series one, a Claisen–Schmidt condensation reaction in the presence of base was used to synthesise compounds **1A**, **1B** and **1C**.

One compound from series one was not successfully synthesised despite conducting several experiments utilising different reaction conditions (**Table 1**). One of the starting materials would precipitate on addition of the base, forming a white precipitate. For the starting materials to react they both have to be in solution and the solution has to be basic. The precipitation of

the starting material could be the reason why the reaction was not successful. The atoms of the starting materials could therefore not react because one starting material was solid and the other in solution.

Table 1: Different reaction conditions employed in an attempt to synthesise the diol derivative of series 1.



Attempted reaction	**Yield**
1) Ethanol, NaOH (1 N), rt 2 hrs, refluxed for 8 hrs	0 %
1) 2) Ethanol, piperidine, stirred at 95˚C for 48 hrs	0 %
3) Microwave reactor (65 W, 65˚C, 2 hrs), ethanol, Al_2O_3	0 %

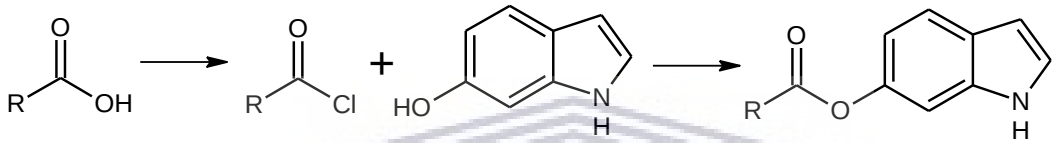
For series two; substituted cinnamic acid and 6-hydroxy-indole had to be conjugated to form curcumin-derived indole hybrids. A couple of reactions were attempted and failed to produce the final product. Direct conjugation of the indole and cinnamic acid using tetrahydrofuran (THF) as solvent and H_2SO_4 as catalyst (reaction one in **Table 2**) was unsuccessful. A thick hard-paste that could not be dissolved in several solvents formed 30 minutes into the reaction. The paste could not be elucidated by NMR.

The second and third attempt was to break down the reaction into two steps, shown in Table 2. The substituted cinnamic acid had to be activated for it to react with hydroxy-indole. The OH group on the substituted cinnamic acid has poor leaving capacity; good leaving groups are weak bases. The substituted cinnamic acids were, therefore, reacted with $SOCl_2$ to form activated acid halide intermediates. The conversion of cinnamic acid resulted in meagre yields, which then ultimately contributed to the low yield of the final compound.

Inert reaction conditions inside the flask are encouraged when reacting $SOCl_2$ because it's a very reactive solvent. The $SOCl_2$ may have reacted with oxygen or water from dichloromethane (DCM**)** which might not be completely dry while inside the three-neck flask, leaving a small

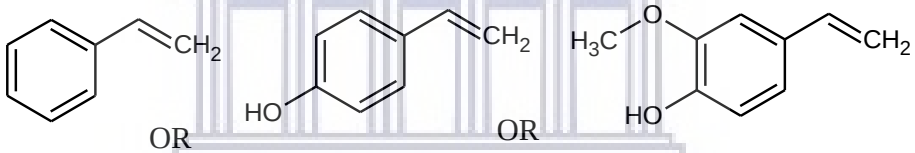
amount of $SOCl_2$ to react with the poor leaving group OH. Purging with helium gas to create inert conditions inside the three-neck flask was unsuccessful and might be because the three-neck flask was not Schlenk designed meaning it had no standardized air-tight quick-fit joints. The other two substituted cinnamic acids have a collective OH group on position number six of the benzene ring. Reacting these compounds with $SOCl_2$ resulted in multiple activated intermediates, which later led to a complex mixture of by-products which chromatography failed to isolate.

Table 2: Synthesis route and attempted reactions for the synthesis of series 2.



intermediate final product

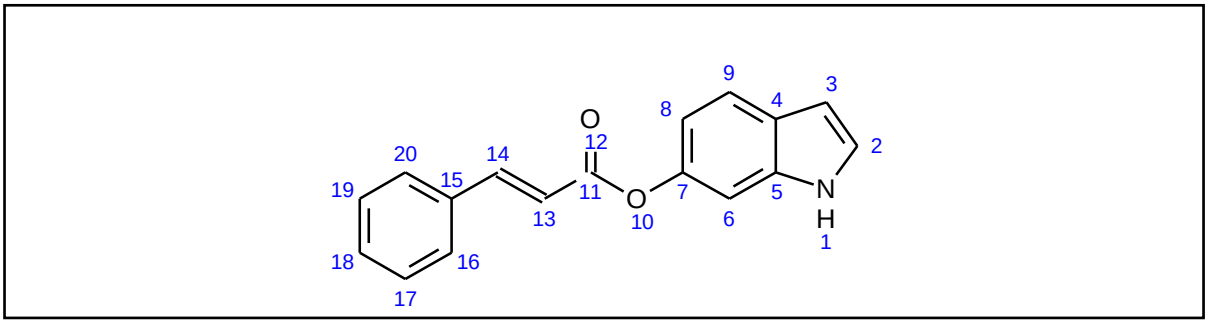
Where R =



Attempted reaction	**Yield**
1) THF, concentrated H_2SO_4, reflux 4-5 hrs	0%
2) $SOCl_2$, DMF 4 hrs; dry pyridine stirred at rt for 2 hrs	0%
3) $SOCl_2$, DMF, DCM refluxed for 2 hrs	0-9%

Only one compound within series 2, 1*H*-indol-6-yl (2*E*)-3-phenylprop-2-enoate (**3A**), was successfully synthesised in a low yield of 9 % (Section 3.5.1, **Table 2**). This compound was also included in the biological evaluations as it may lead to the identification of a new lead compound for future studies. The other 4 compounds of series 2 (**Table 1.1**) that contain the propargylamine group could not be synthesized be the precursor compounds had a low yield to synthesize it or the precursor compound could not the synthesized at all.

3.5.1. 1*H*-Indol-6-yl (2*E*)-3-phenylprop-2-enoate (3A)



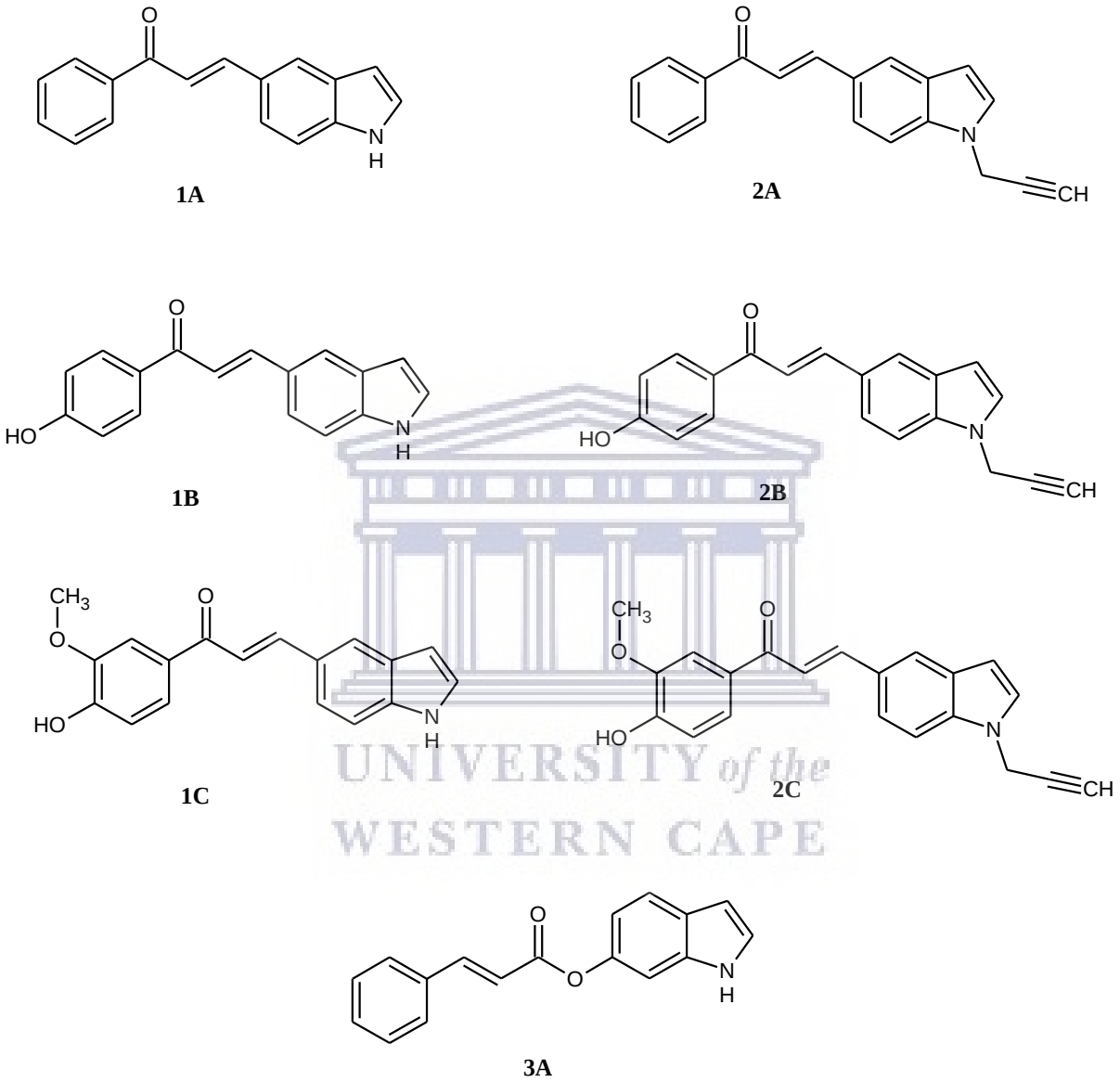
Synthesis: To a solution of cinnamic acid (148 mg, 1 mmol) in dry DCM (5 ml), thionyl chloride (725.4 µL, 10 mmol) and DMF (25 µL) were added. The reaction mixture was then refluxed for 4 hours. After that, the solvent was evaporated under vacuum to get a solid residue. Acetonitrile (10 ml) and K_2CO_3 (166.6 mg, 1.2 mmol) was added to the solid residue and stirred for 2 hours at 20 ˚C. After 2hrs, K_2CO_3 was filtered and the solvent discharged *in vacuo*. An extraction with DCM (15 ml x 3) was carried out, and then the organic phase washed with brine and H_2O. Column chromatography was performed to purify the compound with hexane/ethyl acetate (4:1).

Physical data: $C_{17}H_{13}NO$; **mass**:12 mg ; **yield**: 9%; **mp**: 179-182˚C ; **^{1}H NMR** (400 MHz, DMSO-d_6) δ_H (Spectrum 25): 8.28 (s, 1 H), 7.88 (d, *J* = 16.0 Hz, 1 H), 7.62 (d, *J* = 8.4 Hz, 1 H), 7.61-7.59 (dd, 4.2; 1.6 Hz, 2 H), 7.44-7.42 (t, *J* = 3.2 Hz, 3 H), 7.20-7.19 (t, *J* = 2.8 Hz, 1 H), 6.94-6.92 (dd, *J* = 8.8; 1.6 Hz, 1 H), 6.67 (d, *J* = 16.0 Hz, 1 H), 6.55 (bs, 1 H); **^{13}C NMR** (100 MHz, DMSO-d_6) (Spectrum 26): 166.17, 146.49, 146.29, 135.66, 134.29, 130.62, 128.99, 128.29, 125.93, 124.90, 121.09, 117.63, 114.15, 104.06, 102.66; **IR** (FT-IR, cm^{-1}) (spectrum 27): 3367.92, 2918.86, 1712.88, 1633.90; **MS** (EI, 70 eV) m/z (spectrum 28): 263.09 (M^+); **HR-ESI [M+H]$^+$**: calc. 264.1024, exp. 264.1019.

3.6. Conclusion

A total of seven compounds were successfully synthesized using the described pathways and confirmed by NMR, IR, and MS (Scheme 3.2). The yields of the compounds generally ranged from high to low, and the lower yields could be as a result of by-product formation during the reactions as noticed on the TLCs of some compounds. The compounds had varied substituents

such as OH, OCH_3 and propargylamine groups to evaluate their effects on these structures when subjected to biological evaluations in the next chapter (chapter 4).



Scheme 3.2: Successfully synthesised compounds for biological evaluation.

Chapter 4

4. *In silico* and biological results

4.1. Introduction

The study required the biological validation of chalcone- and curcumin-derived indole hybrids of propargylamine as agents for delaying or halting the death of neurons (Bar-am *et al.*, 2015). The compounds are centred primarily on literature review however, their binding interactions with hMAO-A and -B enzyme was unknown so *in silico* studies had to be computed. MAO oxidatively breaks amines such as bioactive amines of the brain thus generating an imbalance (Cai, 2014). The *in silico* studies were computed after synthesis to add more information on the synthesised compounds, according to literature the compounds are supposed to have some monoamine oxidase activity. A considerable number of reports indicate that inhibiting the hMAO enzyme, hMAO type B to be specific, might contribute to a neuroprotective effect (Tabakman *et al.*, 2004). Free radicals also contribute to the neuronal death, thus compounds capable of neutralizing radical species may also be neuroprotective agents (de Lau and Breteler, 2013). Cytotoxicity screenings are critical in determining the harmful effects of compounds prior to neuroprotection assessment can be performed. Several assays which are cytotoxicity, neuroprotection, anti-oxidant and monoamine oxidase assays were conducted to validate the postulates of the introduction (chapter 1). The findings of these assays are reported and presented in detail.

4.2. Molecular modelling

The academic and pharmaceutical industry sectors have been reshaped by the use of computer software in medicinal chemistry studies (Sansom and Smith, 1998). Molecular modelling software is mainly utilised to comprehend whether a compound binds to an enzyme (Sansom and Smith, 1998). Molecular Operating Environment (MOE) 2015 was used to simulate the feasibility of binding interactions between the compounds and both hMAO-A and -B and show the interactions likely formed with amino acids appropriate for the function. The outcomes of computations were shown as pictures with potential forms of probable bonds or conformational postures within the active-site cavity of monoamine oxidase.

4.2.1. Molecular modelling method

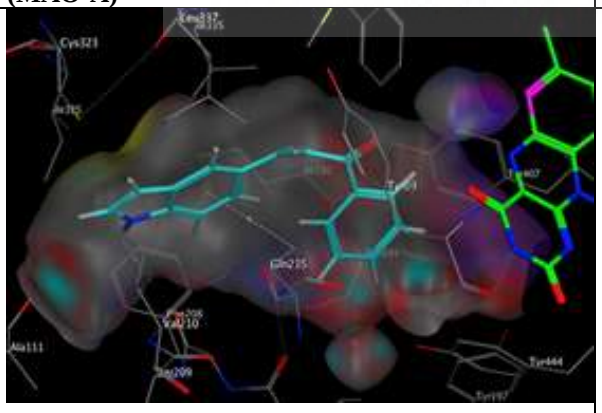
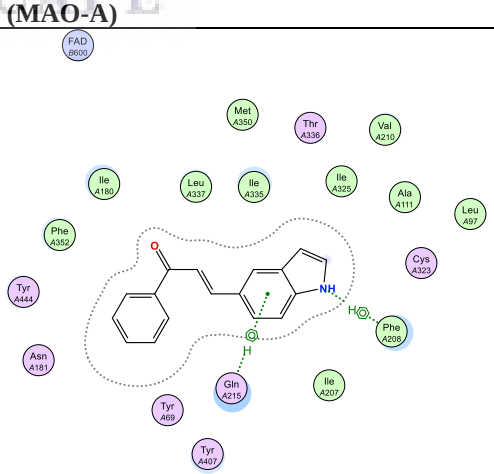
Enzyme complexes from PSLIO / PDB database with hMAO-A co-crystallized with clorgyline (PDB with product number: 2BXS) and hMAO-B co-crystallized with safinamide (PDB with product number: 2V5Z) was used (Binda *et al.*, 2007). The hMAO-A and -B enzymes were ionized to give positive charge and minimized utilizing MMFF94x forcefield. The co-crystallized ligand (clorgyline (for hMAO-A) and safinamide (for hMAO-B) was then selected to determine the enzymes' binding pocket (Binda *et al.*, 2007). Chemsketch® 2016 was used to draw the compounds and saved as V3000 MDL molfiles. The induced fit procedure was applied when docking the compounds.

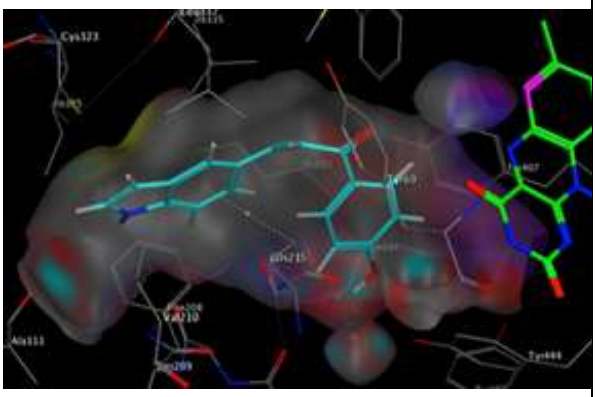
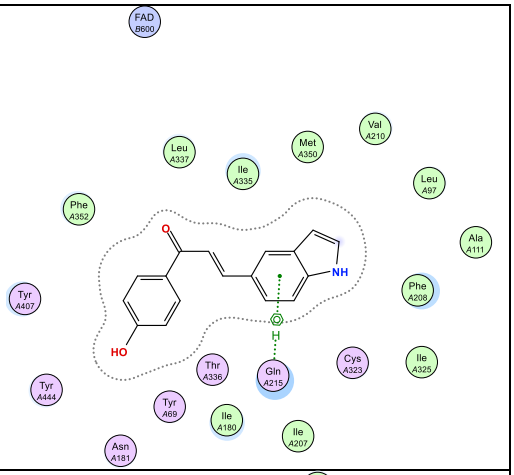
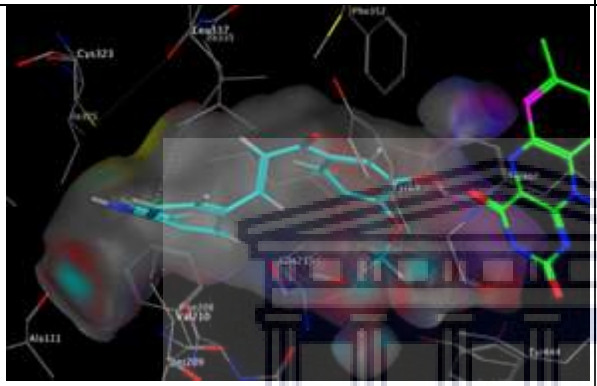
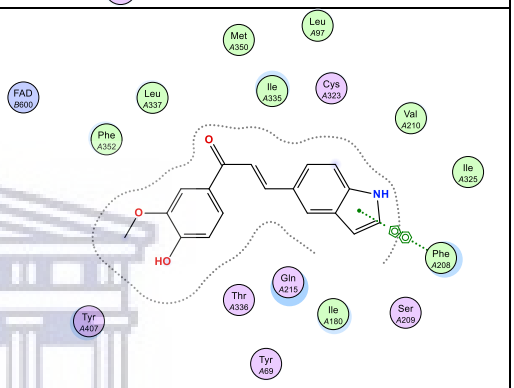
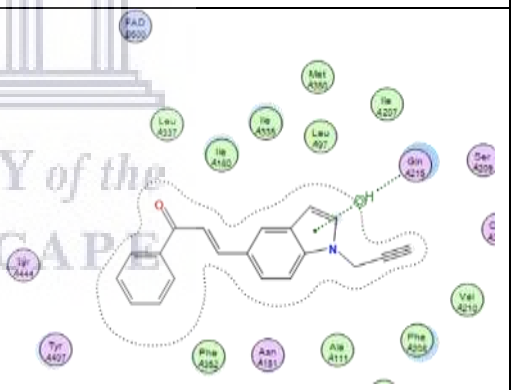
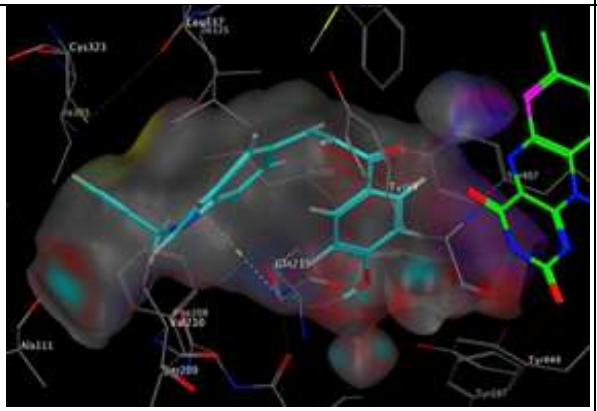
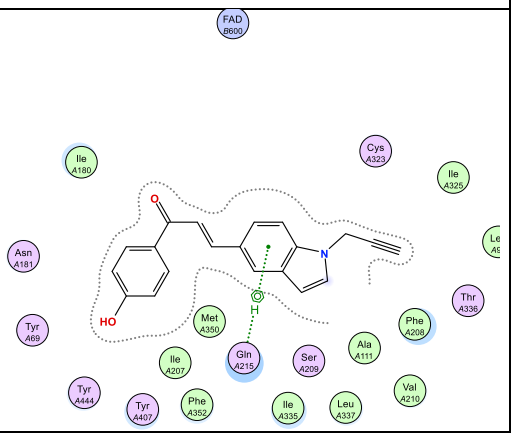
4.3. MAO docking results

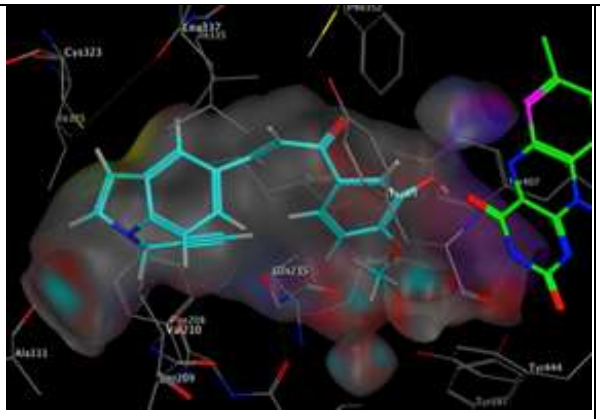
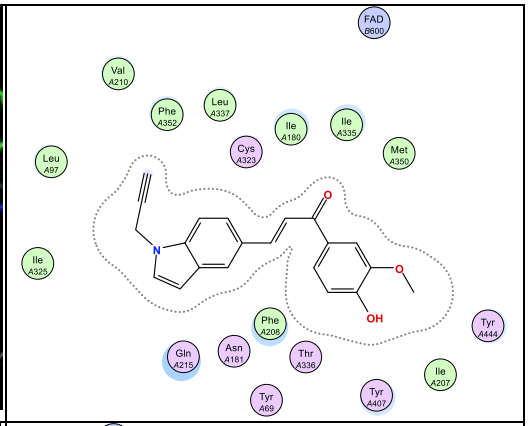
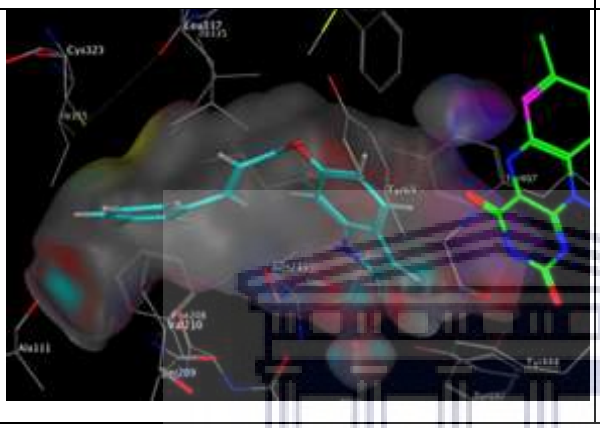
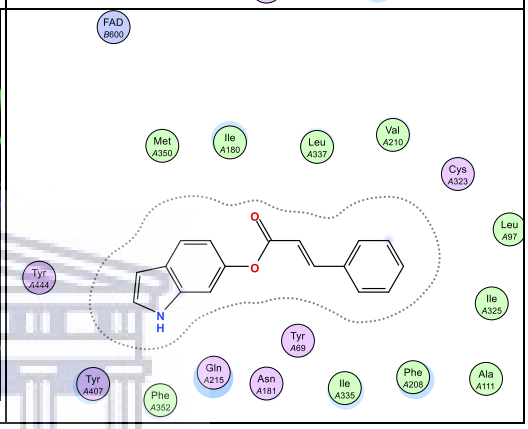
4.3.1. MAO-A results

The hMAO-A docking simulated findings are for compounds in **Figure 3.2**. All compounds interacted or appeared to have some type of binding interaction with the important amino acid(s) that are on the binding site of the MAO-A enzyme. Phe-A208 and Glu-A215 are important amino acids in the moulding of the MAO-A enzymes binding site (Veselovsky *et al.*, 2004). The findings of the MAO-A docking are presented in **Table 4.1.**

Table 4.1: Findings of compounds **1A, 1B, 1C, 2A, 2B, 2C** and **3A** (shown in blue on each simulation while the cofactor (FAD) is in green) after hMAO-A modelling.

Name	Docking simulation (MAO-A)	Ligand interactions (MAO-A)
1A		

1B		
1C		
2A		
2B		

2C		
3A		

Compounds **1A, 1B, 1C, 2A** and **2B** bind to one or more of the amino acids essential for binding sites formation, proposing that compounds might reach the hMAO-A enzyme's active site. The binding interaction between compounds and residues of amino acids is primarily due to the indole scaffold making it consistent with the literature (Shimazaki *et al.*, 2009). The NH moiety located on the pyrrole ring and the benzene ring of indole scaffold is reported to have binding interactions supporting molecular modelling findings (Shimazaki *et al.*, 2009).

All propargylamine-free chalcone hybrids (**1A, 1B, 1C**) bind to the residue Glu-A215 apart from **1C**, which only had arene-arene interactions with Phe-A208. Compound **1A** is predicted to be more active relative to **1B** and **1C** since it produces arene-hydrogen interaction with residues Phe-A208 and Glu-A215. Specific moieties on the benzene ring of the chalcone-derived section of compounds tend to provide no boosting impact on the compounds' ability to create specific interactions.

Human MAO type A activity of propargylamine-containing compounds **(2A, 2B, 2C)** was assumed to be equal to hybrids that are propargylamine-free because no additional interactions were observed. At least one interaction between arene-hydrogen and amino acids occurred at the binding site of hMAO-A enzyme with propargylamine-containing compounds was

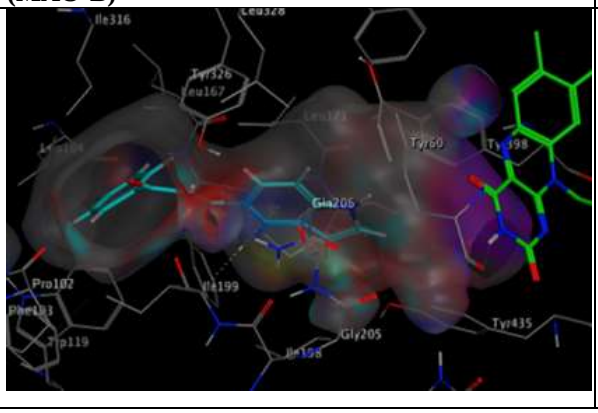
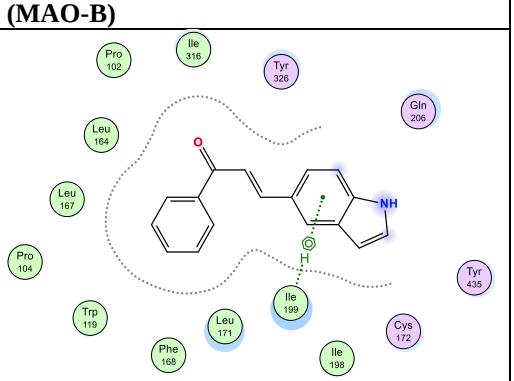
observed. Compound **2C** deviated from **2A** and **2B**, no interactions were observed except for activity recommendation due to the proximity of exposed ligand to Glu-A215 and Phe-A208. From the modelling results, the interaction of the propargylamine with the FAD seemed unlikely.

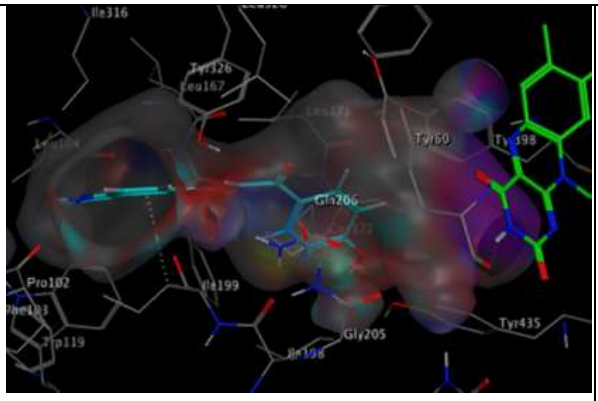
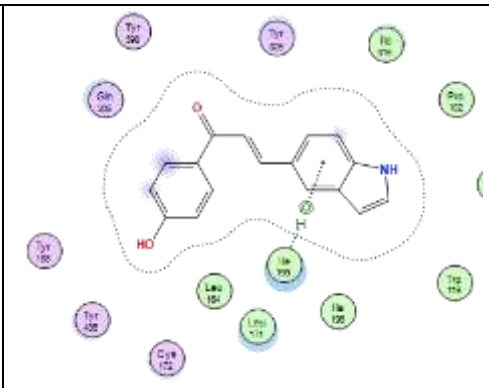
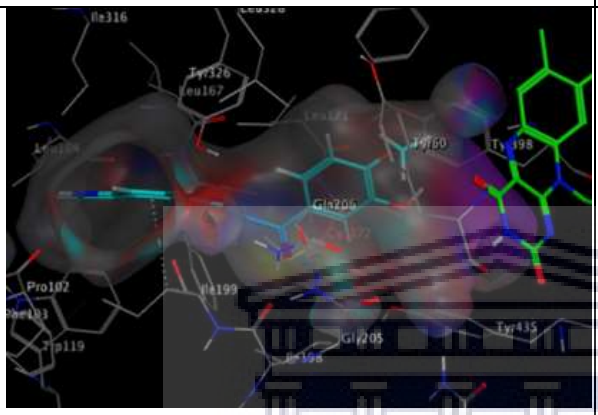
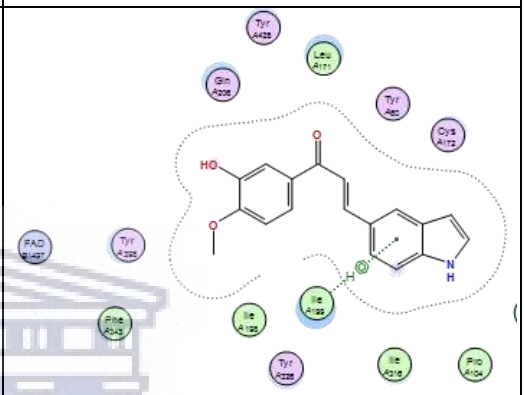
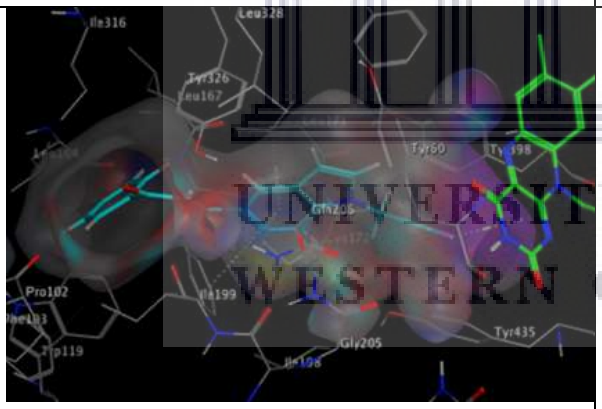
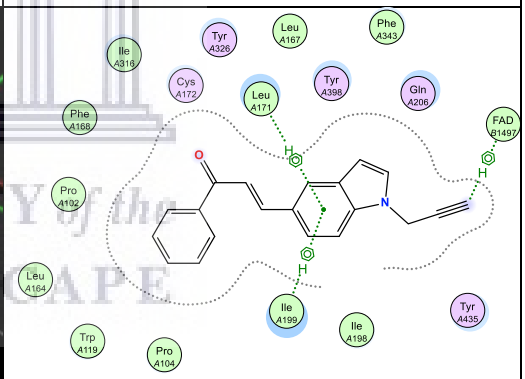
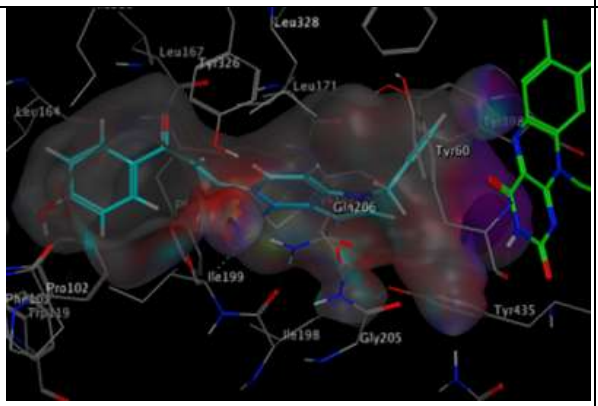
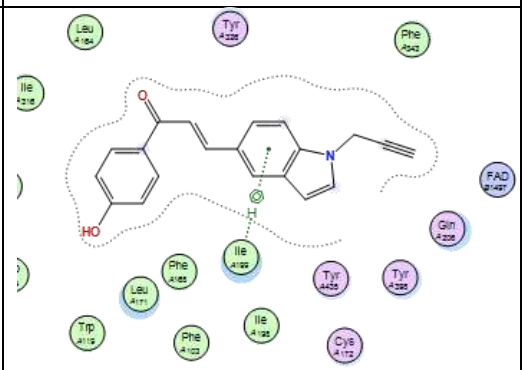
The curcumin-derived indole ester (**3A**), the lowest possible activity among all test compounds was expected. Compound **3A** indicated activity potential because it was located close to Glu-A215 leaving it exposed but no strong interactions were identified. The phe-A208 residue was also exposed by a lower degree in comparison to all other compounds. Curcumin-derived indole ester hybrid (**3A**) is also predicted to possess comparatively low action relative to the unsubstituted chalcone-derived hybrid (**1A**) that possesses Phe-A208 and Glu-A215 interactions.

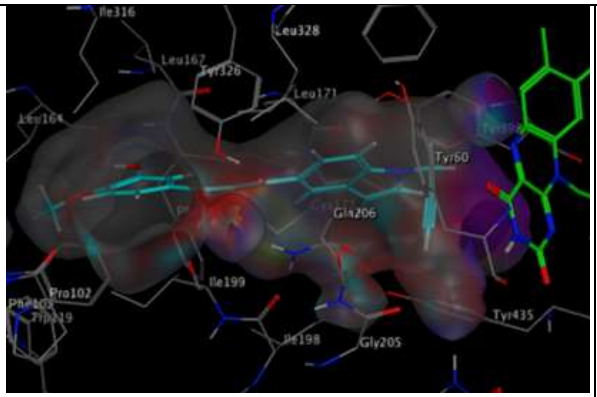
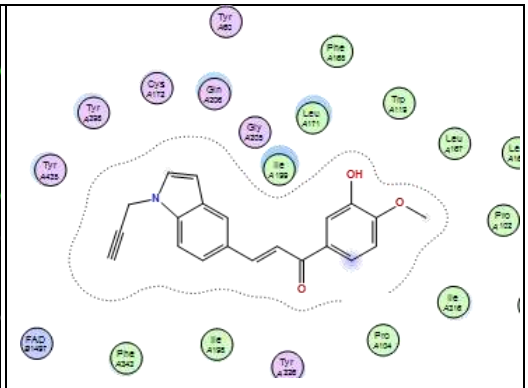
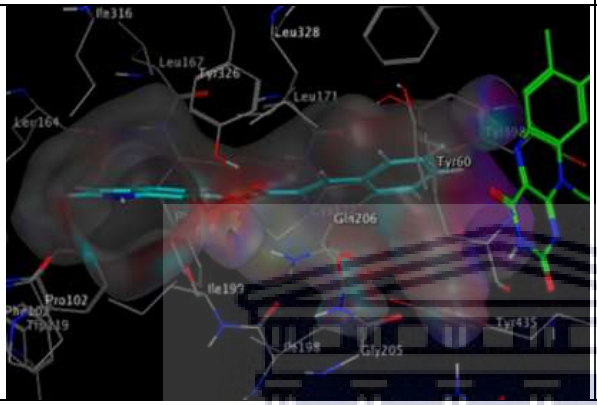
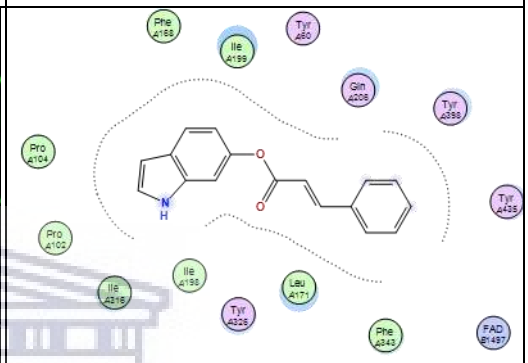
4.3.2. MAO-B docking results

All propargylamine-free chalcone-based hybrids **(1A, 1B, 1C)** interacted with at least lle-199 amino acid residue. lle-199 residue is essential in hMAO-B enzyme since it acts as a systemic indicator for substrate and inhibitor recognition (Hubálek *et al.*, 2005a). As reported above, most binding interactions between amino acid residues and indole scaffold were identified (Shimazaki *et al.*, 2009). The results of hMAO-B docking were included in **Table 4.2.**

Table 4.2: MOE images of hMAO-B enzymes with chalcone-based hybrids (**1A, 1B, 1C, 2A, 2B and 2C)** and curcumin-derived indole ester hybrid **(3A)**. Blue -test compounds, green -FAD co-factor.

Name	Docking simulation (MAO-B)	Ligand interactions (MAO-B)
1A		

1B		
1C		
2A		
2B		

2C		
3A		

Compounds (**1A, 1B** and **1C**) creates arene-hydrogen interactions with lle-199 residue and thus are anticipated to also have similar activity since the number of interactions is similar. Modification of benzene ring on the chalcone derived segment with specific moieties seemed to impact the binding affinity of the compounds. Generally, amino acid residues that range from 50 to 400 are crucial for substrate specificity of MAOB (Tsugeno *et al*., 1995). In **1B**, the hydroxyl group seemed to activate the benzene ring to which the hydroxyl group is attached to leaving the group exposed thereby making it feasible for interactions to form with amino acid residue Gln 206.

Propargylamine-containing compounds (**2A, 2B** and **2C**), showed similar binding interactions in comparison to propargylamine-free (**1A, 1B** and **1C**). Compound **2A** showed exceptional binding interactions and may have the best hMAO-B inhibitory effect as it interacts with two vital amino acids residues (lle-199 and Leu-171) and the propargylamine moiety interacted with FAD co-factor. In **2B,** the propargylamine moiety is close to the FAD co-factor thereby suggesting that the compound might have some interaction with the FAD.

Curcumin-derived indole ester (**3A**) is projected to have reduced hMAO-B activity in contrast to the chalcone-derived hybrid series. The curcumin-derived indole ester (**3A)** did not exhibit strong interactions with the crucial amino acids, however, some residues including lle-199,

Gln-206 and Leu-171 were exposed proposing an opportunity of activity. Compound **3A**'s indole scaffold was identified as a potential interaction site. Contrasting **3A** with **1A** (unsubstituted chalcone-derived hybrid), **3A** is also projected to have reduced action as it generates fewer interactions with significant amino acids.

4.4. Cell viability studies

4.4.1. Introduction

The MTT (3-(4,5-dimethylthiazol-2-yl)-2–5-diphenyltetrazolium bromide) technique was performed to determine compounds ' cytotoxic effects (Itano and Nomura, 1995). Human neuroblastoma SH-SY5Y cells are often used in neurodegeneration studies (An *et al.*, 2019). The assay examines biochemical markers to assess cell metabolism (Datki *et al.*, 2003). Once the MTT reaches the viable cells, it is converted into formazan by enzymes including the NADH reductase (Datki *et al.*, 2003). Formazan concentration is detected spectrophotometrically and it indicates the reduction potential inside the SH-SY5Y cells and cell viability (Liu and Schubert, 1997).

4.4.2. Cell line and treatment

Colleagues in Molecular Biology and Human Biology, University of Stellenbosch, Tygerberg, Cape Town sponsored the SH-SY5Y human neuroblastoma cell line for the assay. Dulbecco Modified Eagles Medium (DMEM, Gibco, Life Technologies Ltd) combined with 10 per cent (v / v) fetal bovine serum (FBS, Gibco, Life Technologies Ltd), 1 per cent 100 U / ml of penicillin, and 100 µg / ml of streptomycin (Lonza Group Ltd., Basel Switzerland) were used to grow monolayer cells. At 37 °C, humid conditions and 5 per cent CO_2, the cells were incubated. Media replacement took place every 24 hours to 72 hours, and cells were sub-cultured by trypsin splitting (Lonza Group Ltd., Basel Switzerland).

4.4.3. SH-SY5Y cytotoxicity assays

SH-SY5Y cells were plated into level surface 96 well plates at 7,500 cells per well and incubated in the culture media as outlined in 4.4.2. The cells were left for a day to rest on the plate's surface, thereafter, new media with test compounds having concentrations of 100 µM, 50 µM and 10 µM was used to substitute used media. DMSO was used to make up the concentrated solution of the test compounds and it was also administered as control where it

was used in quantities equivalent to that of the largest concentration of the test compounds. After 48 hours, 10 µL of MTT solution (5 mg/mL) was added to each well plate and it was incubated for 4 hours at 37 °C. DMSO (100 µL) was used to solubilize the formazan (purple in colour) and transferred into 96 well plates which were then read using a POLARstar Omega Plate Reader- Multi-Mode set an absorbance wavelength of 570 nm.

4.4.4. Results and discussion

The cytotoxicity data collected was analyzed in percentage with the untreated cells(control) set at 100% cell survival. The data is displayed in **Figure 4.1**.

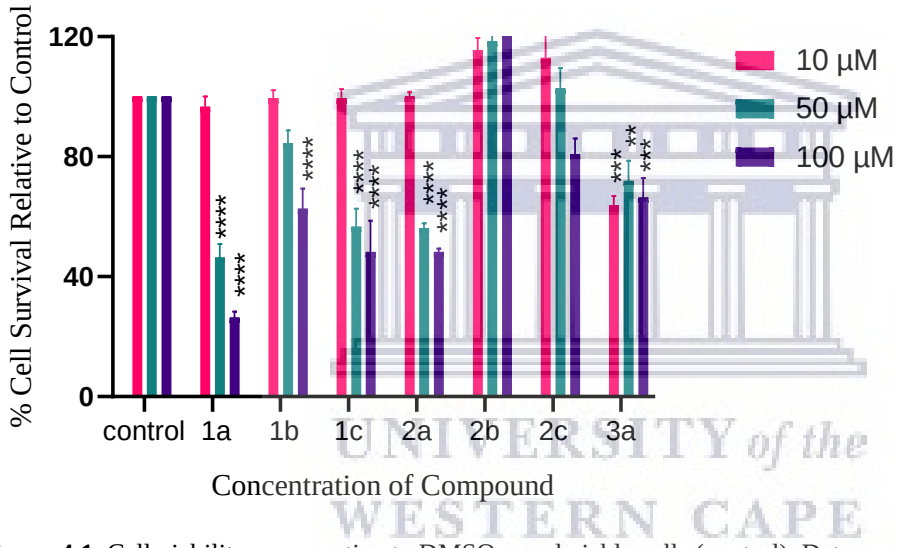


Figure 4.1. Cell viability comparative to DMSO cured viable cells (control). Data mean ± SEM (n= 3). ANOVA data analysis and significance was used to evaluate results[(**) p<0.01,(* **) p<0.001, (* * * *) p<0.0001].

In general, higher concentrations of chalcone-derived compounds appeared to be toxic to cells because of the low percentage of cell viability compared with control cells observed at 50 µM and 100 µM concentrations. Compounds **1A, 1B, 1C,** and **2A** at concentration levels of 100 µM and 50 µM had a cell percentage survival relative to control that ranged from 26.3% to 62.6% and 46.3% to 84.6%, respectively. The data indicated that SH-SY5Y cells were viable at 10 µM of the test compound. Therefore, 10 µM concentration would thus be acceptable for neuroprotective studies because higher concentrations are toxic to cells. The data indicated that compounds that are propargylamine-containing triggered a rise in percentage cell survival, for instance, compounds **1B** (propargylamine-free) but this was not the case for **2B** (propargylamine-containing). After cells treatment with **2B** (propargylamine-containing), cell

proliferation with cell viability of 115.49% more than that of the ones in **1B** (cell viability of 99.58%) was detected. Addition of different functional moieties on the benzene ring of the chalcone-derived section in compounds **1A, 1B** and **1C** did not seem to impact cell viability. The curcumin-derived indole ester (**3A**) exhibited significant cytotoxicity at all three cell concentrations tested. At 100 µM, 50 µM and 10 µM concentrations, the percentage cell survival relative to control ranged from 63.8% to 71.9%. Compound 3A was excluded from neuroprotective studies because it would be toxic to the SH-SY5Y cells at all three different concentrations.

4.5. Neuroprotection

4.5.1. Introduction

Cytotoxicity data of the compounds suggested use of 10 µM concentration for the neuroprotection assay hence the neuroprotection experiments were done with 10 µM of compounds. The SH-SY5Y cells are treated with 1-methyl-4-phenyl pyridinium (MPP^+) which is neurotoxic and cause cell death (Presgraves *et al*., 2003). Any chemicals or substances that reduce MPP^+ may be considered as neuroprotective and can be developed to potential drug therapy of neurodegenerative disorders (Presgraves *et al*., 2003). MPP^+ has been documented and found to trigger parkinsonism in humans, taking note of programmed cell death is one of the suggested processes by which SH-SY5Y cells undergo apoptosis once subjected to MPP^+ (Itano and Nomura, 1995).

4.5.2. Neuroprotection assay

The SH-SY5Ycells were spread into each well of the 96-well plate accompanied with 10 µM of synthesized compounds and rested for 2 hrs. MPP^+ (2 mM) was included in cell treatment and rested for 48 hours. Then, cell survival was calculated utilizing the above-described colourimetric MTT test.

4.5.3. Results and discussion

Cell survival of DMSO only treated cells (control) was fixed at 100%. The neuroprotection percentage was created by subtracting the cell survival of cells treated by only MPP^+ from the final cell survival treated with tested compound and MPP^+. The findings are displayed in **Figure 4.2.**

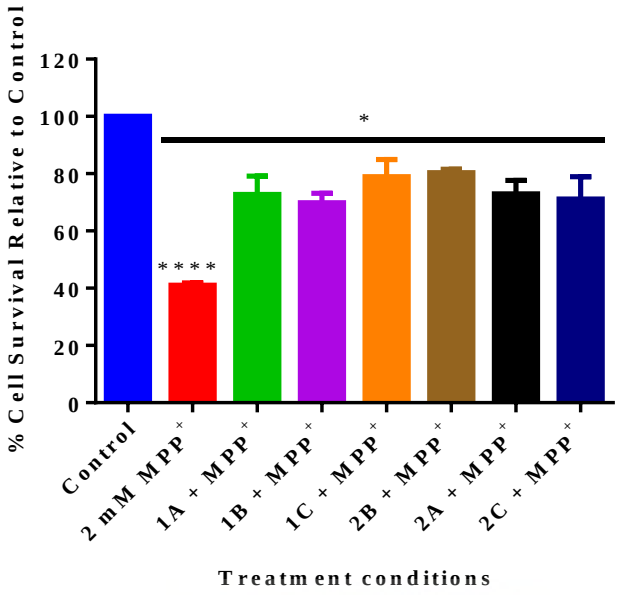


Figure 4.2. The SH-SY5Y was subjected to the toxic substance, MPP^+. The test compounds were compared to control. Data mean ± SEM (n= 3). Tukey's multiple used with significance level= [(*) $p < 0.05$ and (* * * *) $p < 0.0001$].

The cell survival in comparison to the control declined significantly to 41.03% in cells treated with 2 mM MPP+ from 100% in control. The decline supported MPP^+'s well-documented reports of triggering cell death (Itano and Nomura, 1995). The SH-SY5Y cells containing both test compound and 2 mM of MPP^+ seemed to cause cell proliferation when contrasted with cells treated with 2 mM MPP^+ only. SH-SY5Y cells survival varied from 80.37% to 69.81% in cells containing both 2 mM of MPP^+ and test compound. The findings indicate that generally, the compounds possess neuroprotective activity because they were efficient in decreasing the death rate in cells, providing cell survival means considerably better than treated with 2 mM MPP^+ only. The assay data seemed not to supply evidence relating to structure-activity of compounds since the data was in that same ranges.

4.6. Monoamine Oxidase Assay

4.6.1. Introduction

A fluorometric approach was employed to assess recombinant human MAO activity with kynuramine being hMAO-A and -B substrate (Holt *et al.*, 1997; Weissbach *et al.*, 1960). The technique focuses on the oxidation of kynuramine to an intermediate that becomes cyclized to 4-hydroxyquinoline (Weissbach *et al.*, 1960) displayed in **Figure 4.3**. Using a spectrophotometer the 4-hydroxyquinoline can be quantified (Holt *et al.*, 1997). An inhibitor

of MAO prevents kynuramine conversion to 4-hydroxyquinoline resulting in lower fluorescence relative to an inhibitor-free assay (Matsumoto *et al.*, 1985). From fluorescence measurements, the percentage of enzyme inhibitory activity can be generated from a dose-response graph (Morinan and Garratt, 1985). IC_{50} concentrations of test compounds are calculated from the graph (Erol and Bilgin, 2003) and the P-value, using GraphPad Prism®.

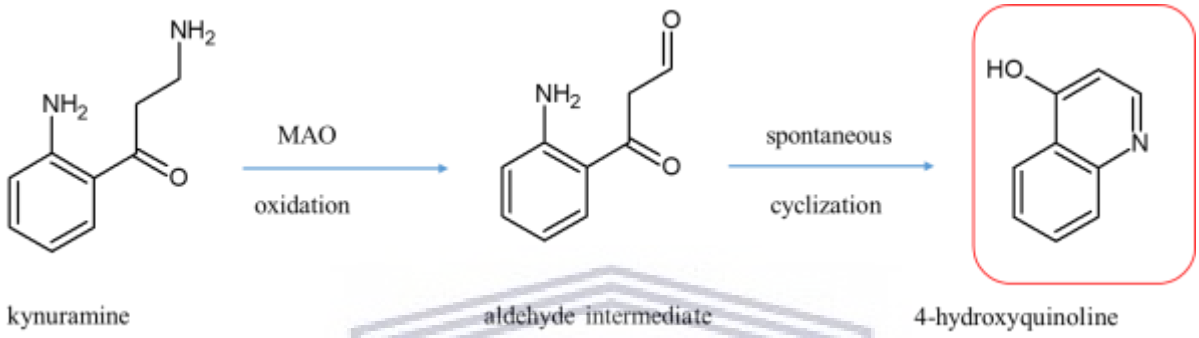


Figure 4.3: The enzyme-deamination of kynuramine(Weissbach *et al.*, 1960).

4.6.2. Method and materials

Sigma-Aldrich® was used to procure all consumables; unless otherwise stated. A 10 mM stock solutions of clorgyline, rasagiline and test compounds were made, light-protected and stored in the fridge. Four separate concentrations (0.001 mM, 0.01 mM, 0.1 mM and 1 mM) were produced by diluting stock solution with DMSO. These concentrations then transformed to 0.001 µM, 0.01 µM, 1 µM, 10 µM, 100 µM in the final assay volume. A 100 mM potassium dihydrogen phosphate buffer with pH 7.4 (adjusted with 0.9% w/v NaOH) was made and kept in the fridge. The recombinant human MAO-A/-B enzymes were reconstituted by adding the buffer and aliquoted into 2 ml centrifuge vials and kept at -80 ˚C. Two different stock solutions of kynuramine were made (750 µM for MAO-A and 500 µM for MAO-B) and these kynuramine concentrations translated to 45 µM and 30 µM in the final assay volume. The fresh substrate was prepared every day to guarantee repeatable findings.

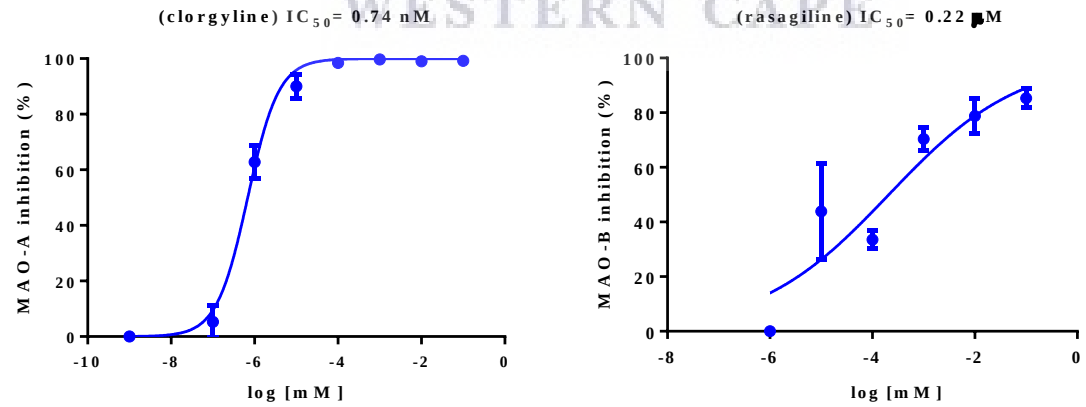
The assay was done in three replicates for each concentration of positive control (containing inhibitor), test compounds and negative control (inhibitor-free). Phosphate buffer (207.5µL) and corresponding test compound (2.5 µL) were transferred to each 2 ml vial and purged two times with fresh tip on every vial to assure uniform distribution. In periods of 10 seconds, 25

µL of the enzyme was transferred into each vial and incubated at 37˚C for 10 mins. Taking note that concentrations of 750 µM were for hMAO-A and 500 µM was for hMAO-B, kynuramine (15 µL) was added in each vial in periods of 10 seconds and incubated for 20 mins. A volume of 150 µL (2 N NaOH) was used to stop the reaction. The mixture (100 µL) was moved into each well of the 96-well plates and read at 400 nm emission and 310 nm excitation wavelengths on a spectrophotometer. Graphpad Prism® software was utilized to assess the results.

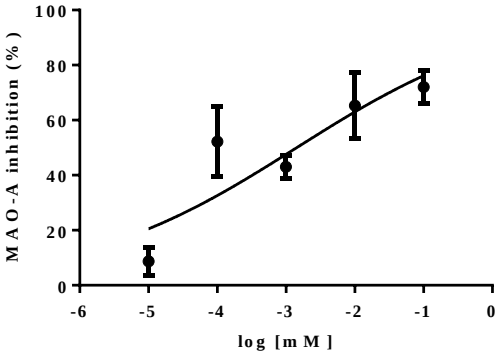
4.6.3. Results and discussion

Compounds **3A, 2C, 2B, 2A, 1C, 1B, 1A** and controls (clorgyline and rasagiline) were assessed for MAO-A/ -B activity and the findings are presented using Graphpad Prism®. The compounds' IC_{50} values are plotted in **Figure 4.4**.

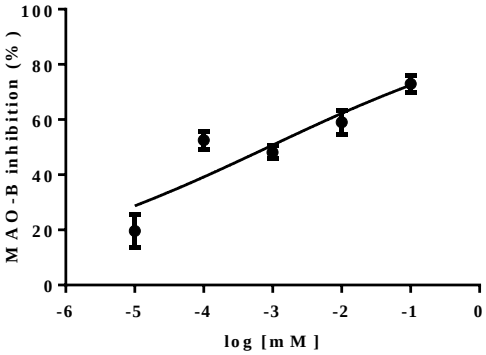
The assay showed that all compounds tested have a certain type of anti-MAO enzyme activity. In summary, the compounds inhibited the MAO enzyme at concentrations of less than 13 µM except for compounds **1C** (MAO-B inhibition IC_{50}= 33.11 µM) and **3A** (MAO-B inhibition IC_{50} more than 100 µM). The cytotoxicity research indicated non-toxic effects at 10 µM concentration of compounds thus implying that the IC_{50} concentration of compounds at 10 µM or below is desirable.



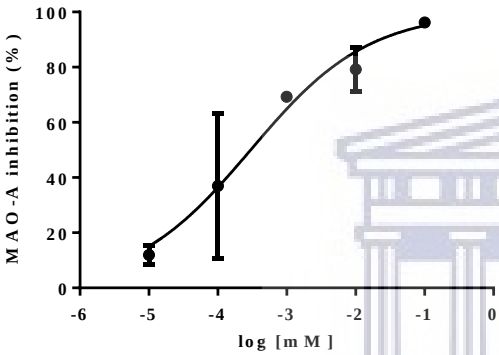
(1A) IC_{50}= 1.10 μM



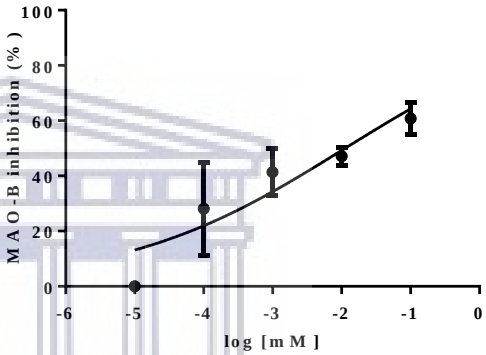
(1A) IC_{50}= 0.91 μM



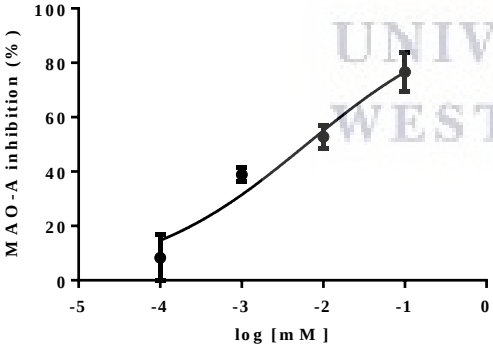
(1B) IC_{50}= 0.33 μM



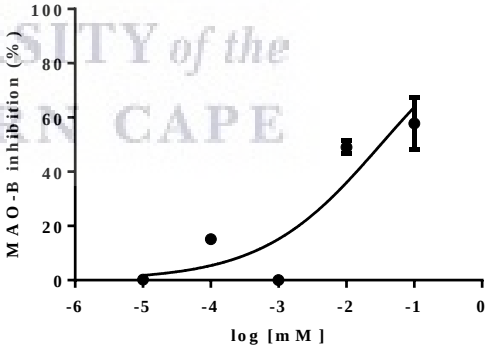
(1B) IC_{50}= 12.02 μM



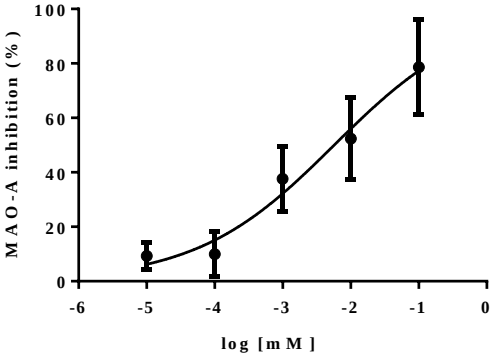
(1C) IC_{50}= 6.61 μM



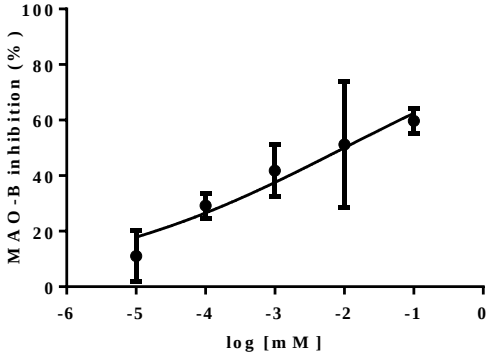
(1C) IC_{50}= 33.11 μM



(2A) IC_{50}= 5.75 μM



(2A) IC_{50}= 9.55 μM



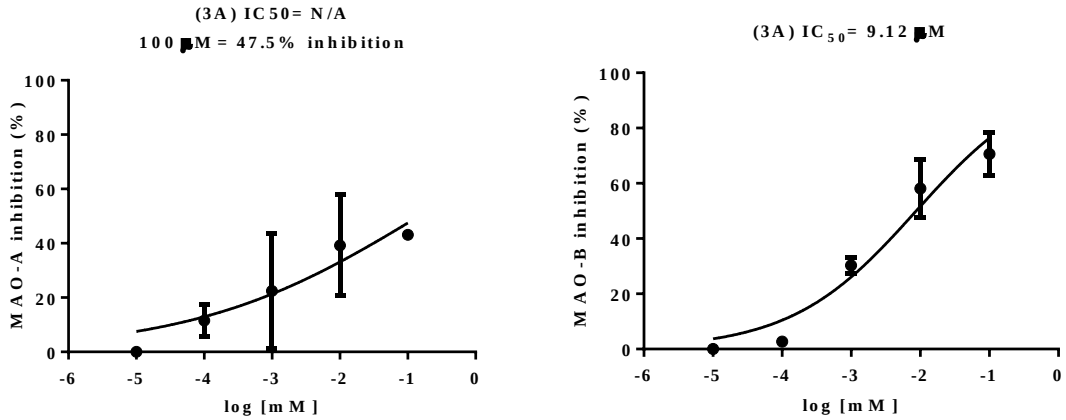


Figure 4.4: Nonlinear regression projections for the compounds' inhibitory effect of hMAO-A (left) and hMAO - B (right). The P-value per graph is less than 0.05 ($p<0.05$).

MAO-A assay findings

Chalcones-derived hybrids (**2C, 2B, 2A, 1C, 1B** and **1A**) seem effective against hMAO-A with IC_{50} values (0.33 µM to 6.61 µM) (**Table 4.3**). These compounds, although active, are not as potent as clorgyline which has an IC_{50} of 0.74 nM. Propargylamine-free compounds (**1A, 1B** and **1C**) have higher potency in comparison to the compounds that are propargylamine-containing (**2A, 2B** and **2C**). A propargylamine moiety is affixed onto the design of the compounds to improve effectiveness (Youdim and Weinstock, 2001), but hMAO-A results revealed differently. The absence of enhancement inactivity could be traced to the incapability of the propargylamine ligand to form interactions with the FAD cofactor within the MAO-A active surface (Mitchell *et al.*, 2001). The propargylamine functional group appeared orientated further from FAD cofactor as per the molecular modelling results. This may be the reason why the propargylamine-containing compounds displayed decreased MAO-A inhibitory ability.

For compounds **1C, 1B** and **1A** the inclusion of specific functional groups on para or meta site of the chalcone-derived section of the hybrid significantly impacts the hMAO-A inhibition effect. The hydroxyl moiety incorporated on para-position of **1B** (IC_{50}= 0.33 µM) enhanced hMAO-A inhibition from IC_{50} =1.10 µM in **1A** (unsubstituted). Adding methoxy moiety to the meta-position in **1C** reduced activity to an IC_{50} of 6.61 µM. Compound **1B** produced the best anti-MAO-A activity of all synthesized compounds. The rise in anti-MAO-A activity after adding the hydroxyl moiety is reinforced by studies suggesting that incorporating a hydroxyl moiety improved the function of related modified compounds (Sulpizio *et al.*, 2016).

Curcumin-derived indole ester (**3A**) has the lowest hMAO-A inhibitory activity (IC_{50} more than 100 µM). Comparing hybrids unsubstituted on the benzene ring, curcumin-derived indole ester **(3A)** with chalcone-derived compound **1A**, compound **3A** has a lower hMAO-A inhibitory effect. The curcumin-derived indole ester **(3A)** is also the least effective hMAO-A inhibitor in comparison to all synthesized compounds in this study. Compound **3A** has relatively low potency which can be attributed to the ester bond that binds the hybrid together. In **3A**, the ester bond binding the hybrid can easily disintegrate contrary to the hydrocarbon bond in chalcone-derive hybrid thereby rendering **3A** less potent (Schoenmakers *et al.*, 2004).

MAO-B assay results

Compounds **(3A, 2C, 2B, 2A, 1C, 1B** and **1A)** had hMAO-B inhibitory effect with IC_{50} concentrations varying from 0.9 µM to 33 µM relative to 0.22 µM of rasigiline (control) shown in **Table 4.3.** Compounds that are proparylamine-containing had improved hMAO-B inhibition than proparylamine-free compounds. Proparylamine-containing compounds **2B** and **2C** with IC_{50} of 5.25 µM and 3.02 µM, respectively had higher inhibitory capacity in comparison to propargylamine-free compounds **1B** (IC_{50} = 12.02 µM) and **1C** (IC_{50} = 33.11 µM). Compound **2A** (IC_{50} = 9.55 µM) deviated from expected activity because compound **1A** (IC_{50}= 0.91 µM) was more potent.

Specific substituents on the chalcone-derived portion of compounds **1C, 1B** and **1A** lowered the hMAO-B inhibitory effect. Unsubstituted compound **1A** (IC_{50} = 0.91 µM) has the highest hMAO-B inhibitory effect that is comparable to the control (rasagiline, IC_{50} = 0.22 µM). MAO-B enzymes favour smaller substrates to larger ones (Hong and Li, 2019). The decrease of activity in case of compounds **1B** and **1C** when compared to **1A** could be due to the bulking caused by the addition of each substituent thereby reducing their preferential affinity for MAO-B.

Compound **3A** (the curcumin-derived indole ester with IC_{50} of 9.12 µM) has an hMAO-B inhibitory effect in the same range as all other test compounds. Compound **3A** hMAO-B inhibitory effect is 10-fold less that of **1A**. The relatively low activity of compound **3A** in contrast with **1A** might also be attributed to the ester bond that links the hybrid together in **3A**.

The selectivity index (SI) shown in **Table 4.3**, of **3A, 2C, 2B, 1C, 1B** and **1A** indicate the compounds are relatively non-selective inhibitors of both MAO isoenzymes. Compounds **1A, 2B** and **2C** have SIs of 1.21, 1.32 and 6.31, respectively but these compounds are qualified as non-selective because the SI is not sufficient to be classified as selective towards hMAO-B inhibition. MAO-B is reported to be involved in causing neurodegenerative disorders thus MAO-B inhibitors are perceived as a therapeutic option (Grimsby *et al.*, 1991). MAO-A may be equally important in combating mood disorder (Lipper *et al.*, 1979). Compound **1B** is 36 times extra selective to MAO-A with an SI of 0.03 and has the best MAO-A inhibitory effect among all the synthesized compounds. Compounds **1C** and **2A** have SI towards MAO-A of 5.00 and 1.66 respectively which is not significant to be labelled as selective MAO-A inhibitors.

Some reports suggest that dual inhibition of both MAO isoenzymes may be more effective in curbing NDs (Delumeau *et al.*, 1994). Patients suffering from NDs often have their prognosis and wellbeing adversely affected by cognitive and behavioural changes (Douven *et al.*, 2018; Hallikainen *et al.*, 2018). MAO-A gene variants were related to a variety of these behavioural changes (Finberg *et al.*, 2016). NDs overlap at numerous levels and behavioural changes are prevalent even in patients with PD and AD. Geriatric patients with Alzheimer's diseases also suffer from an amnestic syndrome where a single non-memory-related cognitive domain is primarily impaired (Trojsi *et al.*, 2018). Neurodegenerative caused by elevated MAO-A behaviours have been reported and was associated with depressed activity in chronically stressed rodents (Lee *et al.*, 2013). **ASS 234** is one of the MTDL being studied for AD with dual MAO activity, it has shown to increase natural memory process (Marco-contelles *et al.*, 2016).

Table 4.3: Compounds' selectivity indices (SI) for MAO activity.

Compound	**MAO-A IC_{50} (µM)**	**MAO-B IC_{50} (µM)**	**SI** $(\frac{MAO-A}{MAO-B})$	**SI** $(\frac{MAO-B}{MAO-A})$
1A	1.10	0.91	1.21	0.82
1B	0.33	12.02	0.03	36.42
1C	6.61	33.11	0.20	5.00
2A	5.75	9.55	0.11	1.66
2B	6.91	5.25	1.32	0.76
2C	19.05	3.02	6.31	0.16
3A	>100	9.12	N/A	N/A

4.7. Antioxidant assay

4.7.1. Introduction

Antioxidants are an agent that effectively reduces or blocks the oxidation of a substance at lower concentrations compared to the sample being oxidized (Halliwell and Gutteridge, 1995). Free radicals are an example of a sample of species which can be oxidized by antioxidants (Betteridge, 2000). Free radicals have an unpaired electron and they can trigger chain reactions which have been documented to cause brain cells damage (Bhat *et al.*, 2015; Halliwell, 1994). Research shows that oxidative damage of cells is implicated as a key or partial pathophysiological cause of diseases such as Alzheimer's, most cancers, Parkinson's and AIDS (Moon and Shibamoto, 2009; Gandhi and Abramov, 2012; Pisoschi and Pop, 2015).

Figure 4.5: DPPH assay reaction

Natural antioxidants exist such as flavonoid, vitamin C and E which are effective at oxidizing free radicals (Nunez-Selles, 2005; Uzum *et al.*, 2006). There are various methods to check compounds' antioxidant effectiveness but in this study, 2,2-diphenyl-1-picrylhydrazyl (DPPH•) assay was used because it is very sensitive and uncomplicated to perform (Moon and Shibamoto, 2009). The test is based on the concept that hydrogen donors are oxidants (Moon and Shibamoto, 2009). DPPH• is a purple free radical, which transitions to yellow DPPH-H if it gains a hydrogen atom, causing a lower UV absorption (MacDonald-Wicks *et al.*, 2006) shown in **Figure 4.5**. The DPPH-H formation is measured by Ultraviolet spectrophotometry with absorption fixed at 517 nm

4.7.2. Method and materials

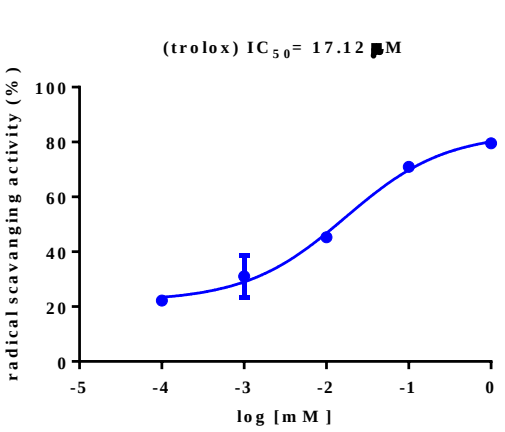
Sigma-Aldrich® was used to procure all chemicals and consumables unless otherwise specified. DPPH• was dissolved in methanol to give a 0.12 mM DPPH• solution and covered against the light. Four concentrations (0.01 mM, 0.1 mM, 1 mM and 10 mM) of Trolox and test compounds were made by diluting with DMSO. DMSO solution was also used as negative control while Trolox was used as a positive control. A volume of 20 µl of the test compounds was transferred into corresponding wells of the 96-well plate and incubated in a darkened room for thirty minutes. The absorbance was quantified spectrophotometrically at 517 nm wavelength by using Polarstar ® Omega.

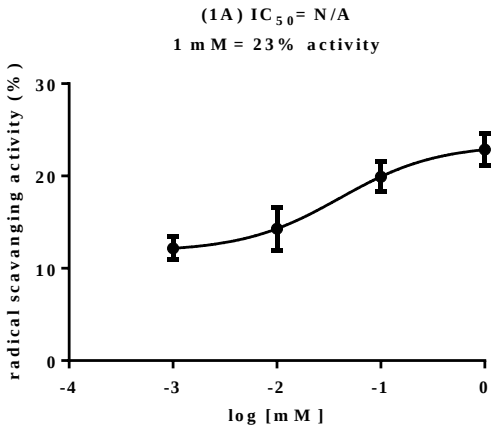
4.7.3. Results and discussion

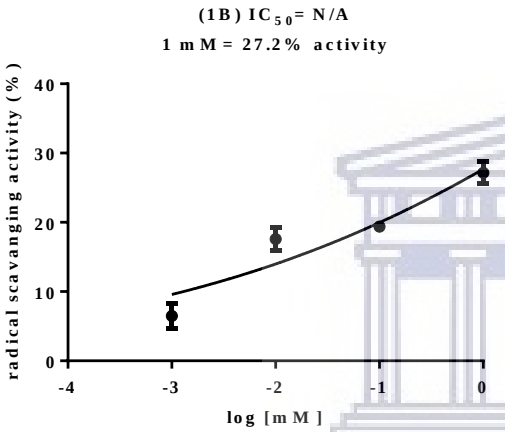
The test compounds' and control's activity were calculated as a percentage using the below equation.

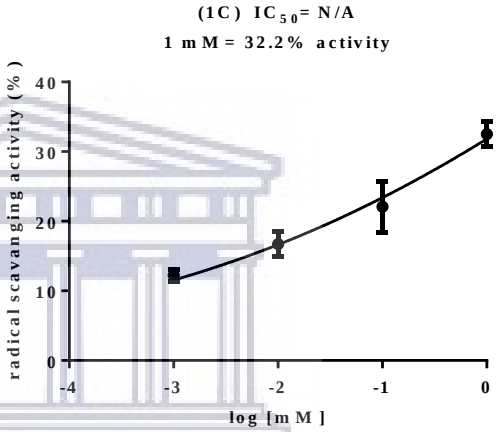
$$\text{Percentage activity} = \frac{(\text{absorbance of blank} - \text{absorbance of test compounds})}{\text{absorbance of blank}} \text{ X } 100$$

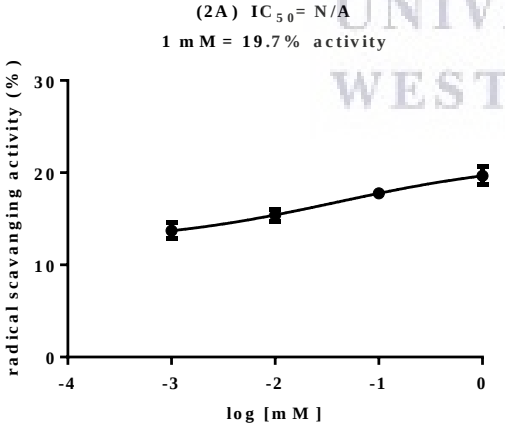
Compounds **3A, 2C, 2B, 2A, 1C, 1B** and **1A** were assessed for free radical scavenging through the DPPH method and their IC_{50} concentrations were determined utilizing GraphPad Prism® software. Trolox (shown in blue in **Figure 4.6**) was the control because it is known for its potent antioxidant/free radical scavenging properties (Lúcio *et al.*, 2009; Hamad, 2010). Each of the test compounds had poor radical scavenging activity which resulted in compounds **1A, 1B, 1C, 2A, 2C** and **3A** not showing more than 50% scavenging ability at the maximum concentration tested (1000 µM). Compound **2B** (IC_{50} = 127.9 µM) had the best activity of all test compounds but relative to Trolox (IC_{50} =17.12 µM) the activity was 7.5 times weaker.

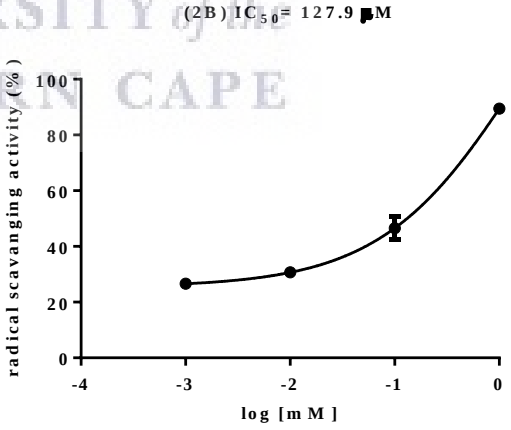












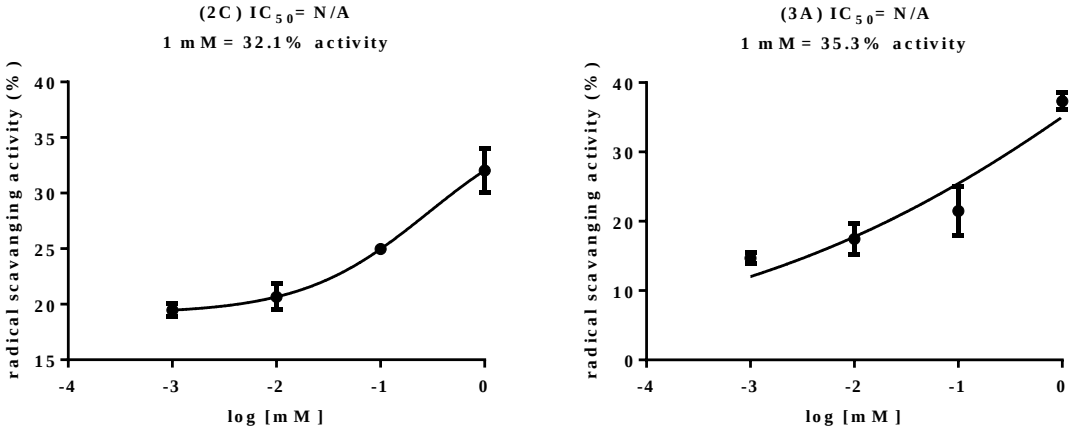


Figure 4.6: Nonlinear regression graphs of free radical scavenging of test compounds and Trolox. IC50 concentrations are given and the p-value for each graph is below 0.05 ($p < 0.05$)**.**

4.8. Conclusion

In silico studies on test compounds using Molecular Operating Environment was performed to compute binding interactions between the compounds and both hMAO isoenzymes, predicting compound-binding interactions with hMAO enzymes. After analyzing the computed compounds, interactions that are significant for receptor binding were observed. The compounds were then assessed for cytotoxic effects, neuroprotection, hMAO-A/ -B and free radical scavenging activity. Three different concentration (10 µM, 50 µM and 100 µM) to every compound was used in the cytotoxicity assay. At 10 µM concentration, the compounds were non-toxic with an exception of compound **3A** that was cytotoxic at three tested concentrations.

Compound **2B** strongly reinforce the feasibility of MTDL that could be used in slowing or halting PD and other NDs. Compound **2B** was found to the most effective lead compound of all the synthesized because it is the least toxic compound that even encourages cell proliferation, has the best neuroprotective activity and exhibits good towards MAO-A/B inhibition. However, compounds **1A, 1B, 2A** and **2B** should be studied further because they have good neuroprotective activity, are not toxic and have MAO-A/B inhibition activity that is low (below12.02 µM) which could help in finding new therapies for ND and increase natural memory processes as demonstrated by other dual inhibitors (Marco-contelles *et al.*, 2016). MAO-A inhibition has been linked to negative side effect profiles (cheese effect) which can result in a hypertensive crisis (Pickar *et al.*, 1981). The compounds (**1A, 1B, 2A** and **2B**) might raise concerns since they inhibit MAO-A, therefore, the same caution on patients taking MAO-

A inhibitors of not taking tyramine rich foods such as cheese is still applicable (Pickar *et al.*, 1981; Marco-contelles *et al.*, 2016). The biological results are summarized in **Table 4.4**.

Table 4.4: Results of all biological assay conducted. The compounds in blue (clorgyline, rasagiline and Trolox) are controls for hMAO-A, hMAO-B and DPPH assay, respectively. For cytotoxicity assay and neuroprotection (MPP+ induced), the control for percentage cell survival was set at 100%.

Compound	MAO-A IC_{50} (µM)	MAO-B IC_{50} (µM)	DPPH activity at 1 mM (%)	Cytotoxicity at 10 µM (%)	Neuroprotection at 10 µM (%)
Clorgyline	0,00074	N/A	N/A	N/A	N/A
Rasagiline	N/A	0.22	N/A	N/A	N/A
Trolox	N/A	N/A	79.5	N/A	N/A
1A	1.10	0.91	23.0	96.65	72.78
1B	0.33	12.02	27,2	99.57	69.81
1C	6.61	33.11	32.2	99.40	78.93
2A	5.75	9.55	19.7	100.04	72.99
2B	6.91	5.25	89.5	115.49	80.37
2C	19.05	3.02	32.1	112.96	71.14
3A	>100	9.12	35.3	63.81	74.78

Chapter 5

5. Summary and conclusion

5.1. Introduction

Neurodegenerative disorders are a group of disorders that causes loss of function or death of the neuronal network of the brain (Przedborski *et al.*, 2003). Global recognition of NDs is necessary because the burden of these diseases is on the rise even in Sub-Saharan Africa where communicable diseases such as malaria, tuberculosis and HIV dominate (Gouda *et al.*, 2019). NDs do not have any cure and treatment is based on symptomatic relief. PD is one of the ND that is characterised by loss of dopaminergic neurones and its treatment includes, but is not limited to monoamine oxidase inhibitors and antioxidants (Halliwell, 2007; Youdim *et al.*, 2006). The complex pathophysiology of NDs has led researchers to turn to the multitarget directed ligand (MTDL) approach to prolong or halt the development of these disorders.

In this study, three different groups namely; chalcone- or curcumin derived-, indole- and propargylamine groups, have been combined to produce MTDLs that could be used to tackle NDs. The chalcone derived- or curcumin-derived group was added to the MTDL for antioxidant activity. The indole group acts as a scaffold to which the other functional groups can be added and the indole scaffold has been reported in several studies as having monoamine oxidase activity. The propargylamine moiety has a neuroprotective activity which has been proven in drugs such as selegiline, rasagiline and ladostigil (Weinreb *et al.*, 2009, 2012; Bar-am *et al.*, 2015).

5.2. Synthesis

The curcumin-derived indole ester and chalcone derived indole hybrids were synthesized and the indole moiety acted as a scaffold for the compounds. For the chalcone derived series (compounds **1A, 1B** and **1C**), substituted ketones were conjugated with indole-5-carboxaldehyde by a Claisen–Schmidt condensation reaction using catalytic base. The substituents on the ketones were varied so that the effect of groups like hydroxyl or methoxy could be investigated to establish if they enhance the activity of the hybrid compounds. The propargyl moiety was conjugated at the *N*-atom of the indole scaffold by an S_N2 nucleophilic reaction (**2A, 2B** and **2C**). One curcumin-derived indole ester compound (**3A**) was synthesized by reacting cinnamic acid and 6-hydroxy-indole in a two-step reaction. The first step was the

formation of an activated acid halide by reacting cinnamic acid with $SOCl_2$. The activated acid halide was then reacted with 6-hydroxy-indole for 2 hours at room temperature in acetonitrile (10 ml) and K_2CO_3 (1.2 mmol) to give the final cinnamic acid- indole ester.

Column chromatography was used to purify all synthesized compounds while NMR, HRMS and IR spectra were for structural elucidation. Seven hybrid compounds were synthesised with yields ranging from 9% to 77.2% and their biological activity was evaluated.

In silico studies on the novel, compounds were carried out using Molecular Operating Environment® software to assess the compound's binding interactions inside the active sites of both MAO isoenzymes. Based on the docking simulations the novel compounds appeared to fit well inside the active sites and interacted with important conserved amino acids of hMAO-A and hMAO-B. Compounds **2B, 2A, 1C, 1B** and **1A** interacted with at least one of the important amino acid (Phe-A208 or Glu-A215) responsible for the moulding of hMAO-A binding site (Veselovsky *et al*., 2004). Four compounds (**2C, 2A, 1C** and **1A)** formed interactions with residue lle-199, which is crucial for the systemic determination of substrate and inhibitor recognition in the hMAO-B enzyme (Hubálek *et al*., 2005b).

5.3. Biological evaluation

The MTT assay, which is colourimetric based assay, and human neuroblastoma SH-SY5Y cells were used to screen the cytotoxic activity of compounds at three concentrations (100 µM, 50 µM and 10 µM). In overall, all compounds appeared to be cytotoxic at high concentrations (100 µM and 50 µM) except for compounds **2B**. At a concentration of 10 µM, the chalcone-derived compounds **2C, 2B, 2A, 1C, 1B** and **1A** proved to be non-toxic and the percentage cell viability in comparison to cells untreated (set at 100%) varied from 96.7% to 115.5%. The propargylamine-containing compounds (**2A, 2B** and **2C**) seemed to enhance cell proliferation with percentage cell viability compared to untreated cells ranging from 100.0% to 115.5% at a concentration of 10 µM compared to their precursor (**1A, 1B** and **1C**) which ranged from 96.6% to 99.6%. Compound **2B** had the best percentage cell viability relative to untreated cells of 115.5%. Compound **3A** which is curcumin-derived indole ester was toxic at all concentrations with the percentage cell viability relative to untreated cells ranging from 63.8% to 72.0% across the three different concentrations tested.

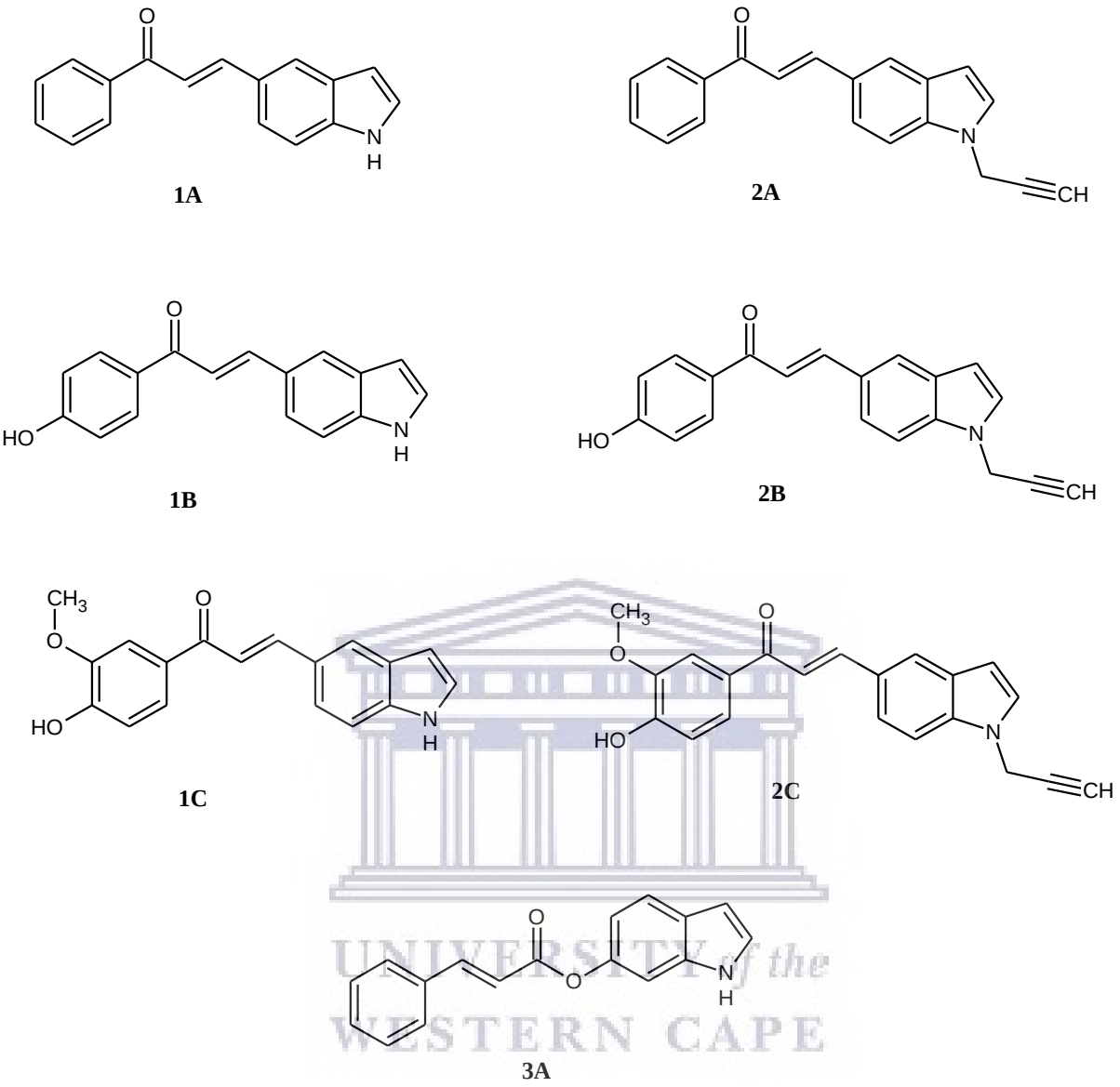


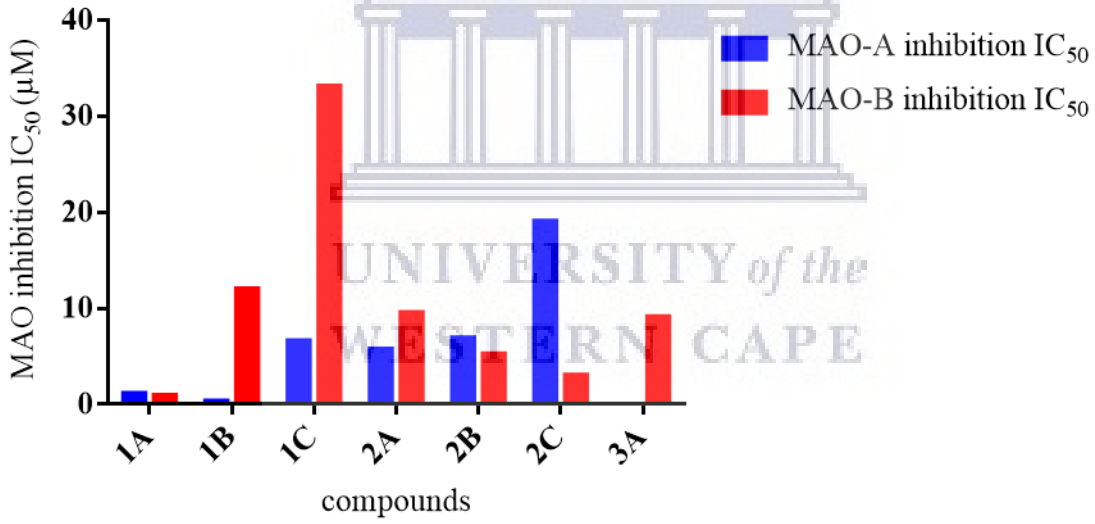
Figure 5.1: Compounds accessed for biological evaluation.

The compounds were also subjected to a neuroprotection assay in which neurodegeneration was induced by the addition of the neurotoxin, MPP^+. The assay was conducted at 10 µM of each compound because this was the concentration deemed non-toxic from the cytotoxicity assay. The findings are reported as percentages and DMSO-treated cells (control) were fixed to 100%. The cells that only had 2 mM MPP^+ had a 40% cell survival. The survival of human neuroblastoma SH-SY5Y cells containing both test compound and 2 mM of MPP^+ varies from 69.81% to 80.37%. Compound **2B** had the best neuroprotective activity with a cell viability mean of 80.37%.

In vitro studies to determine the synthesized compounds' inhibitory capacity on both human recombinant MAO isozymes were also conducted. Generally, the synthesized compounds were

active in inhibiting the hMAO-A enzyme with the IC_{50} concentrations varies from 0.33 µM to 19.05 µM. Compound **3A** which is a curcumin-derived indole ester hybrid was the only exception with an IC_{50} value of more than 100 µM. The addition of the propargylamine moiety (compounds **2A, 2B** and **2C**) did not seem to improve the compounds' hMAO-A inhibition, because the IC_{50} values were higher compared to their precursor compounds (**1A, 1B** and **1C**). Compounds **1A, 1B** and **1C**, that varied at para and meta substituents on the benzene moiety of the hybrid compound showed that para hydroxyl substitution enhances the activity of the hybrid. For **1B** (IC_{50} = 0.33 µM), a para-substituted hydroxyl derivative had the best hMAO-A inhibition activity but was not in a similar range as the control, clorgyline (IC_{50} = 0.74 nM). Compound **1B** only formed hydrogen bonds with amino acid residue Gln-A215 and this residue is significant in the moulding of MAO-A active site (Veselovsky *et al.*, 2004). **Graph 5.1** summarizes hMAO-A and -B inhibition by the synthesized compounds.

Graph 5.1: Monoamine oxidase inhibition



The compounds showed significant hMAO-B inhibition with IC_{50} concentrations varying from 0.91 µM to 33.11 µM. Compounds with the propargylamine group had better activity (IC_{50}= 5.75 for **2B** and IC_{50}= 3.02 for **2C**) when compared with the precursor compounds (IC_{50}= 12.02 for **1B** and IC_{50}= 33.11 for **1C**). Compound **1A** (IC_{50} = 0.91 µM) had the best activity that is comparable with control, rasagiline (IC_{50} = 0.22 µM). *In silico* studies showed that 1A forms hydrogen bonds with amino acid residue IIe 199 which is crucial as a systemic indicator for substrate and inhibitor recognition, had potential interactions with Gln 206 and Leu 171 amino acid residues which have been reported to be important for substrate activity (Hubálek *et al.*, 2005a). The unsubstituted curcumin-derived indole ester hybrid (**3A**) has weaker activity when

compared to compound **1A** which is a chalcone derivative with no substituents on the hybrid. Compounds **1A, 2B** and **2C** were relatively non-selective with SI values for hMAO-B inhibition of 1.21, 1.32 and 6.31 respectively. Dual inhibition of MAO has been reported by Delumeau *et al* (1994) to be more effective at curbing NDs.

An antioxidant assay was carried out on the synthesized compounds to determine the compounds' effectiveness in the prevention of oxidation caused by free radicals. The DPPH assay was used and the compounds proved to have poor free radical scavenging activity with IC_{50} values above 100 µM. Compound **2B** had the best anti-oxidant activity with an IC_{50} concentration of 127.9 µM, but when compared to the control, Trolox (IC_{50} = 17.12 µM), the activity is not considered significant.

5.4. Conclusion

The multifactorial nature of NDs have made the treatment of these disorders difficult with single therapy drugs, this, therefore, prompted researchers to turn to MTDL strategies. MAO enzymes are key targets known to be implicated in the development of Parkinson's disease and other NDs. The research's objective was to design and synthesize MTDLs that can delay or stop the progression of the NDs. The synthesized compounds were to be evaluated using four biological assays which included the cytotoxicity assay, neuroprotection assay, MAO assay and antioxidant assay. A total of seven final compounds were designed, synthesized and biologically assessed for activity. The use of *in silico* studies was important in determining the types of possible interactions formed inside the MAO active site cavities by the compounds. In general, the compounds were non-toxic, except for compound **3A** at higher concentrations, and the neuroprotection assay showed that the compounds have a favourable neuroprotective ability. The compounds were also shown to exhibit significant MAO inhibitory properties within the micromolar range. The compounds, in general, showed poor antioxidant activities in the DPPH assay.

The synthesized compounds should be tested using several *in vitro* biological assays to explore their mechanism(s) of neuroprotection in future studies. Furthermore, pharmacokinetic studies to determine if the compounds can cross the blood-brain barrier should also be conducted in future. *In vivo* animal studies, would also improve the current understanding of the neuroprotective abilities of these compounds. The study findings have led to strengthened

confidence in developing agents for NDs and encourages the development of effective selective inhibitors, on specific multiple targets, to avoid adverse effects of non-selective inhibition.

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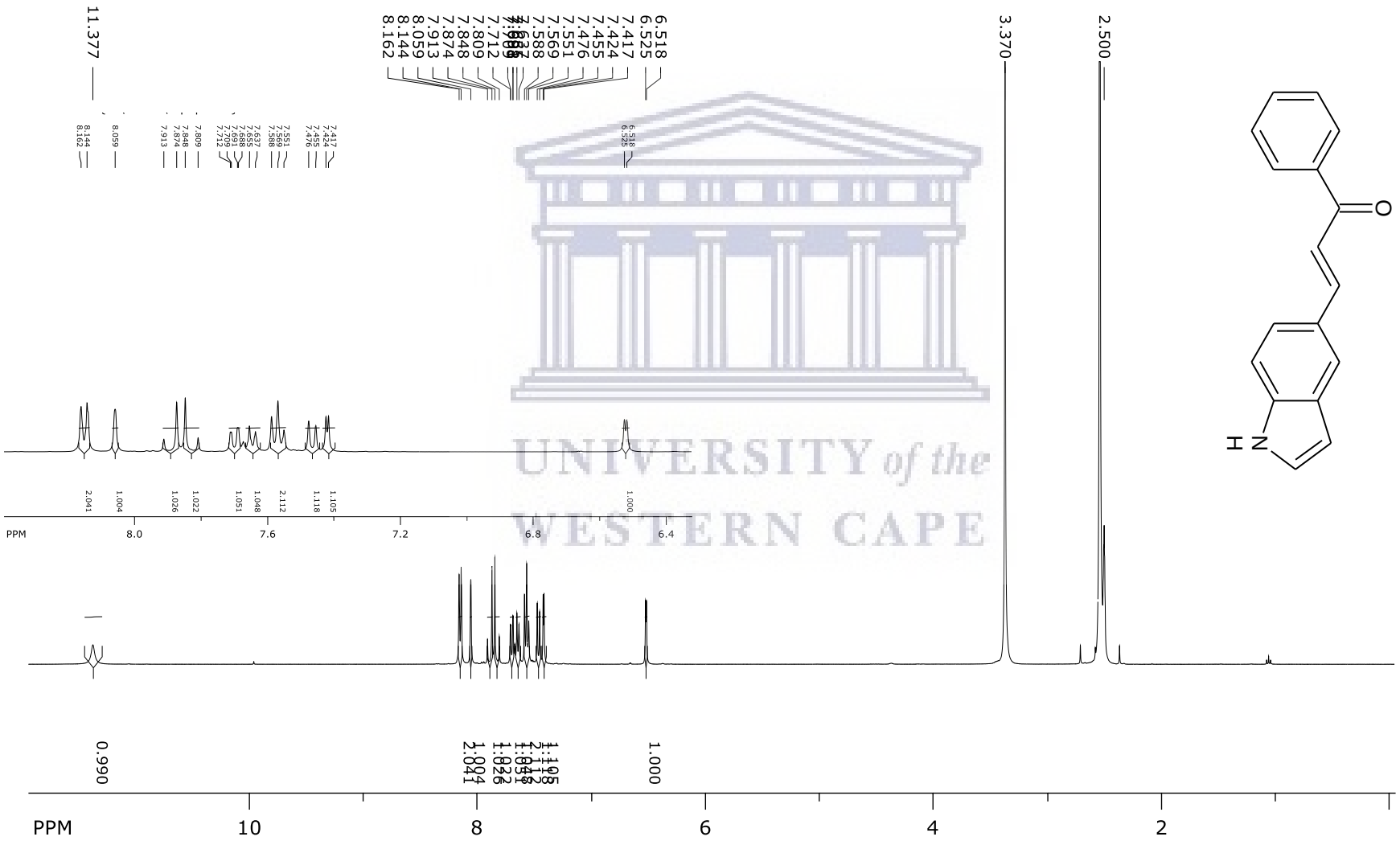
Annexure: Spectral data

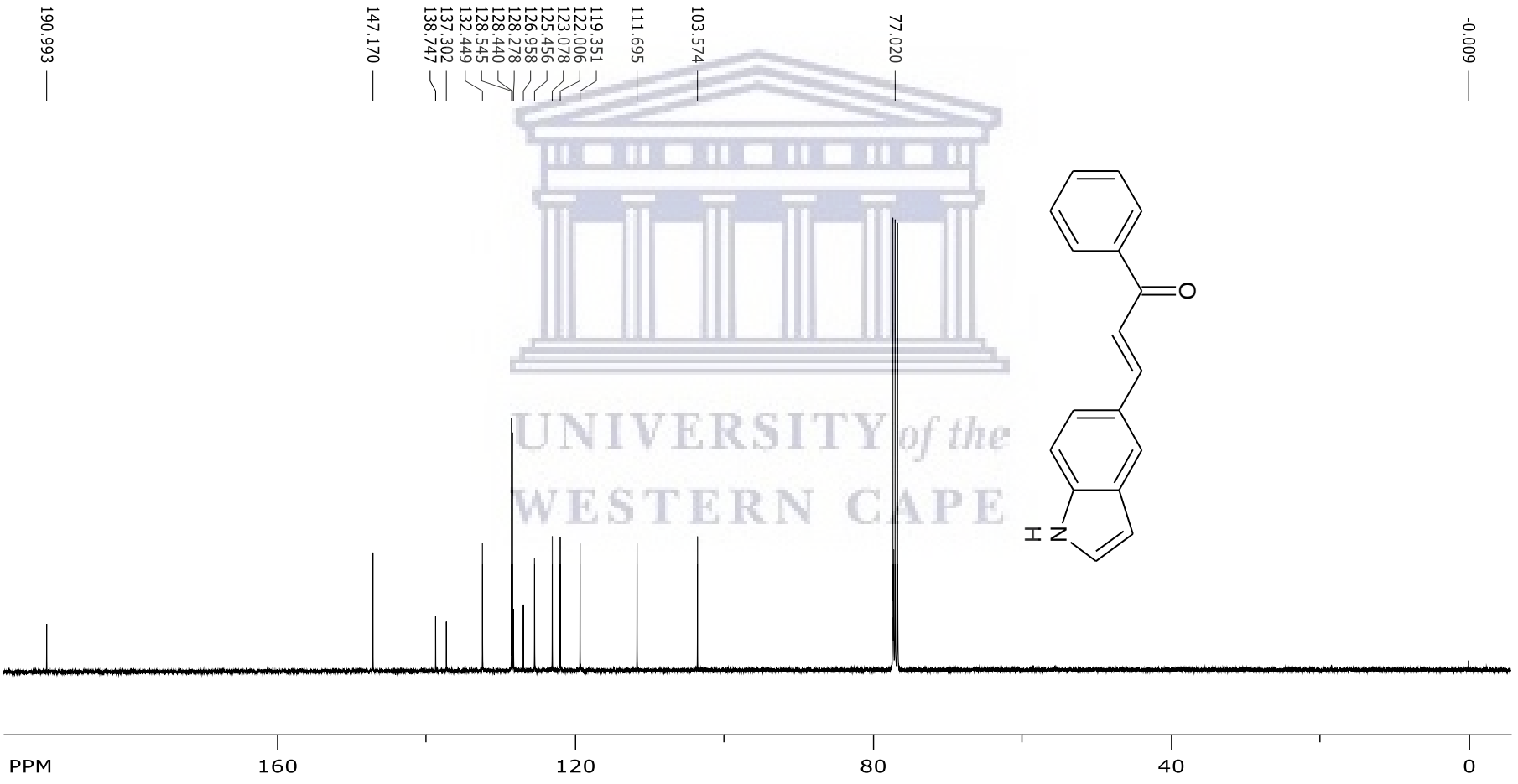
^{1}H NMR, ^{13}C NMR, IR and M

Spectrum 1: ^{1}H NMR Compound **1A**

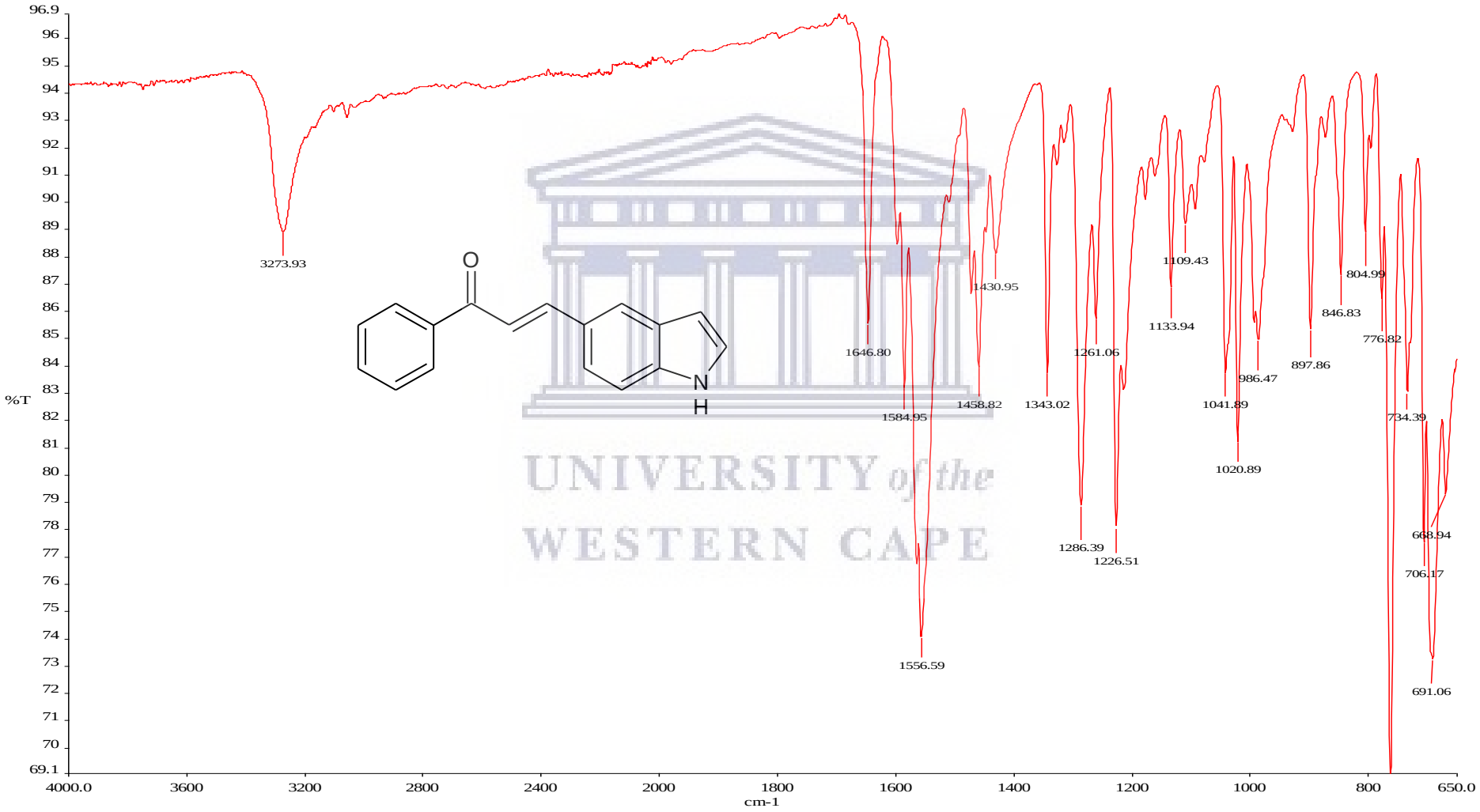
SpinWorks 4:
PROTON DMSO {C:\Bruker\TopSpin3.2} JJ-LovetoneMusakwa 14



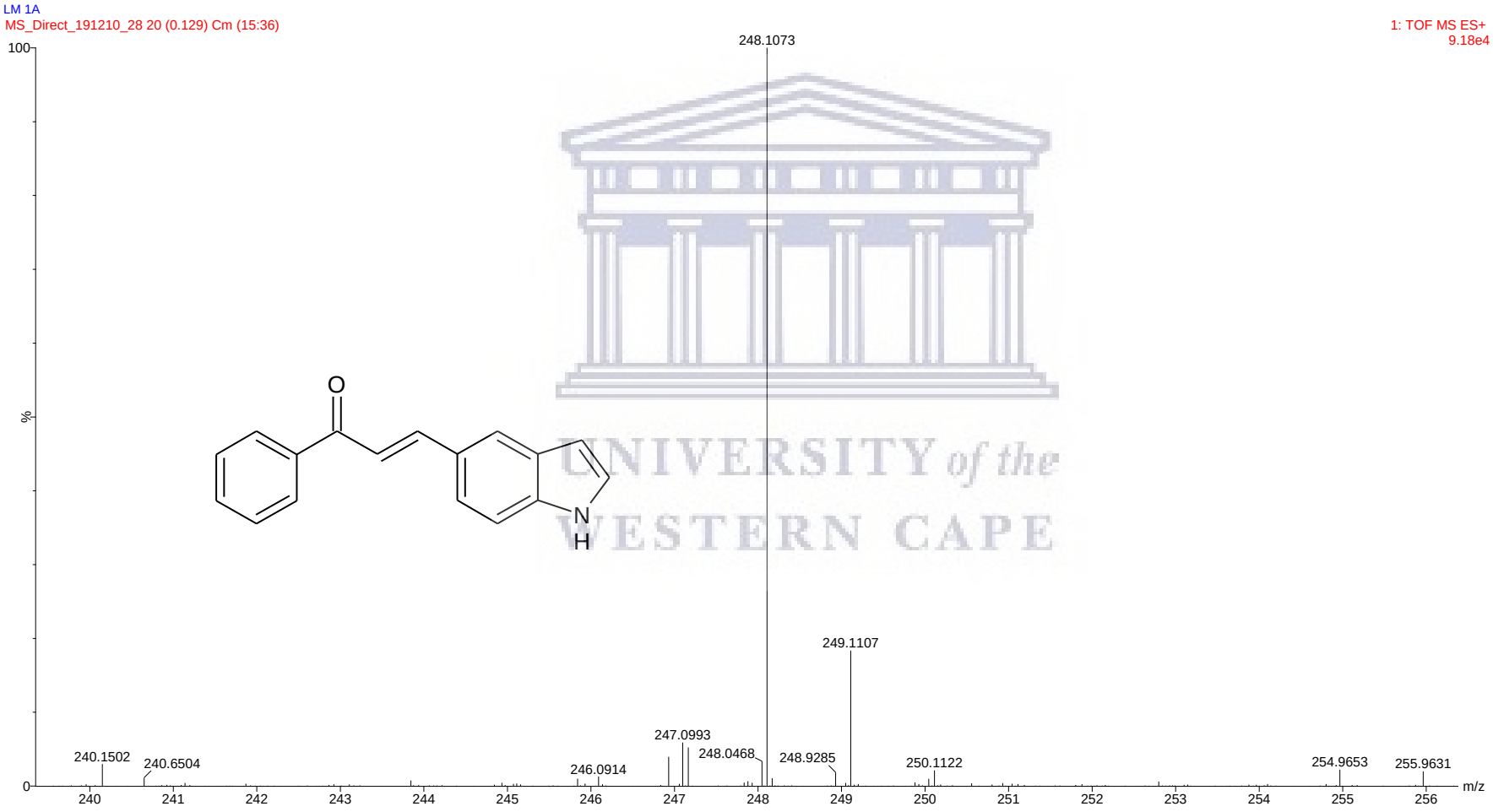
Spectrum 2: ^{13}C NMR Compound **1A**



Spectrum 3: IR compound **1A**



Spectrum 4: MS compound **1A**



Spectrum 5: ^{1}H NMR Compound **1B**

SpinWorks 4:
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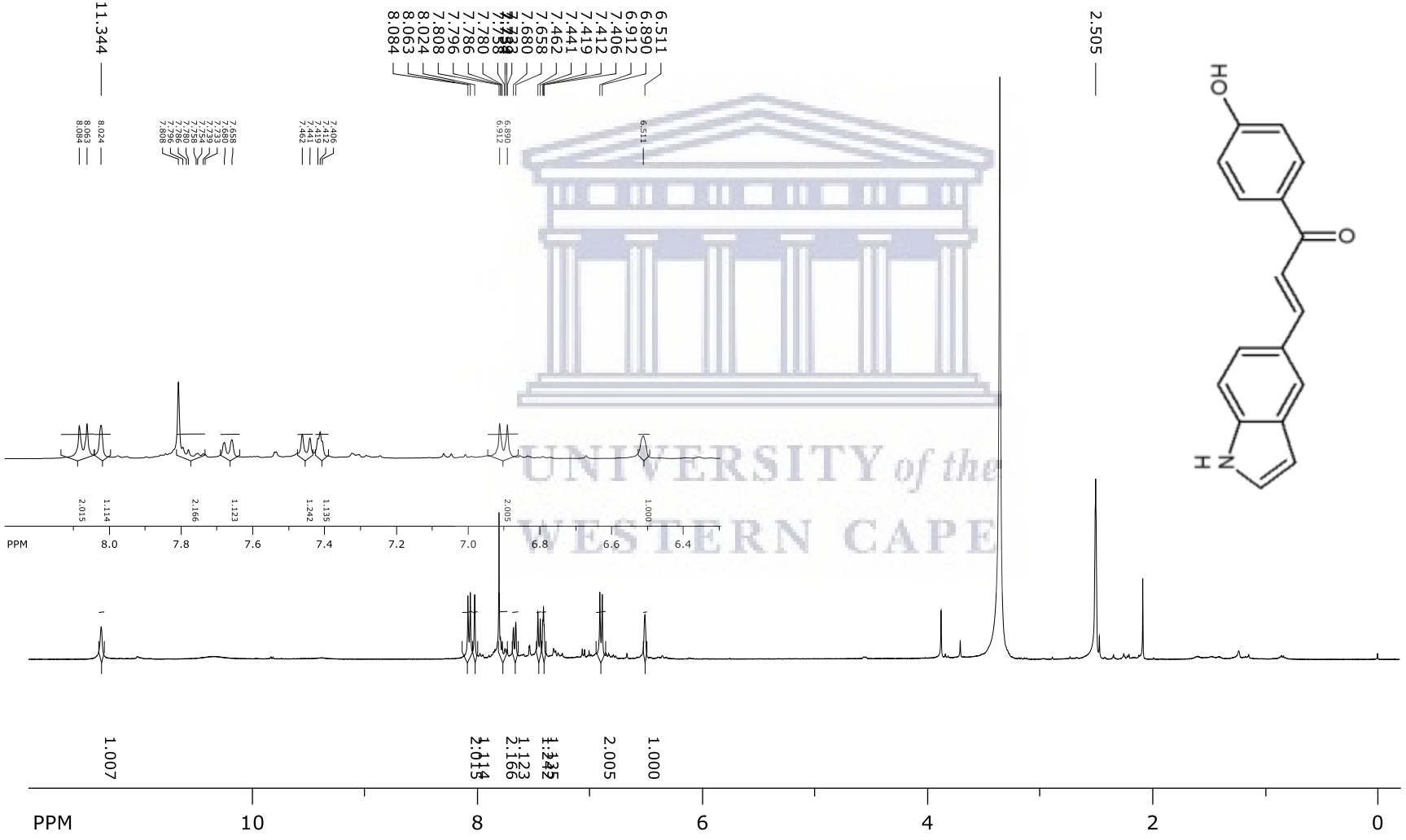
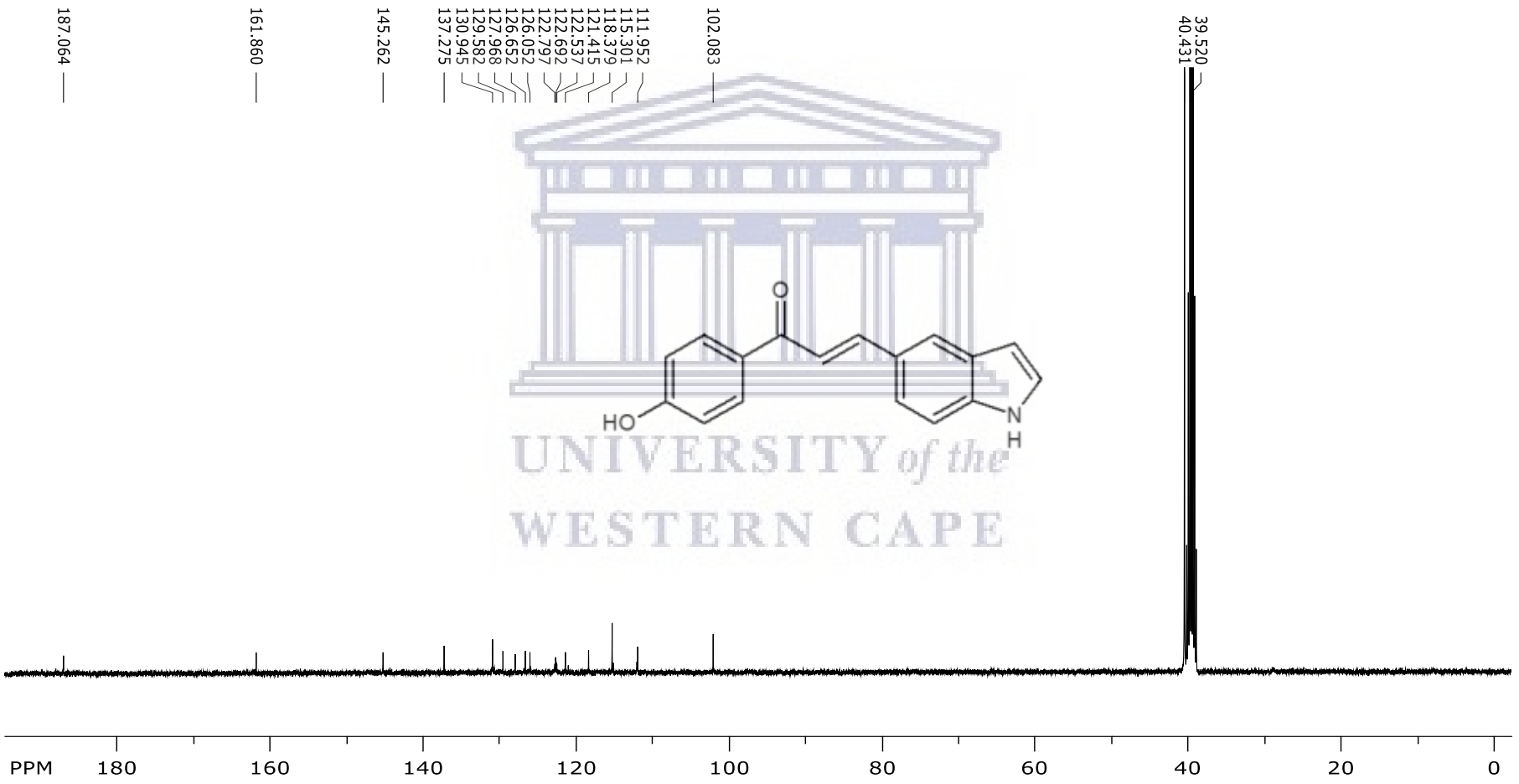
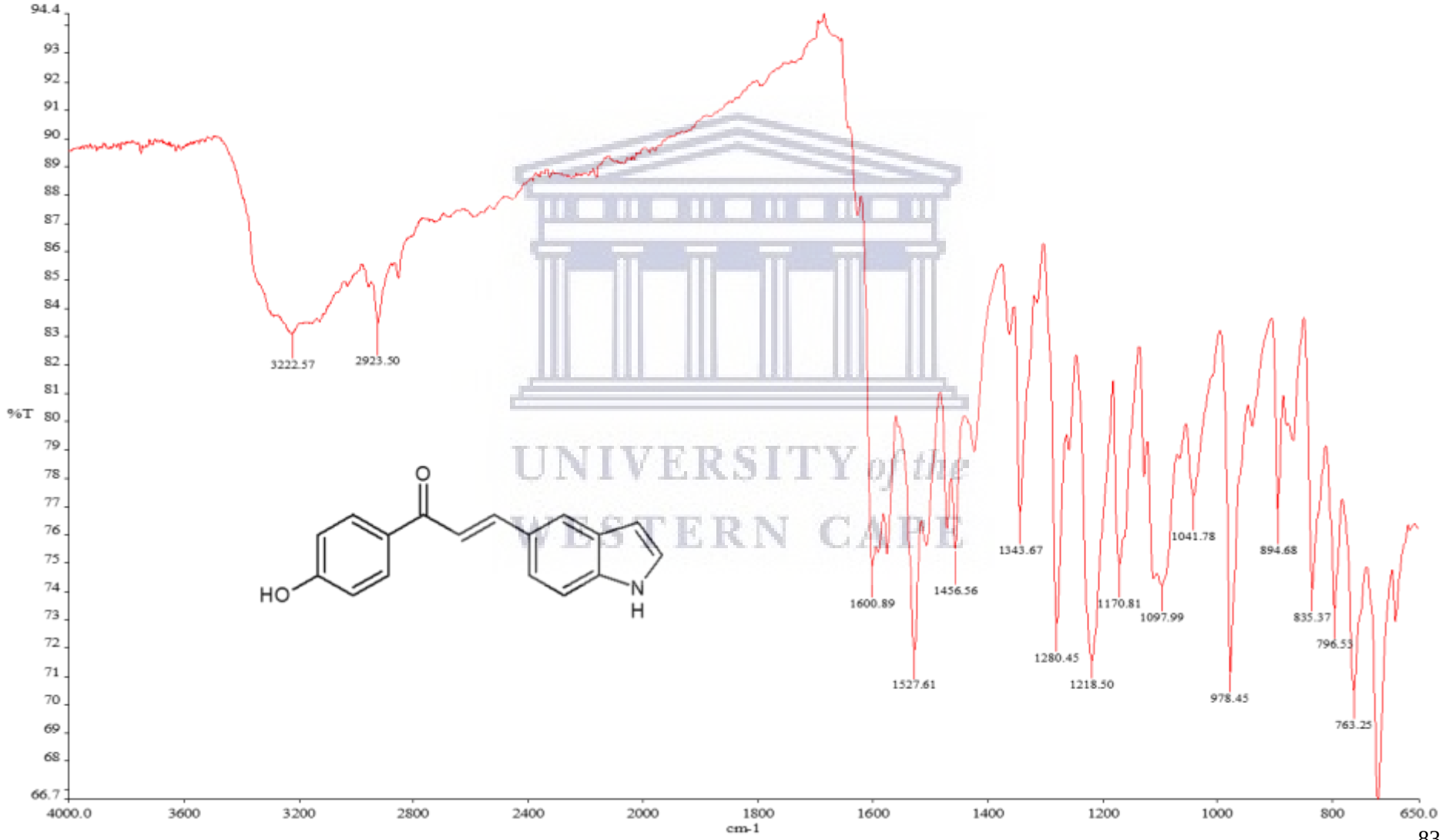


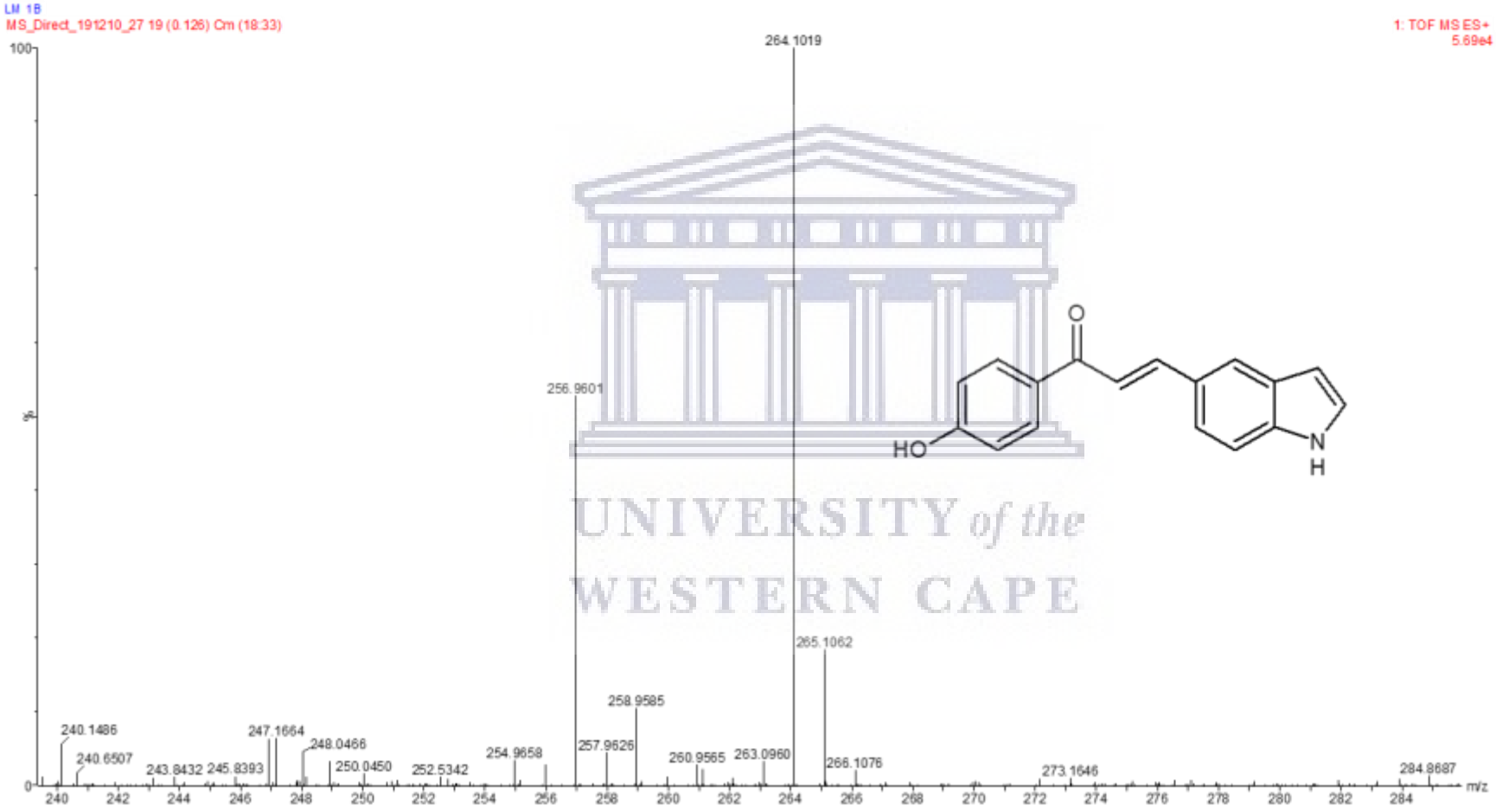
Spectrum 6: ^{13}C NMR Compound **1B**



Spectrum 7: IR Compound **1B**



Spectrum 8: MS Compound **1B**



Spectrum 9: ^{1}H NMR Compound **1C**

SpinWorks 4:
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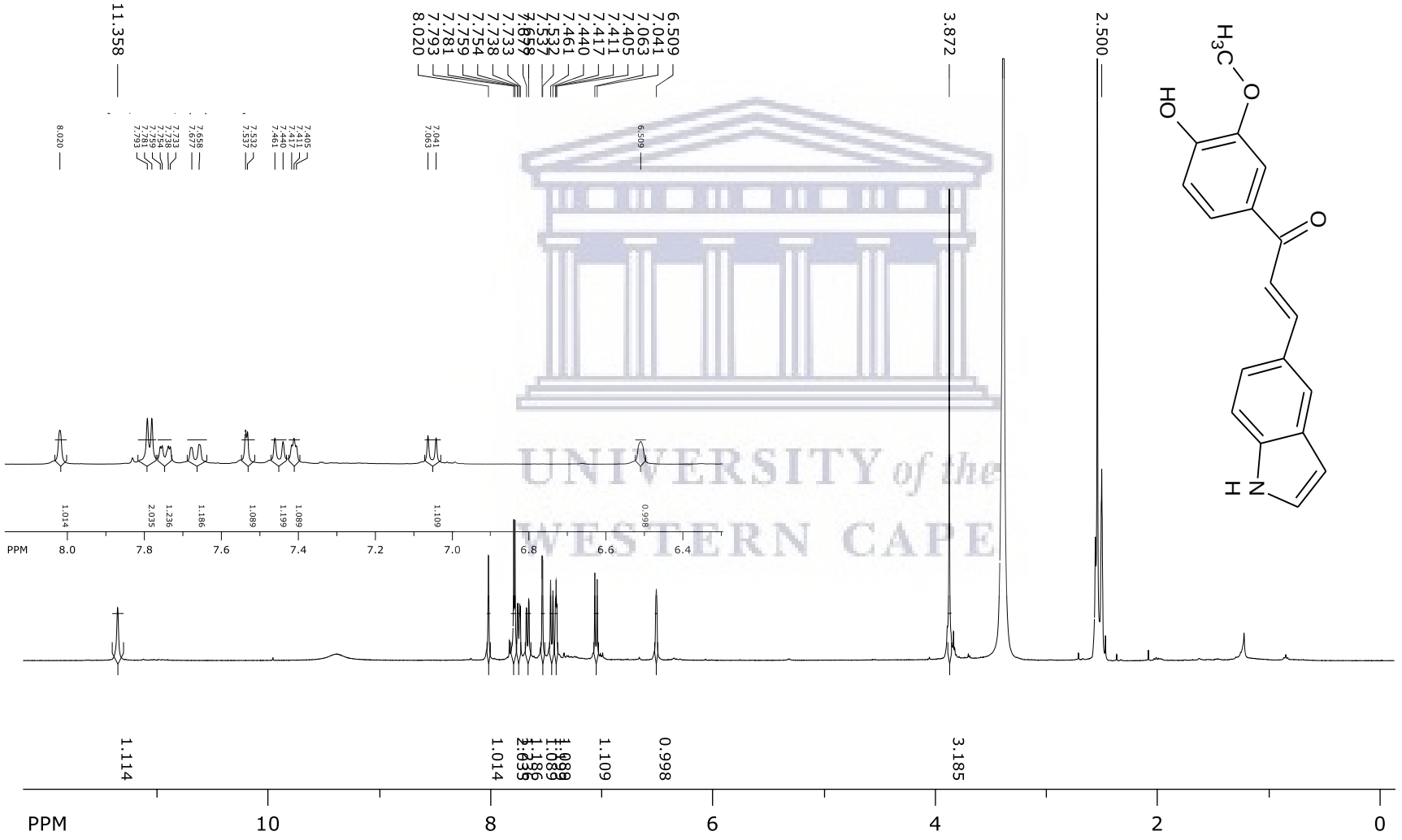
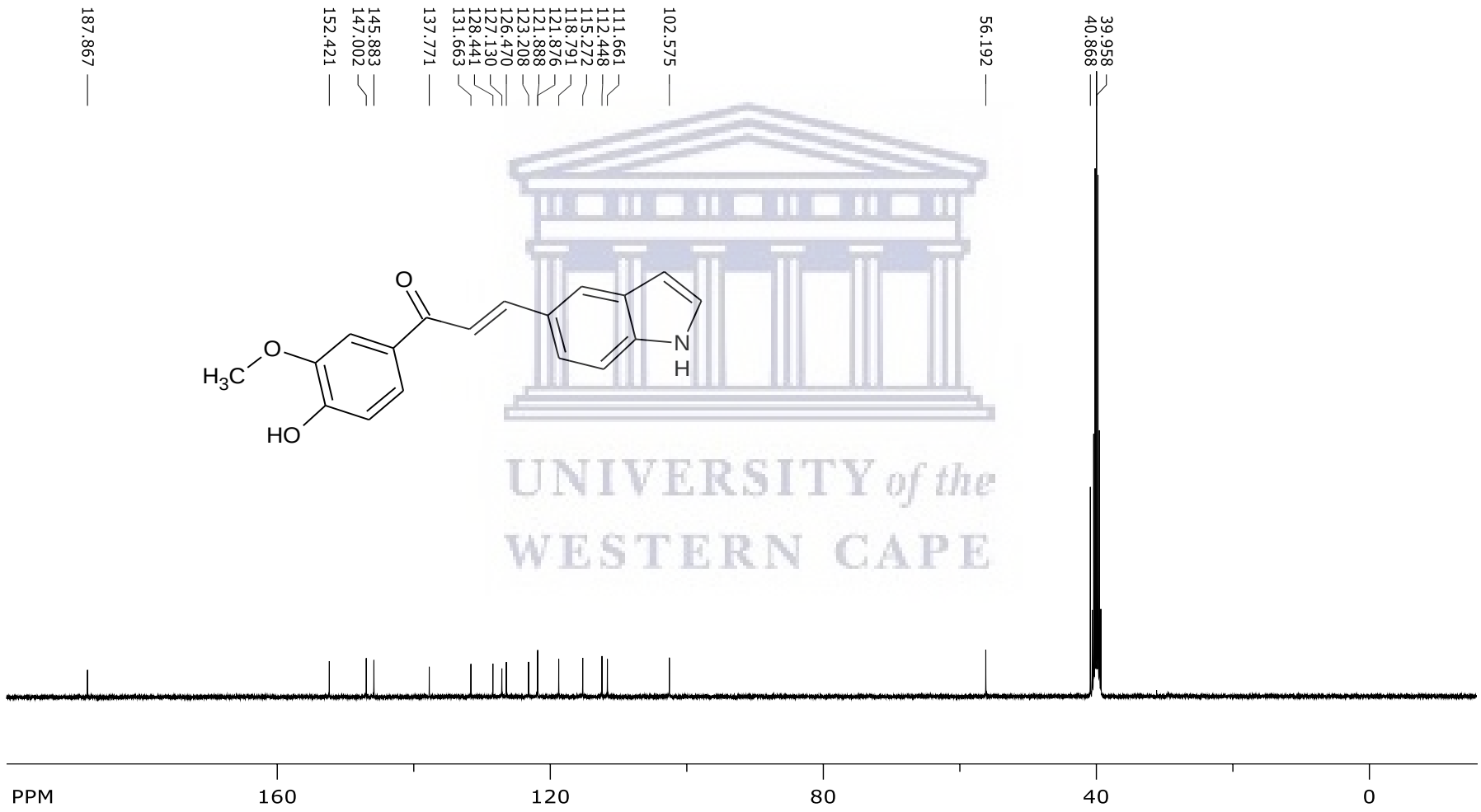
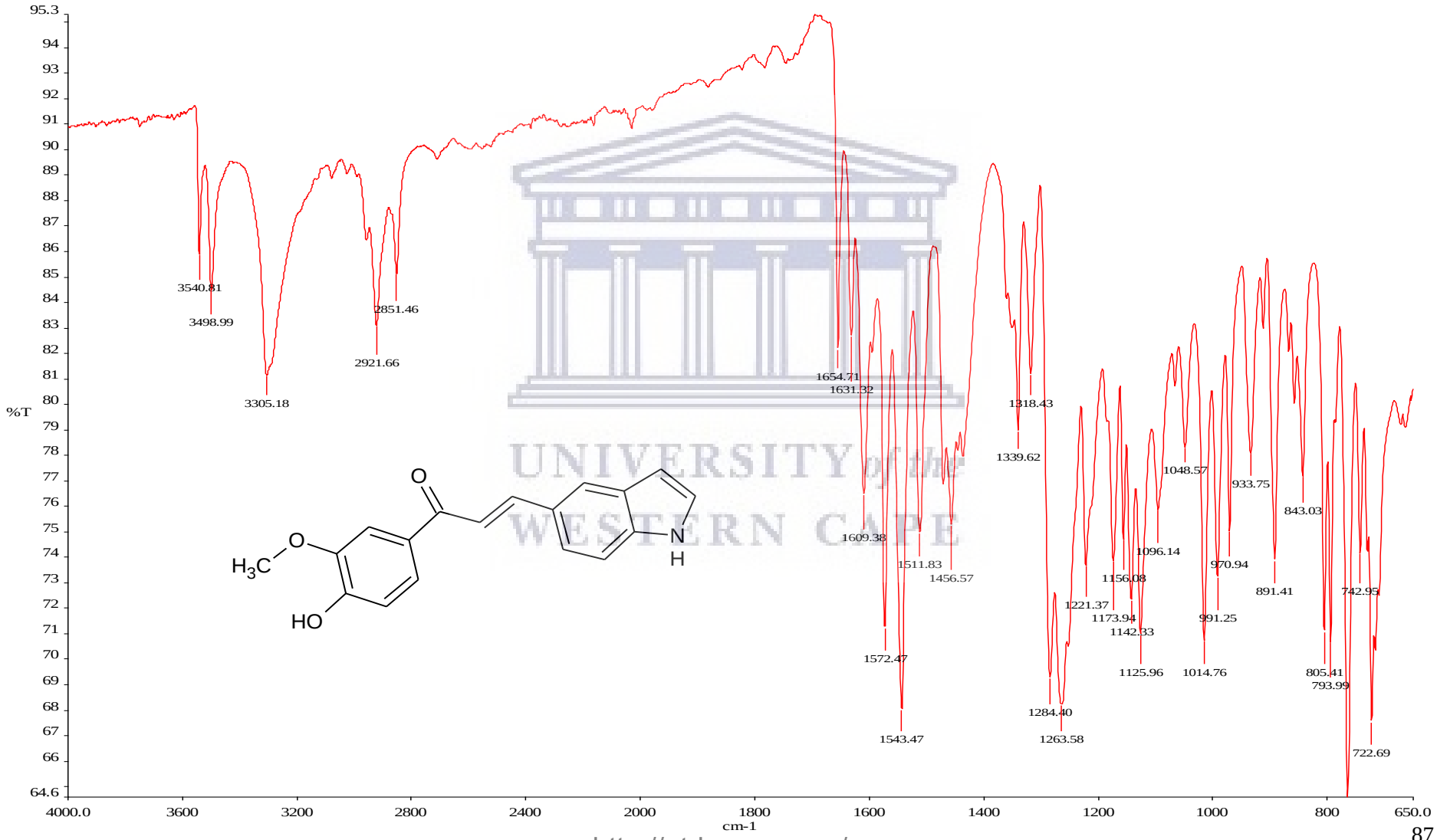


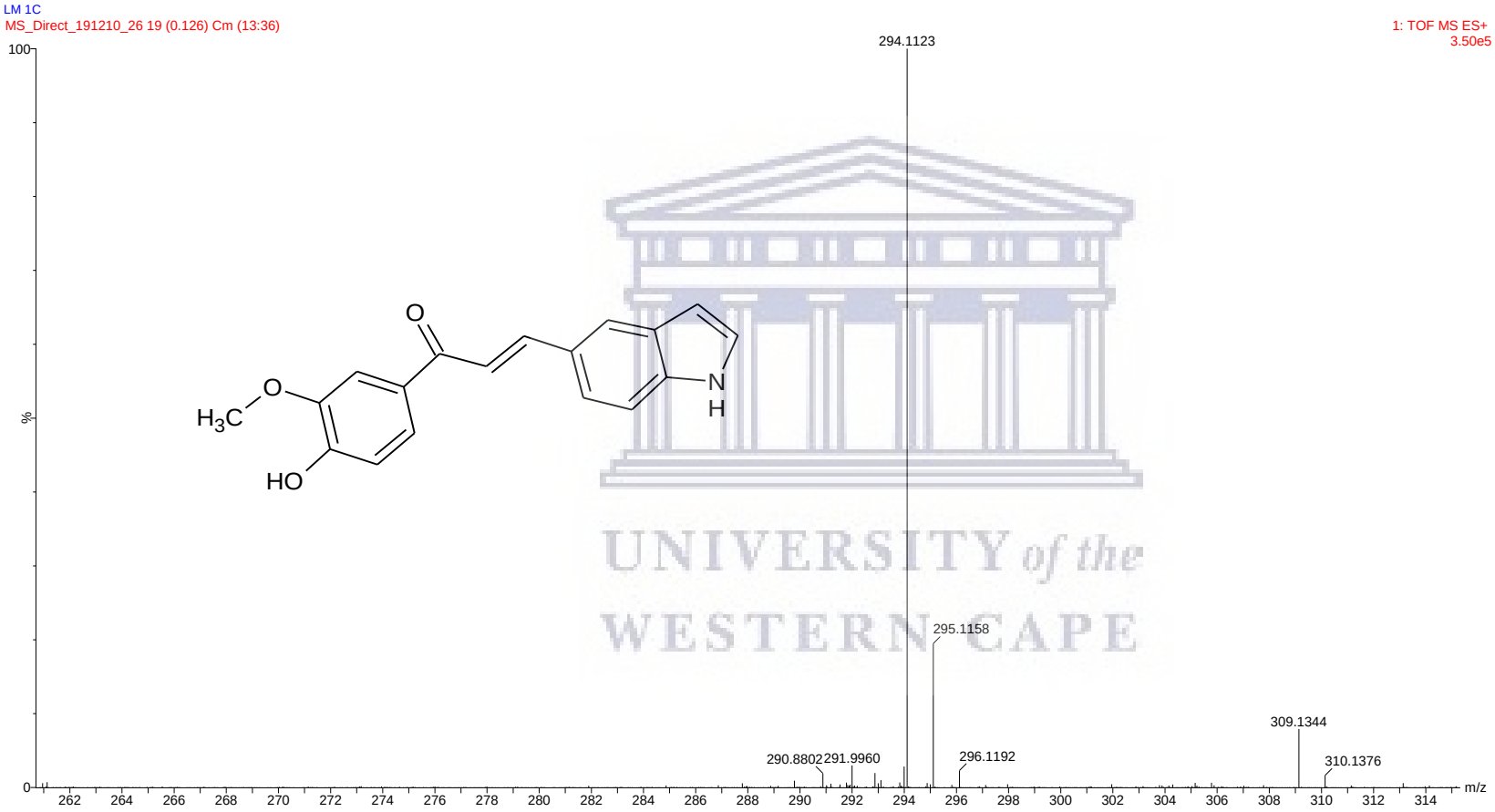
Spectrum 10: ^{13}C NMR Compound **1C**



Spectrum 11: IR Compound **1C**

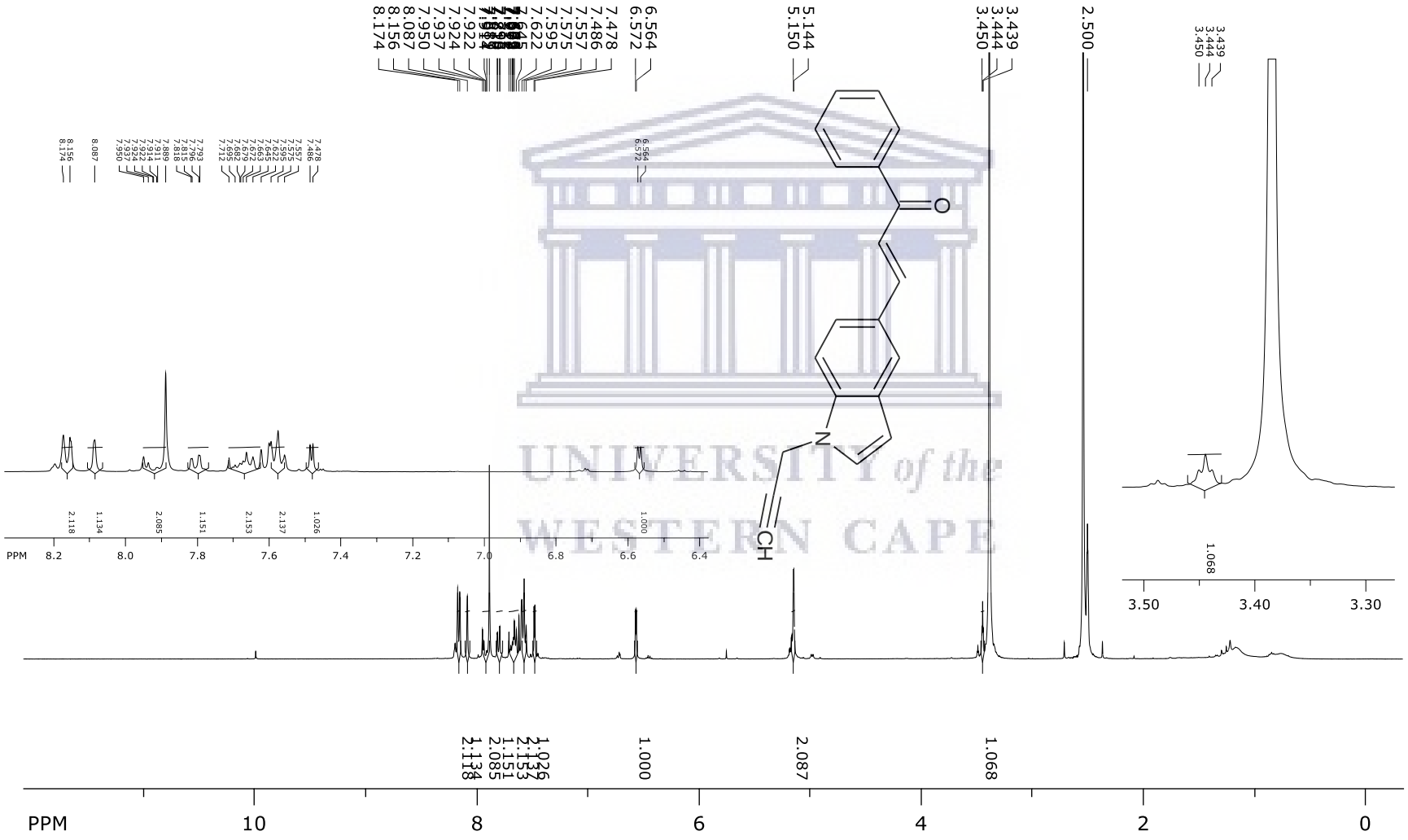


Spectrum 12: MS Compound **1C**

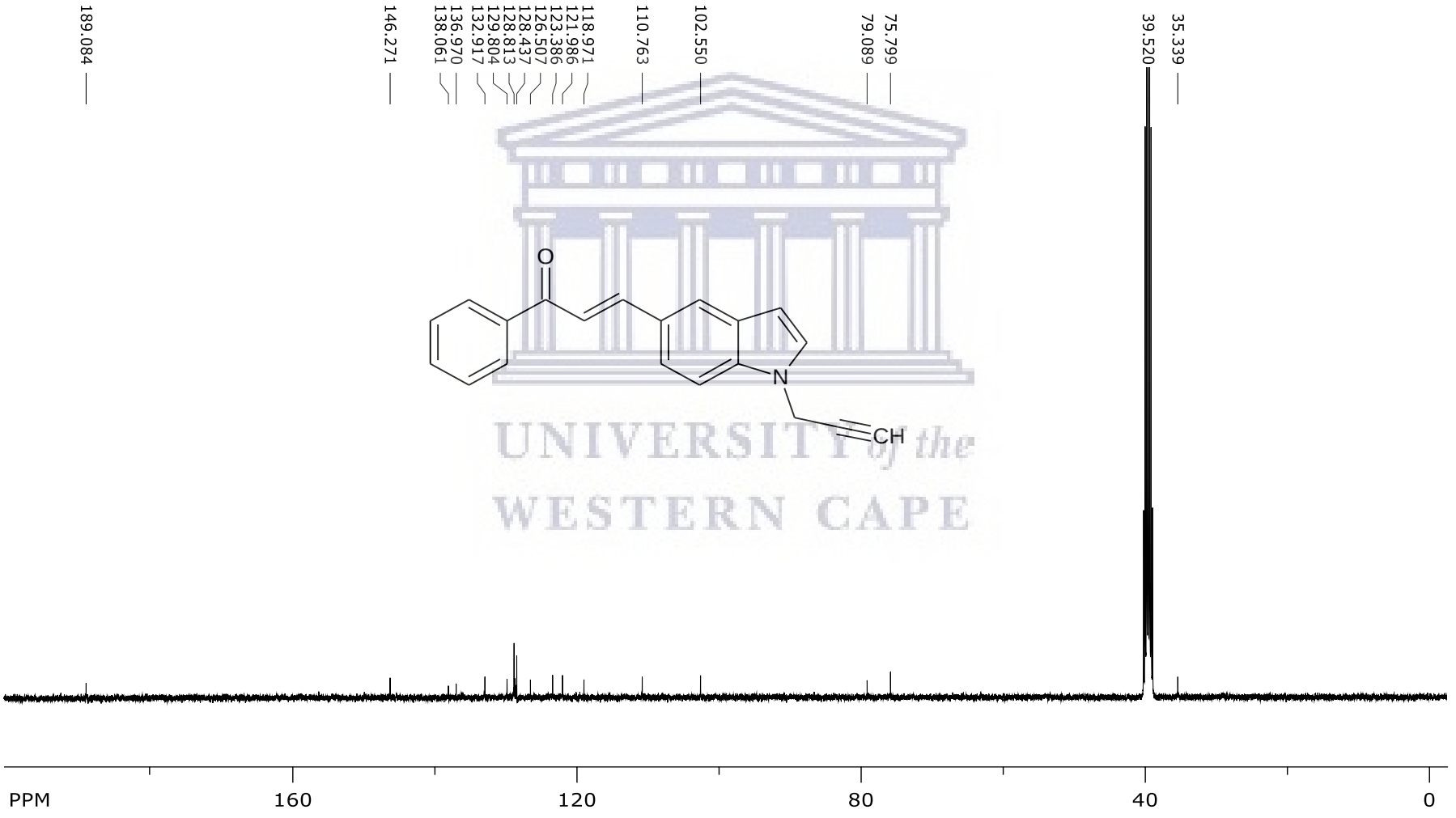


Spectrum 13: ^{1}H NMR Compound **2A**

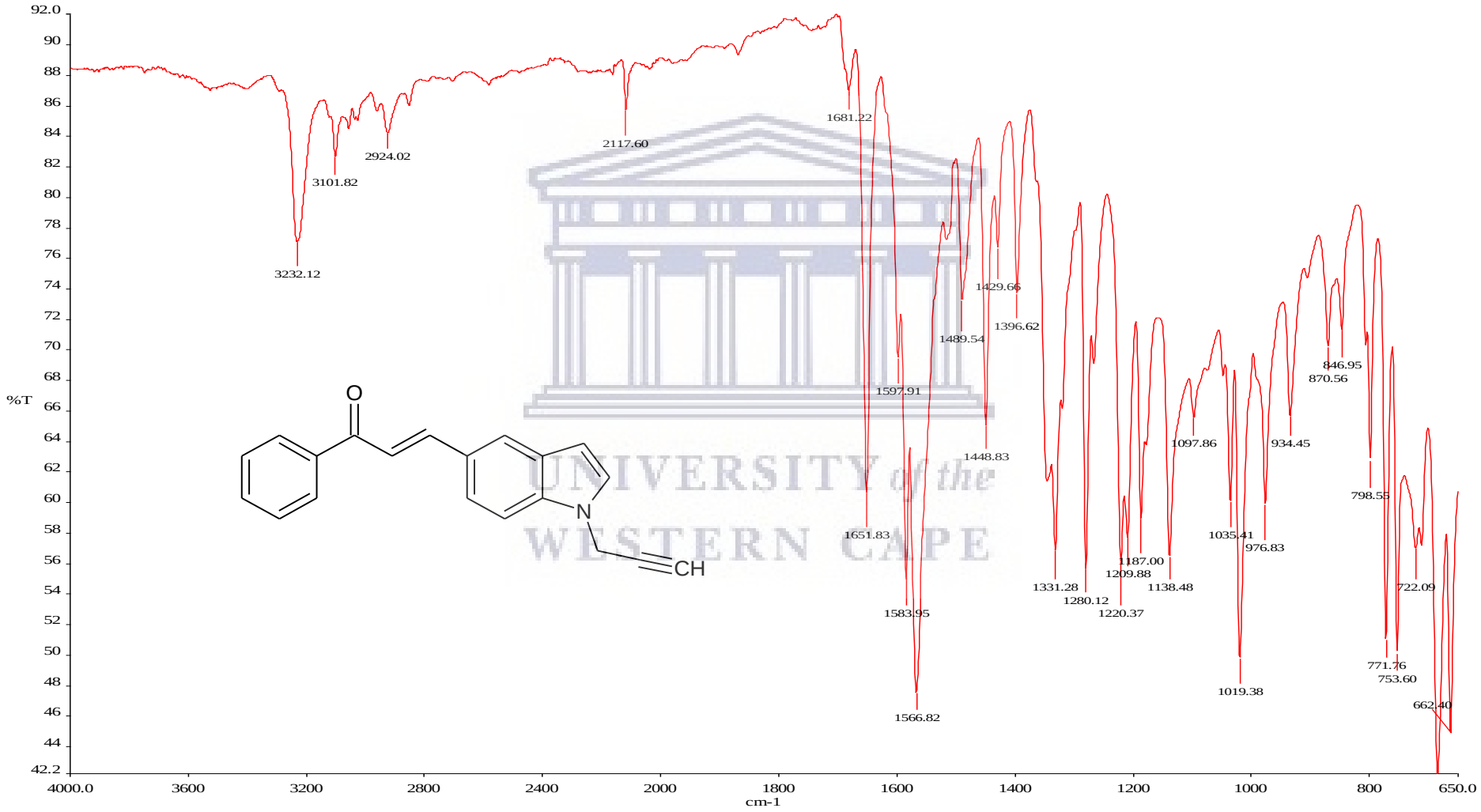
SpinWorks 4:
PROTON DMSO {C:\Bruker\TopSpin3.2} JJ-LovetoneMusakwa 1



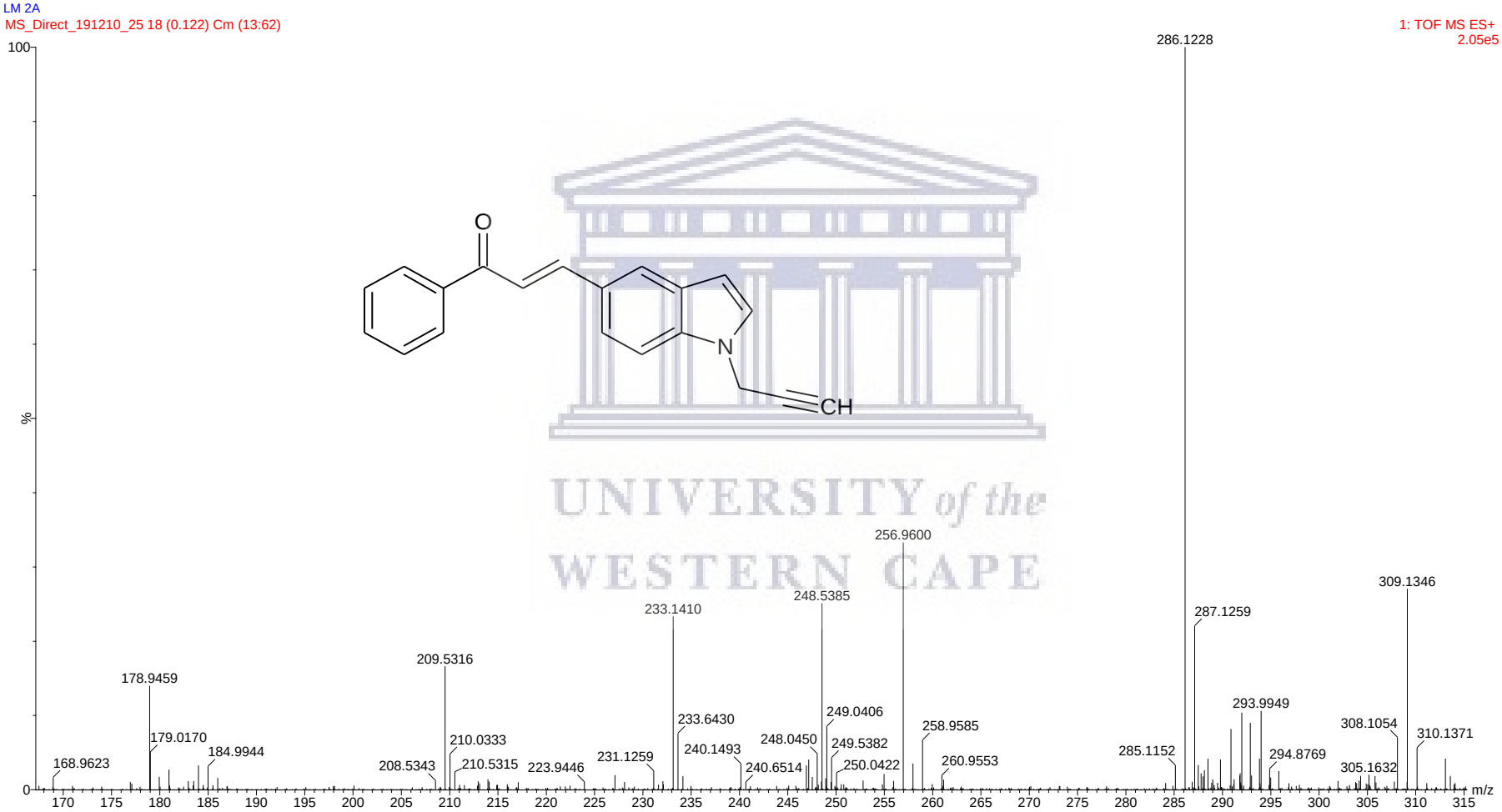
Spectrum 14: ^{13}C NMR Compound **2A**



Spectrum 15: IR Compound **2A**

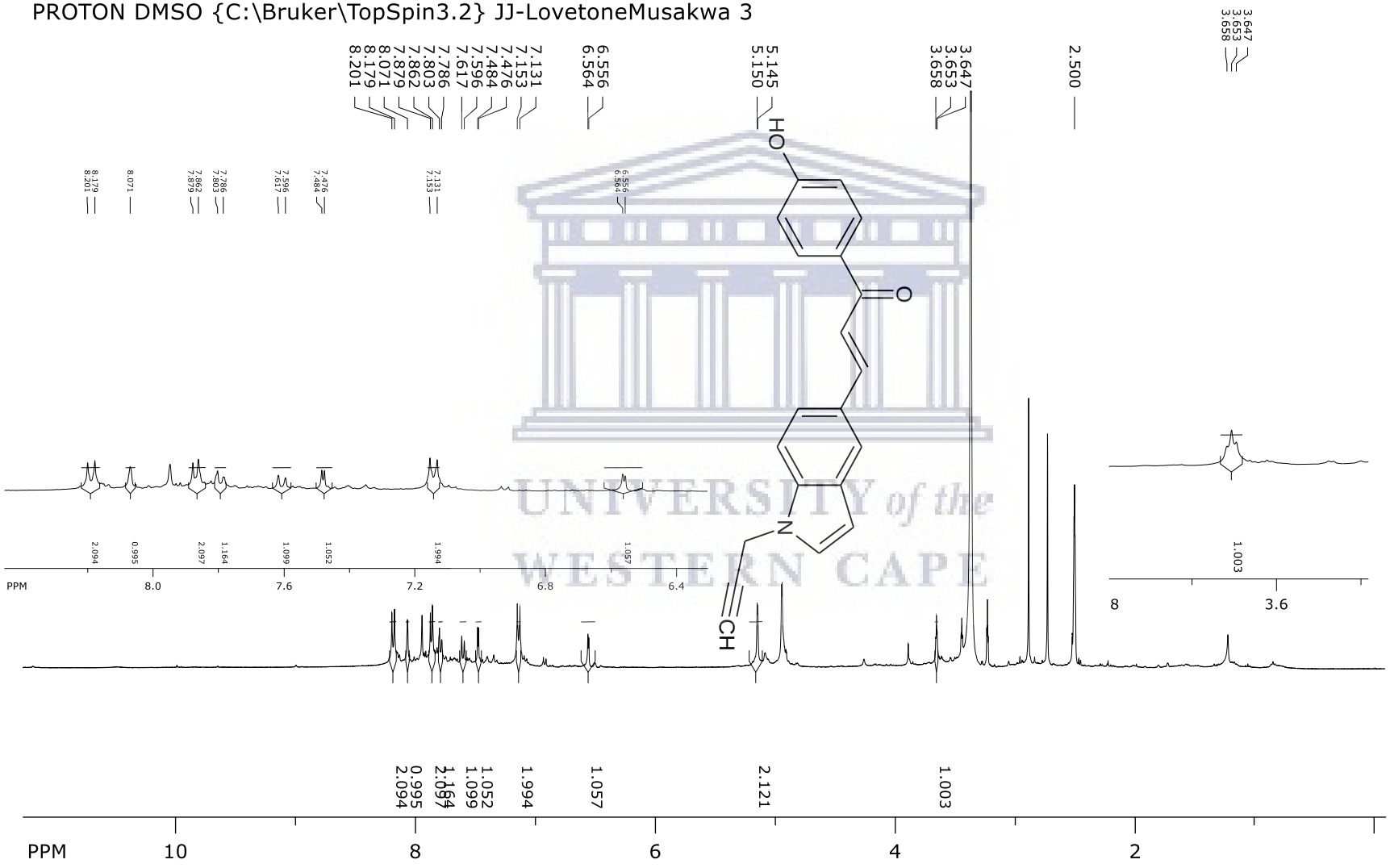


Spectrum 16: MS Compound **2A**

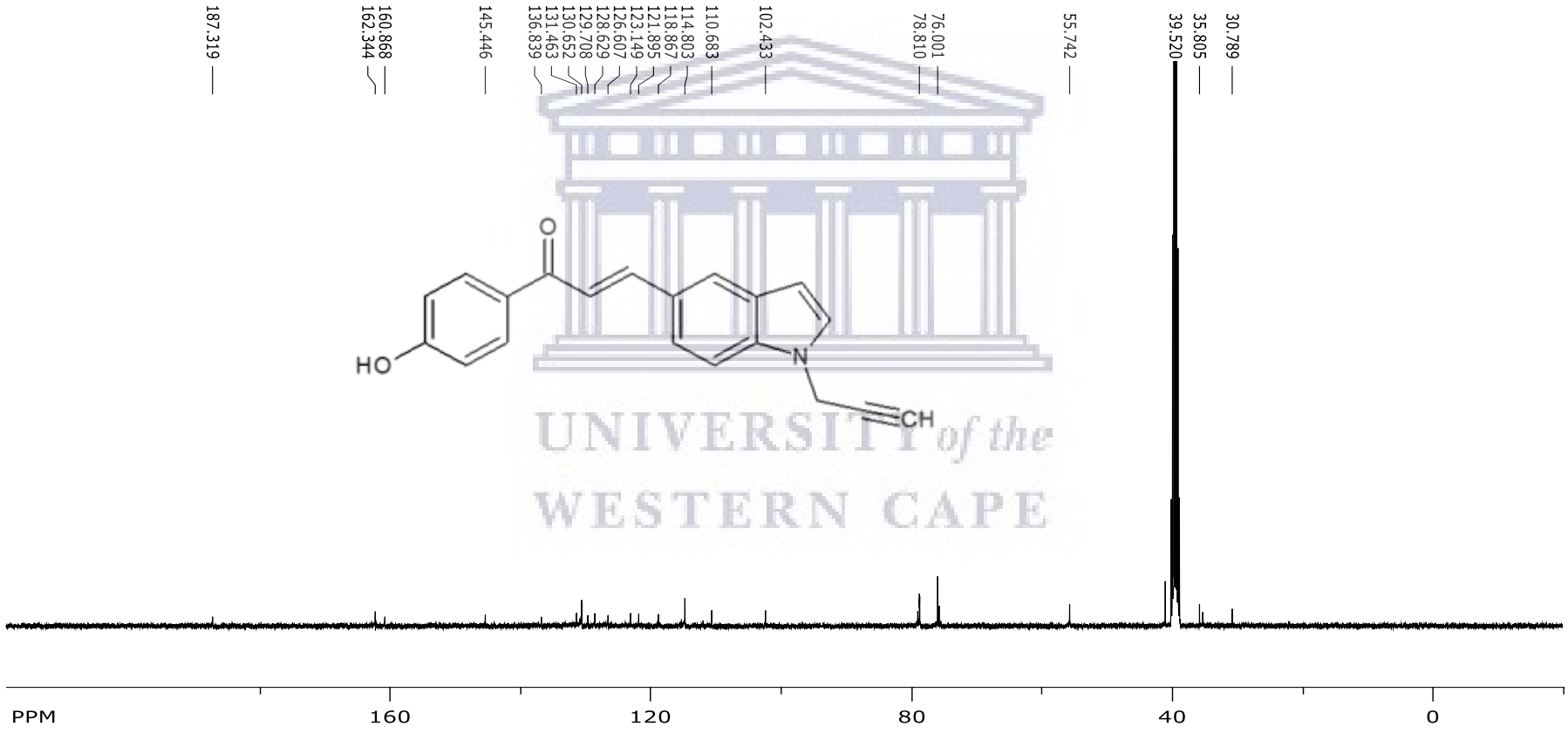


Spectrum 17: ^{1}H NMR Compound **2B**

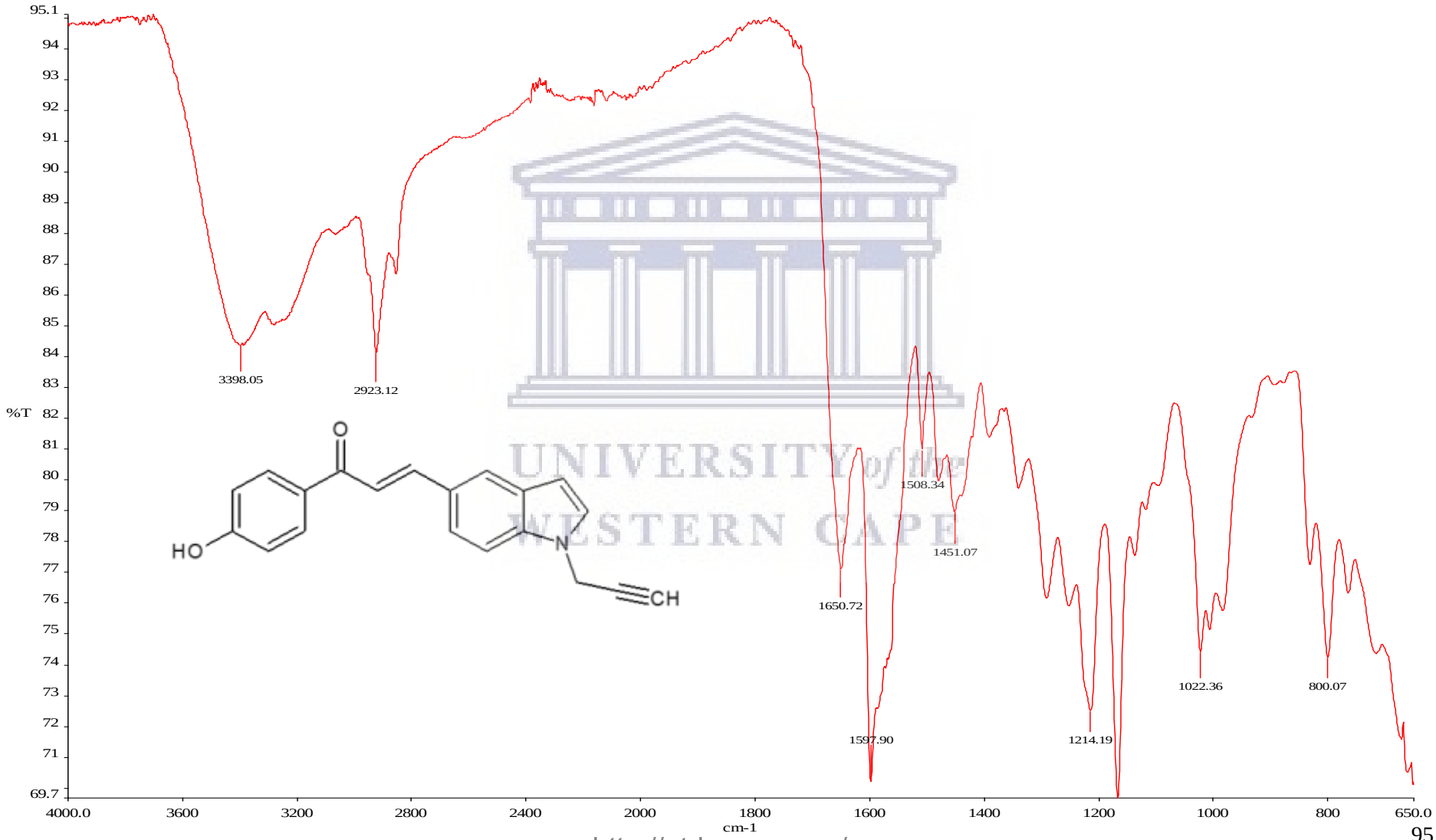
SpinWorks 4:
PROTON DMSO {C:\Bruker\TopSpin3.2} JJ-LovetoneMusakwa 3



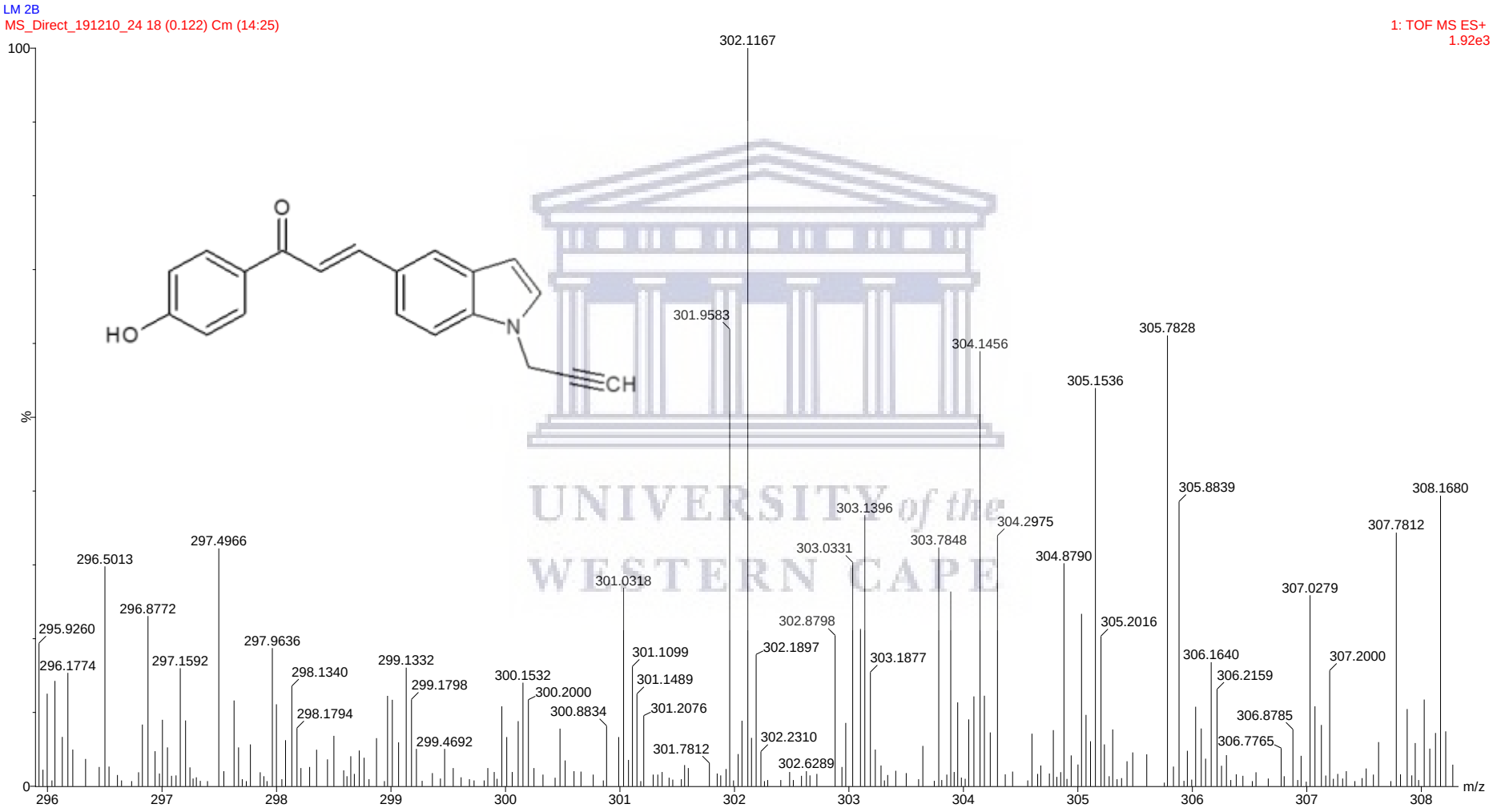
Spectrum 18: ^{13}C NMR Compound **2B**



Spectrum 19: IR Compound **2B**

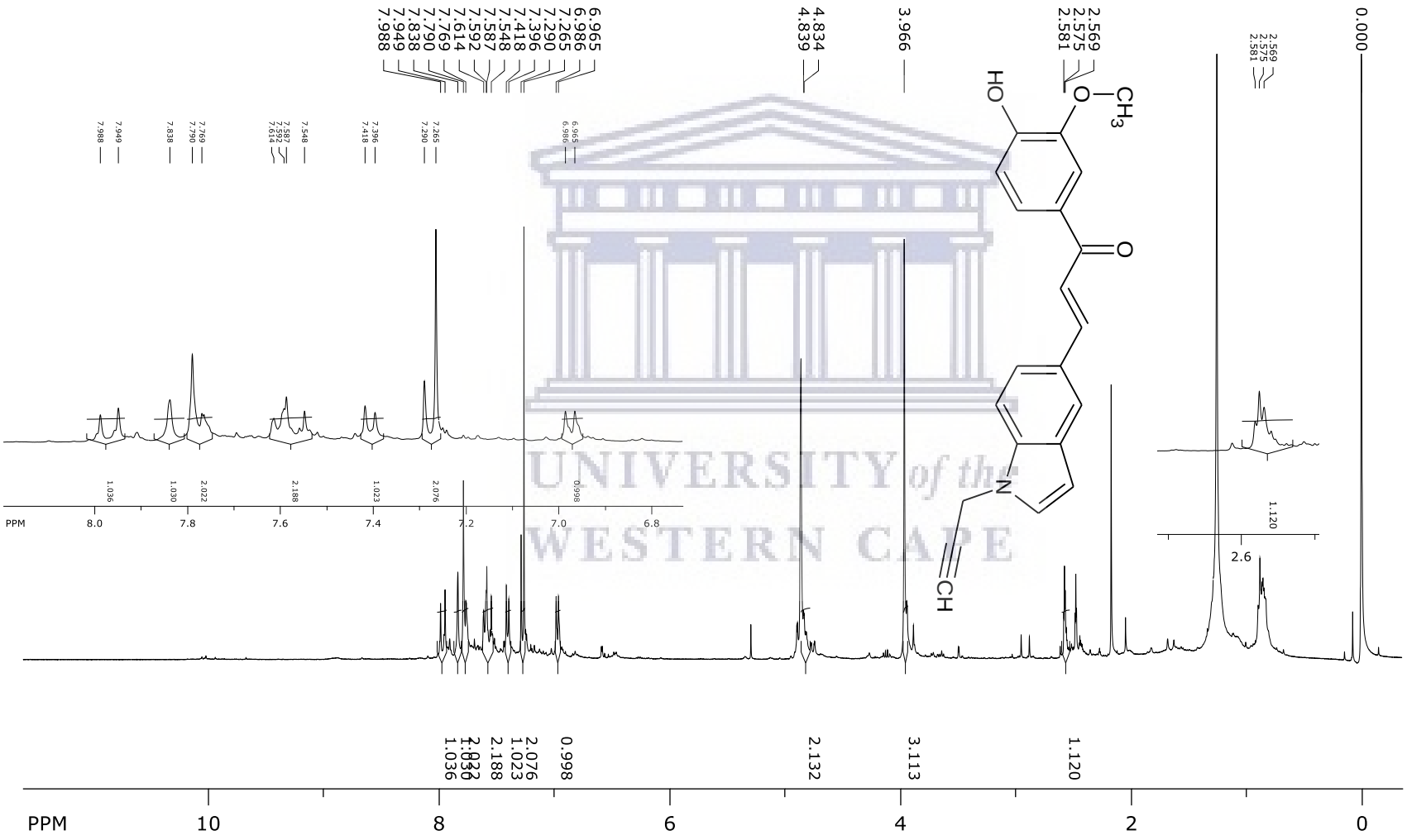


Spectrum 20: MS Compound **2B**

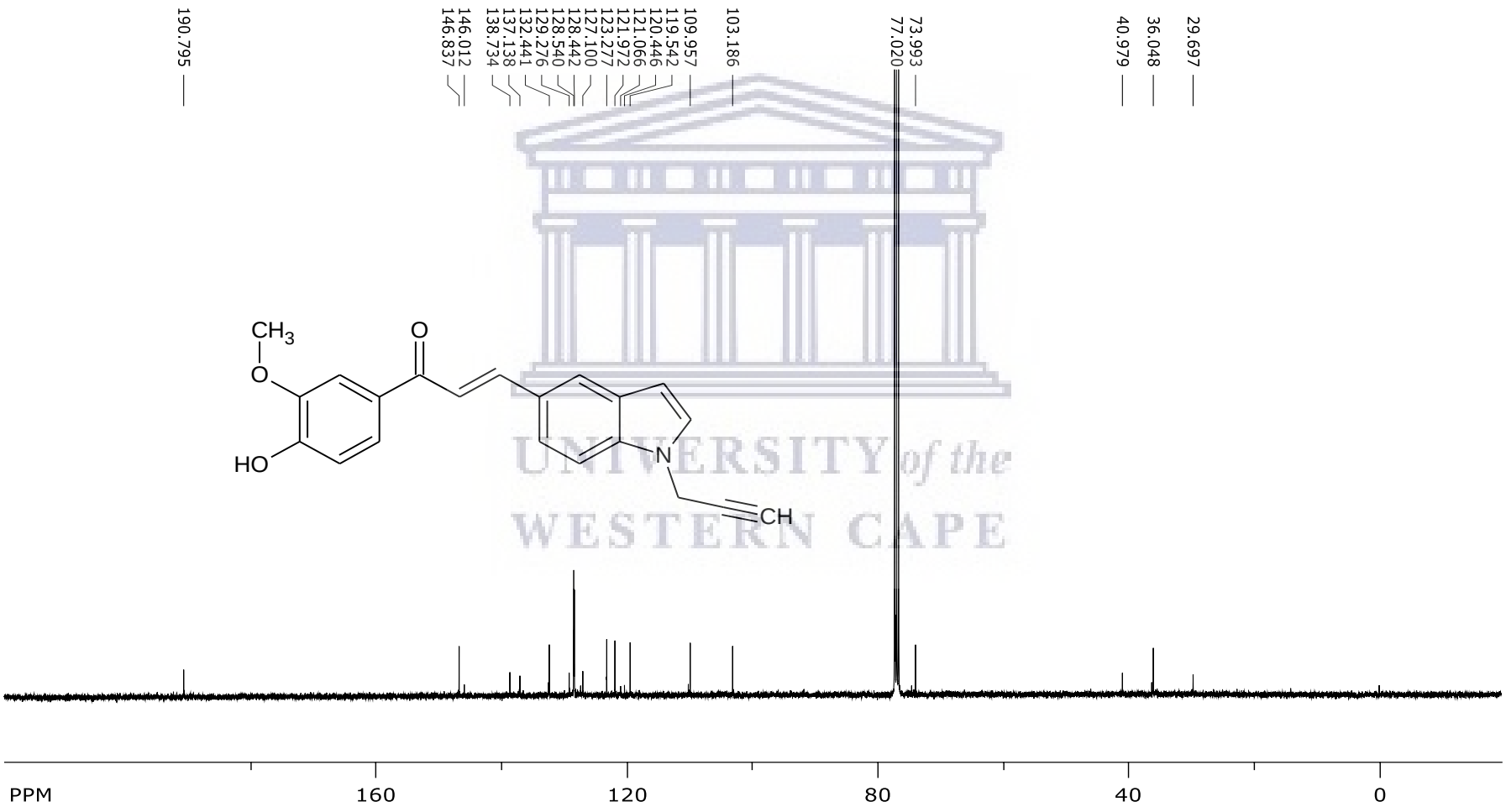


Spectrum 21: ^{1}H NMR Compound **2C**

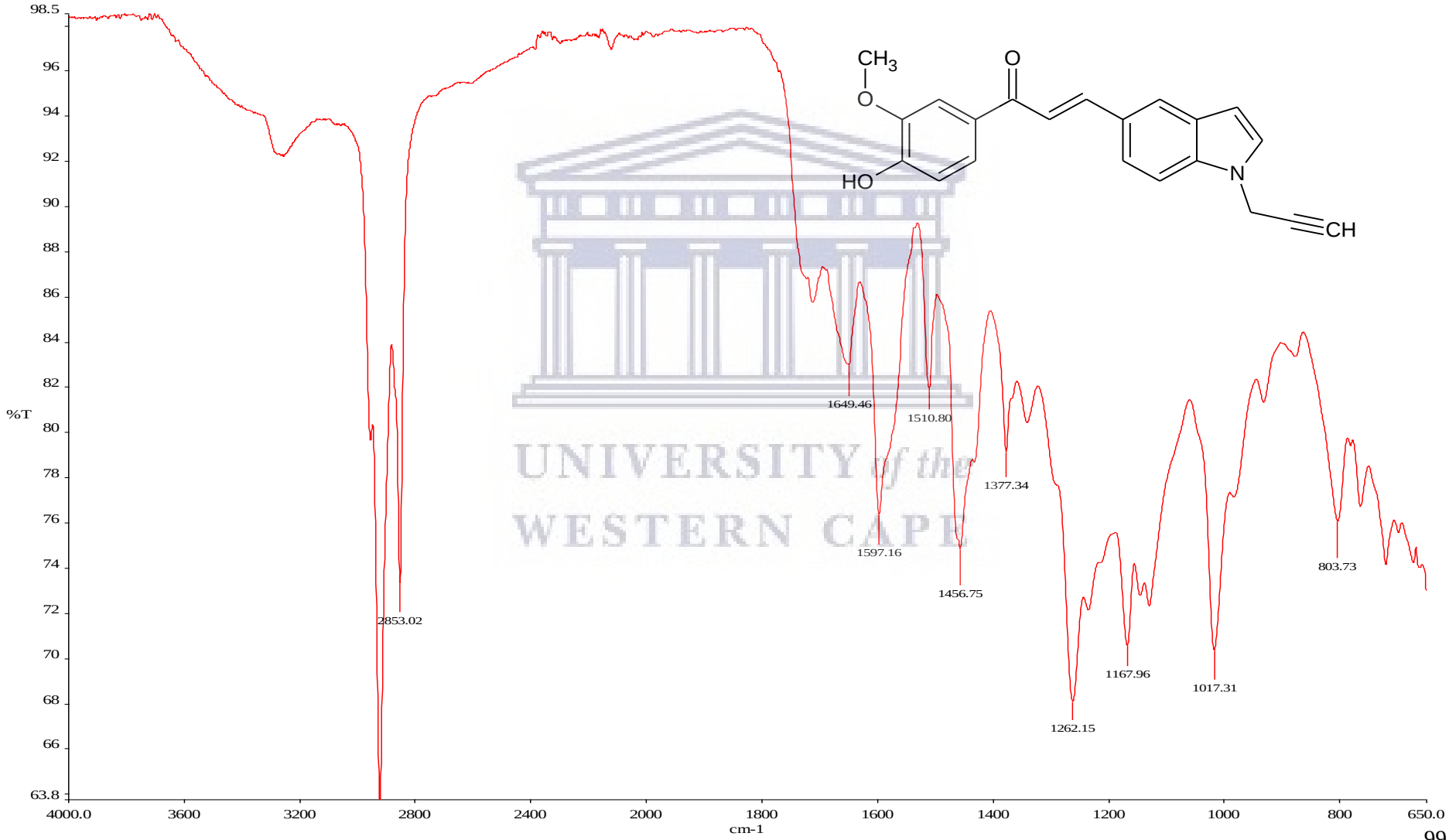
SpinWorks 4:
PROTON CDCl3 {C:\Bruker\TopSpin3.2} JJ-LovetoneMusakwa 22



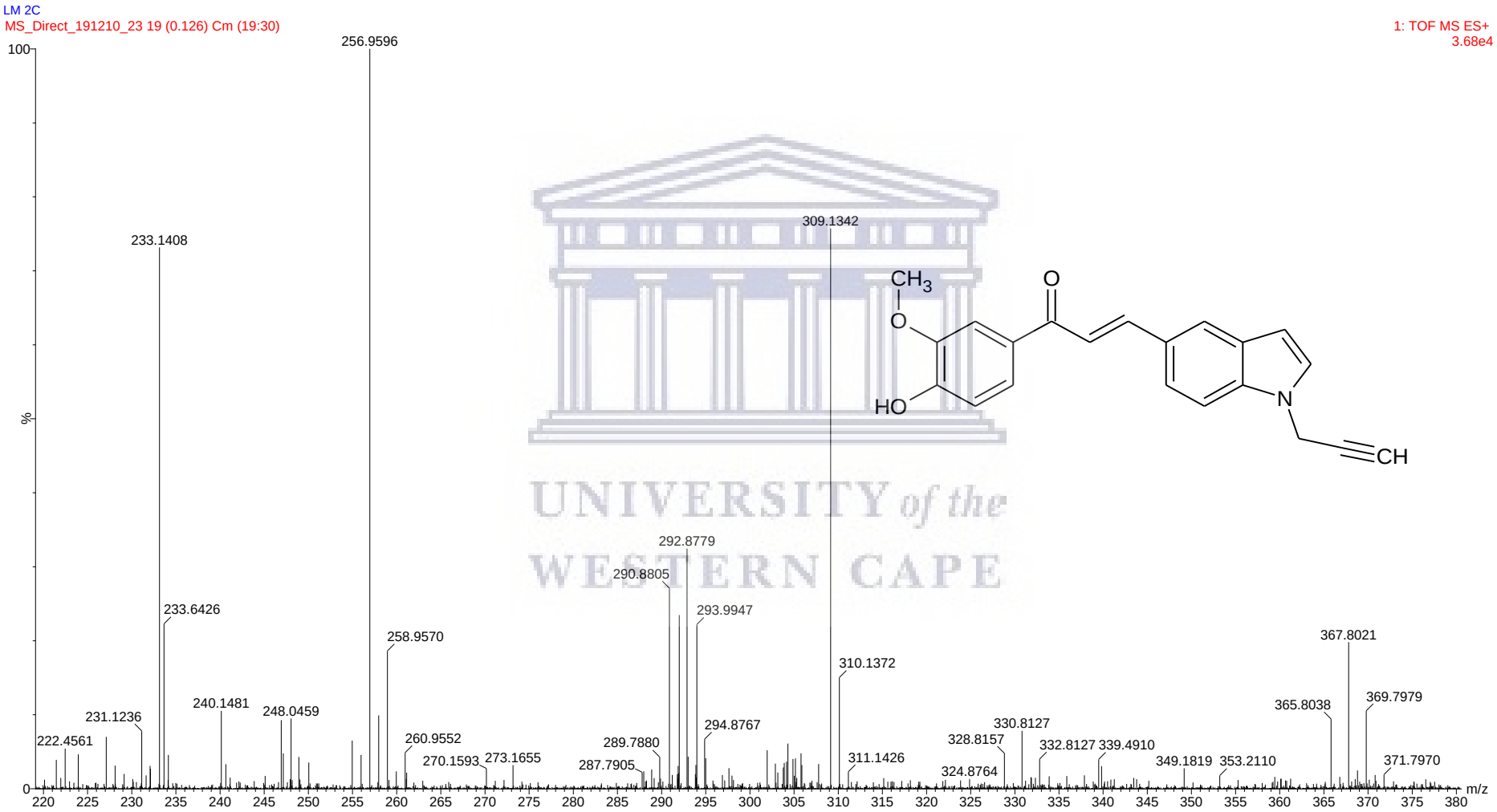
Spectrum 22: ^{13}C NMR Compound **2C**



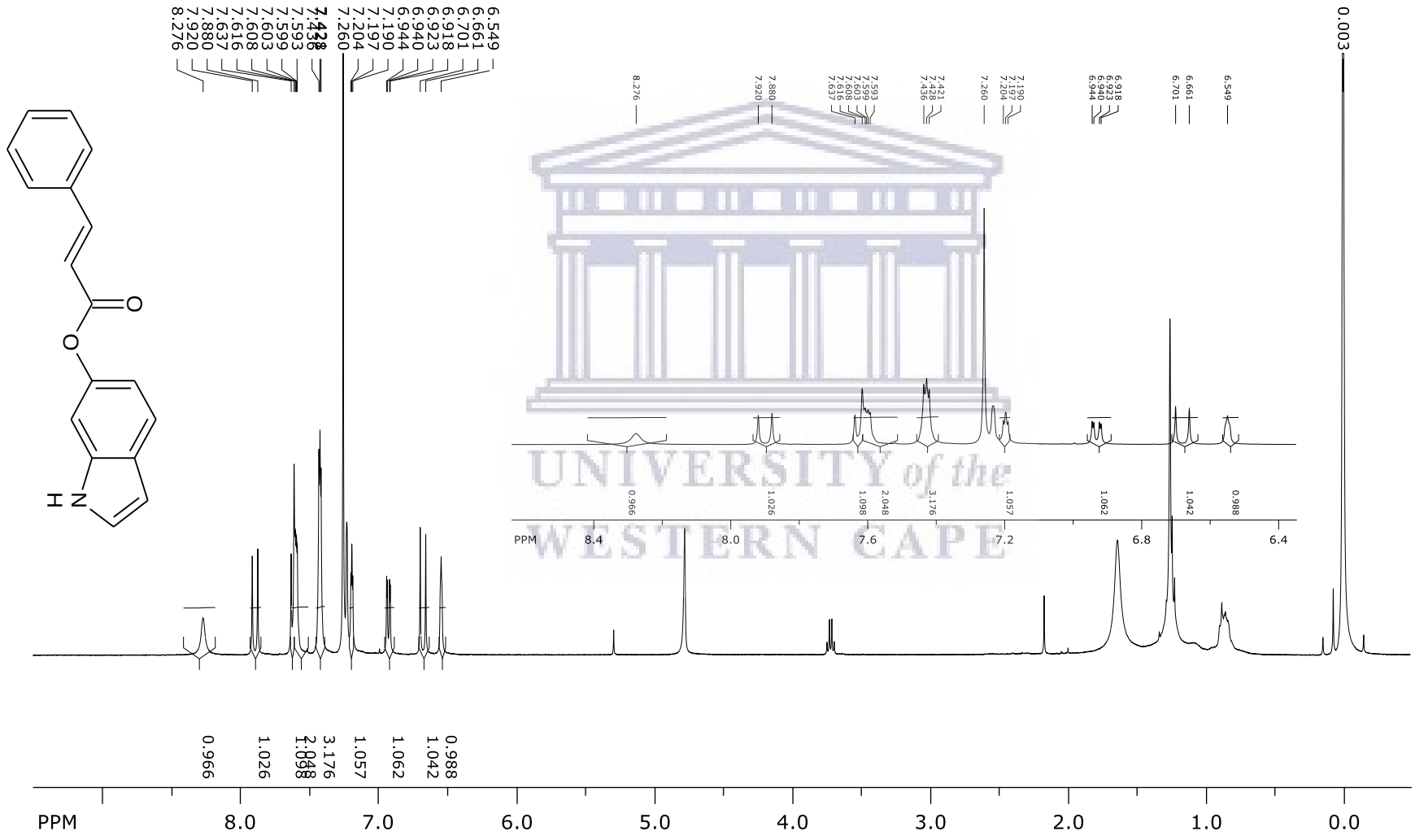
Spectrum 23: IR Compound **2C**



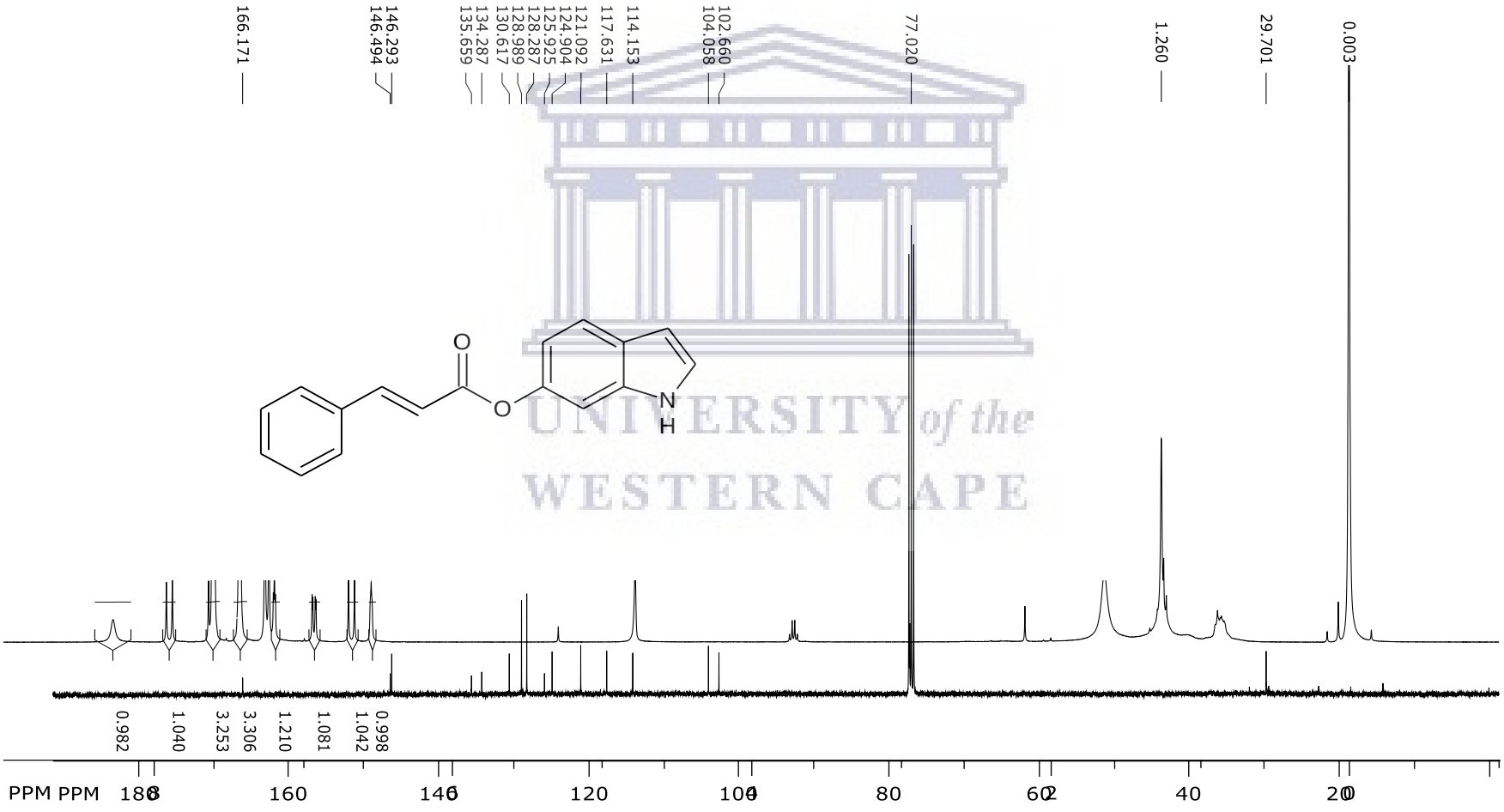
Spectrum 24: MS Compound **2C**



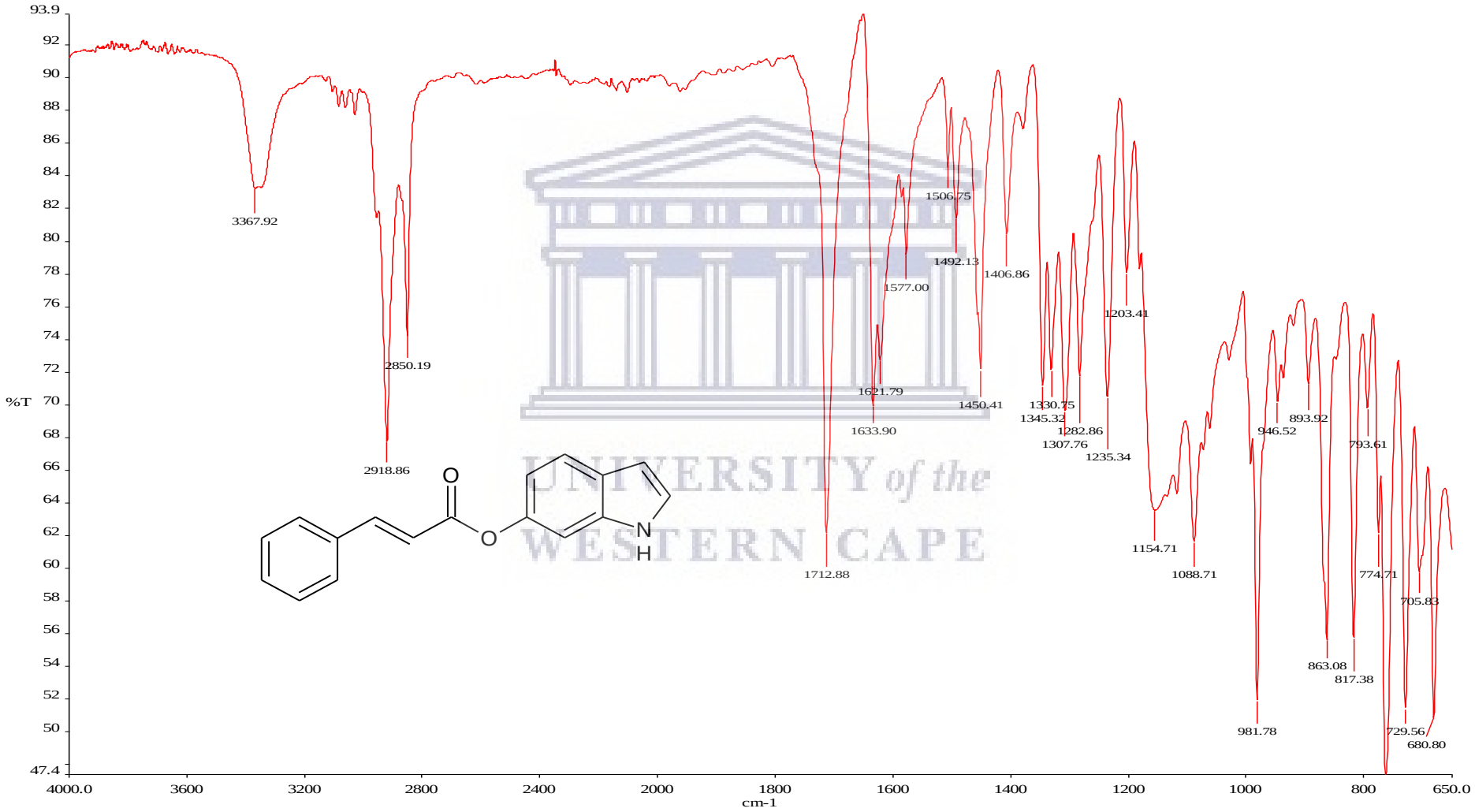
Spectrum 25: ^{1}H NMR Compound **3A**



Spectrum 26: ^{13}C NMR Compound **3A**



Spectrum 27: IR Compound **3A**



Spectrum 28: MS Compound **3A**