Synthesis and evaluation of cyclic chalcones as monoamine oxidase inhibitors

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PREFACE

This thesis is submitted in an article format in accordance with the General Academic Rules (A.13.7.3) of the North-West University.

The document includes three articles which are to be submitted to the following journals, in order: Bioorganic & Medicinal Chemistry Letters, European Journal of Medicinal Chemistry and Bioorganic & Medicinal Chemistry. The articles are prepared according to the author's guidelines, available in the author information pack on the individual journals homepages:

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ABSTRACT

Keywords: chalcones, monoamine oxidase inhibitors, Parkinson's disease, 2-benzylidene-1-tetralone

Parkinson's disease (PD) is the second most-prevalent age-related neurodegenerative disorder following Alzheimer's disease. The manifestation of clinical PD begins after a loss of neurons from the substantia nigra pars compacta (SNpc) which lead to the striatal dopamine (DA) deficiency and dysregulation of the motor circuits that project throughout the basal ganglia. The classical PD symptoms are bradykinesia, rigidity and tremor while non-motor symptoms such as dementia, psychosis, depression and apathy also occur. The majority of the population affected by PD is in the aged group over 65 years.

To date, there is no known cure for PD except for the symptomatic relief of motor symptoms using a variety of therapies such as levodopa (L-3,4-dihydroxyphenylanine or L-Dopa), DA agonists and monoamine oxidase B (MAO-B) inhibitors. These therapeutic agents are often used in combination to ensure effective alleviation of symptoms. However, due to adverse effects arising from combined therapies, research into monotherapies for PD is on-going. Amongst others the MAO enzymes, especially the MAO-B isoform, are of particular interest for PD therapy.

MAO (A and B) enzymes are flavin adenine dinucleotide (FAD) dependent enzymes found in the outer mitochondrial membrane of neuronal, glial and other mammalian cells where they catalyse the oxidative deamination of neurotransmitters. MAO-B is predominant in the basal ganglia where it metabolises DA to yield hydrogen peroxide and aldehydes. These compounds may lead to the accumulation of the hydroxyl radical, formed via the Fenton reaction. Therefore, the inhibition of MAO-B may increase physiological DA levels in the brain and may act as neuroprotector against hydroxyl radicals and oxidative stress. Apart from DA and tyramine, which are metabolised by both MAO isoforms, MAO-B is also responsible for the metabolism of other neurotransmitters such as benzylamine and 2-phenylethylamine. MAO-A on the other hand, selectively breaks down amines such as noradrenalin, adrenalin and serotonin, hence MAO-A is a target for other disorders such as anxiety and depression. It is noteworthy that irreversible inhibitors of MAO-A can pose toxicological threats when combined with serotonin drugs and tyramine rich food diets resulting in the serotonin syndrome and "cheese effect", respectively.

Irreversible MAO inhibitors (especially isoform non-selective inhibitors) may pose undesirable risks. For example, selective irreversible MAO-B inhibitors such as selegiline and rasagiline show good inhibitory MAO activities at low doses although isoform selectivity is lost at high or repeated administration. Thus application of selective reversible MAO-B inhibitors is recommended for PD therapy.

Chalcones have recently attracted attention as potential MAO inhibitors for PD therapy. A study by Chimenti and colleagues (2009) reported promising activities of chalcones as inhibitors of MAO-B with the most potent compound displaying an IC_{50} of 0.0044 μM. This compound also exhibited high isoform selectivity (SI > 11364) in favour of MAO-B. In addition, Robinson, and co-workers (2013) examined the MAO inhibition activity of furanochalcones. The most active compound exhibited an IC_{50} value of 0.174 μM for the inhibition of MAO-B and 28.6 μM for the inhibition of MAO-A. The results demonstrate that these furan substituted chalcones exhibited moderate to good inhibitory activities towards MAO-B, but showed weak or no inhibition of the MAO-A enzyme. Based on the validity of chalcones as potential MAO-B selective inhibitors, this study will explore structure-activity relationships (SARs) of cyclic chalcones, which are conformational restricted forms of chalcones. In this regard, the study will focus on the 2-benzylidene-1-tetralone class of compounds with various substituents (polar and lipophilic) on rings A and B. SARs will also explore 2-heteroarylidene-1-tetralone derivatives and the effects of these substitutions on the MAO inhibition activities. The open chain chalcone will also be compared to the cyclic tetralone derivatives. This is based on the consideration that restricted analogues are envisaged to, at least, retain the activity and to have better isoform selectivity compared to “open-ring” chalcones.

Chemistry: The cyclic (benzylidenes and heteroarylidenes) along with the open-chain chalcone analogues were studied as three separate series and were synthesised via the Claisen-Schmidt reaction. Depending on the chemical behaviour of the reactants in solution, the reactions were carried out in the presence of either an acid (concentrated hydrochloric acid) or a base (potassium/sodium hydroxide or piperidine) as catalysts. The precipitates obtained by addition of water were dried and recrystallised from appropriate solvents. Chemical characterisation of the structures consisted of nuclear magnetic resonance spectroscopy (NMR) and high resolution mass spectroscopy (HRMS), whereas purities in the range 96–100% (with exception of two compounds; **1d** of series 1 at 87.5% and **2c** of series 2 at 89.4%) were confirmed by high performance liquid chromatography (HPLC).

MAO inhibition studies: The inhibition potencies of the test inhibitors of the three different series were expressed as IC_{50} values from which the selectivity index (SI) values were determined. The measurement of IC_{50} values was done by employing the recombinant human enzymes and kynuramine as the substrate. The first series studied the MAO inhibition properties of benzylidene-substituted indanones, tetralones, benzosuberones, chromones, chromanones and thiochromanones. The results indicated that the compounds are moderate inhibitors of MAO with significant selectivity for the MAO-B isoform. The series consisted of 8 compounds, of which 5 exhibited IC_{50} values below 1 μM, while one inhibitor **1h** showed no activity for either MAO isoforms. Compound **1b** (a chromone) exhibited the most potent inhibition activity (IC_{50} = 0.157 μM) and is isoform specific for MAO-B. With regards to the MAO-A isoform, low inhibition potencies were recorded for the series with the most potent inhibitor, **1f** (an indanone), exhibiting an IC_{50} value of 0.346 μM, with a poor isoform selectivity of 0.822. It was concluded that ring expansion to bigger enone rings reduces MAO activity. Therefore, the second series focused on 2-benzylidene-1-tetralones (6-membered ring analogues). The tetralones exhibited relatively moderate and selective inhibition of the MAO-B isoform, with **2u** being the most potent inhibitor with an IC_{50} value of 0.0064 μM. Compound **2p**, the most potent MAO-A inhibitor in this series, displays an IC_{50} value of 0.753 μM. Inhibitor **2t** possessed the highest selectivity (SI: 787). The last series studied investigated 2-heteroarylidene-1-tetralone derivatives (**4**). All 12 compounds explored except for **4g** (MAO-A specific) showed selective inhibition for MAO-B. Amongst the 2-heteroarylidene-1-tetralone derivatives, the non-aromatic cyclohexyl ring (**4a**) yielded relatively potent MAO-B inhibition (IC_{50} 0.895 μM), which is the highest inhibition activity towards MAO-B in the series. Contrary to that, the 2-chloro-3-pyridine derivative (**4g**) was the most potent and selective MAO-A inhibitor of the series with an IC_{50} value of 1.37 μM. This makes it a potential drug for the treatment of depression. It was also observed that non-aromatic ring expansion from cyclopentane (**4b**) to cyclohexane (**4a**) improves MAO activity significantly.

It may be concluded that this study successfully synthesised series of cyclic chalcone derivatives and recorded promising MAO inhibition activities for many of the compounds. Selective and potent MAO-B inhibitors, in particular, may find application in the treatment of PD.

OPSOMMING

Sleutelwoorde: chalkone, monoamienoksidase-inhibeerders, Parkinson se siekte, 2-bensielideen-1-tetraloon

Parkinson se siekte (PS) is die tweede mees algemene ouderdomsverwante neurodegeneratiewe siekte naas Alzheimer se siekte. Die kliniese simptome van PS volg op die verlies van die neurone van die substantia nigra pars compacta (SNpc) wat striatale dopamien- (DA) tekort en wanregulering van die motoriesebane van die basale ganglia veroorsaak. Die klassieke simptome van PS is bradikinese, rigiditeit en tremore, terwyl nie-motoriese simptome soos demensie, psigose, depressie en apatie ook voorkom. Die ouderdomsgroep ouer as 65 jaar is die gedeelte van die gemeenskap waar PS die meeste voorkom.

Tans bestaan daar geen genesing vir PS nie en die motorsimptome word met verskeie geneesmiddels soos levodopa (L-3,4-dihidroksiefenielalanien of L-Dopa), DA-agoniste en monoamienoksidase B (MAO-B) inhibeerders behandel. Hierdie geneesmiddels word baie keer in kombinasies gebruik om effektiewe verligting van simptome te verkry. As gevolg van die newe-effekte van kombinasieterapie word navorsing gedoen vir die ontwikkeling van alleenterapieë vir PS. Onder andere is die MAO-ensieme, veral die MAO-B-isovorm, van belang in die behandeling van PS.

MAO (A en B) ensieme is flavienadeniendinukleotied- (FAD) afhanklike ensieme wat in die mitochondriale membrane van neuronale-, glia- en ander soogdieselle gevind word waar hulle die oksidatiewe deaminering van neuro-oordragstowwe kataliseer. MAO-B kom hoofsaaklik in die basale ganglia voor waar dit DA metaboliseer om waterstofperoksied en aldehiede te lewer. Hierdie verbindings kan lei tot die akkumulasie van hidroksielradikale en oksidatiewestres. Die inhibisie van MAO-B kan dus die fisiologiese konsentrasies van DA in die brein verhoog en as neurobeskermend optree teen hidroksielradikale en oksidatiewestres. Behalwe vir DA en tiramien wat deur beide MAO-isovorme gemetaboliseer word, is MAO-B ook verantwoordelik vir die metabolisme van ander neuro-oordragstowwe soos bensielamien en 2-fenieletielamien. Op sy beurt metaboliseer MAO-A amiene soos noradrenalien, adrenalien en serotonien, en MAO tree dus op as teiken vir die behandeling van siektetoestande soos angs en depressie. Dit is merkwaardig dat onomkeerbare inhibeerders van MAO-A toksikologiese newe-effekte kan veroorsaak as dit met serotonergiesemiddels en tiramien-bevattende voedsel gekombineer word om tot die serotoniensindroom en “kaasreaksie”, onderskeidelik te lei.

Onomkeerbare MAO-inhibeerders (veral isovorm-nieselektiewe inhibeerders) kan onverwagte effekte toon. Selektiewe onomkeerbare MAO-B-inhibeerders soos selegilien en rasagilien toon goeie MAO-inhibisie aktiwiteite by lae dosisse terwyl isovorm selektiwiteit verlore gaan met hoë dosisse en herhaaldelike toediening. Selektiewe omkeerbare MAO-B-inhibeerders word dus aanbeveel vir die behandeling van PS.

Chalkone het onlangs belangstelling ontlok as moontlike MAO-inhibeerders vir die behandeling van PS. 'n Studie deur Chimenti en kollegas (2009) lewer verslag oor die belowende aktiwiteite van chalkone as MAO-B-inhibeerders met die mees potente verbinding wat 'n IC_{50} waarde van 0.0044 µM toon. Hierdie verbinding besit ook 'n hoë mate van selektiwiteit vir MAO-B (SI = 11364). Robinson en medewerkers (2013) het op hul beurt weer die MAO-inhibisie aktiwiteite van furanochalkone ondersoek. Die mees aktiewe verbinding toon 'n IC_{50} waarde van 0.174 µM vir die inhibisie van MAO-B en 28.6 µM vir die inhibisie van MAO-A. Dié resultate wys dat furaan-gesubstitueerde chalkone matig tot goeie inhibisie aktiwiteite teenoor MAO-B besit, maar swak of geen inhibisie van die MAO-A-isovorm toon. Op grond van die selektiewe inhibisie van MAO-B deur chalkone, ondersoek hierdie studie die struktuuraktiwiteitsverwantskappe (SAVe) van sikliese chalkone, wat as die geslote-konformasie analoë van chalkone beskou kan word. Hierdie studie fokus op die 2-bensielideen-1-tetraloon klas verbindings met verskeie substituente (polêr en lipofiel) op ringe A en B. Die SAVe van 2-heteroarielideen-1-tetraloon derivate en die effekte van hierdie substituente op MAO-inhibisie sal ook ondersoek word. Die oop-ketting chalkone sal ook met die sikliese-ckalkoonderivate vergelyk word. Hierdie vergelyking word getref omrede geslote-konformasie chalkone moontlik aktiwiteit sal behou terwyl dit beter isovorm-selektiwiteit mag besit vergeleke met oop-ketting chalkone.

Chemie: Die sikliese (bensielidene en heteroarielidene) en oop-ketting chalkone is as drie verskillende reekse ondersoek en is gesintetiseer deur die Claisen-Schmidt reaksie. Afhangende van die chemiese reaktiwiteit van die reaktante in oplossing, is die reaksies in die teenwoordigheid van óf 'n suur (gekonsentreerde soutsuur) of 'n basis (kalium- of natriumhidroksied, of piperidien) as kataliste uitgevoer. Die presipitate wat verkry is na byvoeging van water is gedroog en geherkristalliseer uit toepaslike oplosmiddels. Die strukture is chemies gekarakteriseer deur kernmagnetieseresonans- (KMR) spektroskopie en hoë-resolusie massaspektroskopie (HRMS), terwyl die suiwerhede met hoëdruk-vloeistofchromatografie bepaal is. Die suiwerhede het gewissel van 96-100%, met die uitsondering van **1d** (reeks 1) en **2c** (reeks 2) wat suiwerhede van 87.5% en 89.4%, onderskeidelik, getoon het.

MAO-inhibisie studies: Die potensies van MAO-inhibisie deur die toetsinhibeerders van die drie verskillende reekse is uitgedruk as die IC_{50} waardes, en hieruit is die selektiwiteitsindeks (SI) bereken. Die IC_{50} waardes is bepaal deur van die rekombinante mensensieme en kinuramien as substraat gebruik te maak. Met die eerste reeks is die MAO-inhibisie eienskappe van bensielideen-gesubstitueerde indanone, tetralone, bensosuberone, chromone, chromanone en tiochromanone ondersoek. Die resultate wys dat dié verbindings matig potente inhibeerders van MAO is, met selektiwiteit vir die MAO-B-isovorm. Hierdie reeks het uit 8 verbindings bestaan waarvan 5 verbindings IC_{50} waardes laer as 1 µM besit het terwyl een inhibeerder, **1h**, geen aktiwiteit vir die MAO-isovorme getoon het nie. Verbinding **1b** ('n chromoon) was die mees potente inhibeerder (IC_{50} = 0.157 µM) en besit ook selektiwiteit vir MAO-B. Met betrekking tot MAO-A is swak inhibisie vir die reeks aangeteken en die mees potente inhibeerder **1f** ('n indanoon) besit 'n IC_{50} waarde van 0.346 µM en swak isovorm-selektiwiteit (SI = 0.822). Daar is tot die gevolgtrekking gekom dat ringvergroting om groter enoonringe te lewer, MAO-inhibisie aktiwiteit verlaag. Om hierdie rede fokus die tweede reeks op 2-bensielideen-1-tetralone (seslidringanaloë). Die tetralone toon matige en selektiewe inhibisie van MAO-B met **2u** as die mees potent inhibeerder met 'n IC_{50} waarde van 0.0064 µM. Verbinding **2p** is die mees potente MAO-A inhibeerder van die reeks met 'n IC_{50} waarde van 0.753 µM. Inhibeerder **2t** besit die beste selektiwiteit (SI = 787). Die laaste reeks bestudeer 2-heteroarielideen-1-tetraloon derivate (**4**). Behalwe vir **4g** (MAO-A-selektief) is die 12 inhibeerders selektief vir MAO-B. Onder die 2-heteroarielideen-1-tetraloon derivate het die nie-aromatiese sikloheksielring (**4a**) die beste MAO-B-inhibisie gelewer. In teenstelling hiermee was die 2-chloro-3-piridienderivaat (**4g**) die mees potente MAO-A inhibeerder in die reeks. Hierdie verbinding is dus 'n potensiële middel vir die behandeling van depressie. Daar is ook aangeteken dat nie-aromatiese ringvergroting van siklopentaan (**4b**) na sikloheksaan (**4a**) MAO-inhibisie aktiwiteit betekenisvol vergroot.

Die gevolgtrekking kan dus gemaak word dat reekse sikliese-chalkoonderivate met sukses gesintetiseer is en dat belowende MAO-inhibisie aktiwiteite vir baie verbindings aangeteken is. Selektiewe en potente MAO-B-inhibeerders kan vir die behandeling van PS aangewend word.

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^{13}C-NMR	Carbon 13 nuclear magnetic resonance
^{1}H-NMR	Proton nuclear magnetic resonance
3-OMD	3-O-Methyldopa
4-HQ	4-Hydroxyquinoline
AD	Alzheimer's disease
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
BBB	Blood-brain barrier
$CDCl_3$	Deuterated chloroform
CNS	Central nervous system
COMT	Catechol-O-methyl transferase
CSF	Cerebrospinal fluid
DA	Dopamine
DCC	Dopa decarboxylase
DMSO-d_6	Deuterated dimethyl sulfoxide
EOPD	Early onset Parkinson's disease
ETC	Electron transport chain
FAD	Flavin adenine dinucleotide
GABA	γ-Aminobutyric acid
GI	Gastrointestinal
GPe	External pallidal segment
GPi	Internal pallidal segment
GSH	Glutathione
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
IC50	50% inhibitory concentration
L-DOPA	Levodopa
LRRK2	Leucine rich repeat kinase 2
MAO-A	Monoamine oxidase A
MAO-B	Monoamine oxidase B
MPP+	1-Methyl-4-phenylpyridinium

MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NMDA	N-Methyl-D-aspartate
PD	Parkinson's disease
PINK 1	Pten-induced putative kinase 1
PQ	Paraquat
ROS	Reactive oxygen species
SARs	Structure activity relationships
SD	Standard deviation
SET	Single electron transport
SI	Selectivity index
SNCA	α-Synuclein
SNpc	Substantia nigra pars compacta
SNpr	Substantia nigra pars reticulata
SOD	Superoxide dismutase
STN	Subthalamic nucleus
TH	Tyrosine hydrolase
WHO	World health organisation

CHAPTER 1: INTRODUCTION AND PROBLEM STATEMENT

1.1 Introduction

1.1.1 Parkinson's disease

Parkinson's disease (PD) was first described in 1817 by James Parkinson and is the second most prevalent age-related neurodegenerative disorder following Alzheimer's disease (AD) (Dauer & Przedborski, 2003). The World Health Organization (WHO) classifies neurological disorders as diseases of the central and peripheral regions with great causal potential of mortality. Statistics indicate that neurodegenerative related deaths constituted 11.84% of deaths globally in 2015 and will be 12.22% by 2030 (Singh *et al.*, 2015). PD is characterised by the depigmentation of the substantia nigra pars compacta (SNpc) as a result of the selective and progressive degeneration of dopaminergic neurons (Lees *et al.*, 2009). The subsequent depletion of dopamine in the central regions leads to abnormal regulation of the motor circuit and the clinical manifestation of PD (Dauer & Przedborski, 2003).

The classical motor symptoms of PD, bradykinesia, rigidity and tremor develop when approximately 50% of the striatal dopaminergic nigrostriatal neurons and approximately 80% striatal dopamine (DA) production are lost (Muller, 2015). Since the discovery of levodopa as therapy for PD 45 years ago, levodopa has been the most effective therapy for the symptomatic treatment of PD. Levodopa constitutes dopamine replacement (Thanvi & Lo, 2004). Although considered the "gold standard" therapy, chronic levodopa usage may result in motor complications which may be associated with the loss of dopamine neurons and post-synaptic changes caused by prolonged intermittent levodopa administration (Agid, 1999). These adverse effects can be as disabling as the disease itself and are often inevitable. This led to the application of other drugs as replacement and/or adjunct therapies to levodopa which are aimed at preventing or treating chronic levodopa complications. These include amongst others dopamine agonists, peripheral dopa decarboxylase (DCC) inhibitors, catechol-O-methyl-transferase (COMT) inhibitors and monoamine oxidase (MAO)-B inhibitors. Unfortunately, some of these alternative therapies may also result in complications; therefore much research is aimed at discovering new potent and safe drugs for PD.

1.1.2 MAO inhibitors

The MAOs are outer mitochondrial membrane enzymes found in neuronal, glial and other mammalian cells (Ferino *et al.*, 2013), and exist as two isoforms, MAO-A and MAO-B. The two isoforms catalyse the deamination of various neurotransmitters in mammals. For example, DA and tyramine are substrates of both MAO enzymes (Ferino *et al.*, 2013). The two isoenzymes

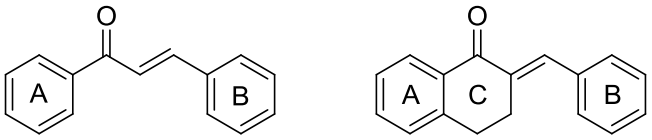
are substrate specific, a feature which plays a role in pathological conditions. MAO-A affect neurotransmitters involved in depression and anxiety, whilst MAO-B plays a role in neurological disorders such as AD and PD (Fowler *et al.*, 2002). MAO inhibitors can be selective/non-selective and reversible/irreversible. Selectivity and reversibility of MAO inhibition are important considerations for the "cheese reaction". This is a hypertensive crisis resulting from the accumulation of tyramine in the periphery due to inhibition of MAO-A (Finberg, 2014). The brain contains mostly the MAO-B isoform and selective inhibitors of MAO-B are used in PD treatment as monotherapy or adjunct to levodopa. MAO-B inhibitors are not associated with the "cheese reaction". Selegiline and rasagiline are examples of MAO-B inhibitors that are used in the clinic for PD therapy (Hubalek *et al.*, 2005; Finberg, 2014).

The physiological function of MAO is to metabolise both endogenous and dietary amines. The by-product of these reactions is mainly hydrogen peroxide, which may lead to the formation of reactive oxygen species (ROS). ROS are known to induce oxidative stress in the central nervous system (CNS) (Stewart, 2008; Macphee & Stewart, 2012). Oxidative stress has been implicated in the neuropathogenesis of PD and the inhibition of central MAO-B may thus have a neuroprotective effect.

1.1.3 Chalcones as MAO inhibitors

The application of MAO-B inhibitors in the therapy of PD makes the discovery of new and effective compounds a worthwhile pursuit. In particular, compounds from the chalcone class have recently been shown to act as MAO inhibitors. Various structure-activity relationships (SARs) studies indicated that chalcones are a general class of MAO-B selective inhibitors. Hence continued effort is being made to discover more potent and selective chalcones. Chimenti and co-workers (2009) used the open chain chalcone scaffold to identify suitable substituents for MAO inhibition while other researchers substituted the phenyl ring A for furan. Although the natural chalcones displayed better MAO inhibition than the furan-based chalcones, both scaffolds possessed selectivity and highest potency for the MAO-B isoform over the MAO-A isoform (Chimenti *et al.*, 2009; Robinson *et al.*, 2013).

The current study further explores the SARs for chalcones as MAO inhibitors by introducing conformational restriction. This will be done by cyclizing the structure of chalcone to yield 2-benzylidene-1-tetralone derivatives (Figure 1.1). Further structure modification will explore substitution of the B-ring of chalcone with heteroaromatic moieties and making modifications to ring C.



Chalcone

2-Benzylidene-1-tetralone

Figure 1.1: Open chain and restricted chalcone scaffolds.

1.2 Hypothesis

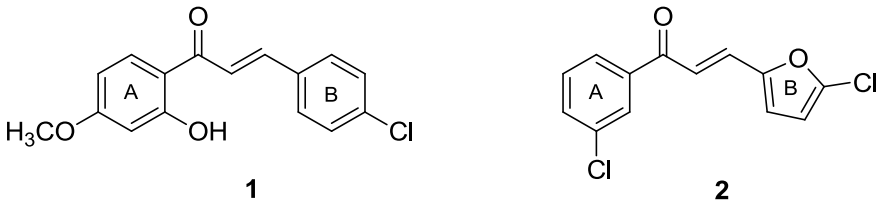
The MAO inhibitory activity of the "open-ring" chalcone scaffold with various substituents on both or either of the aromatic rings A and B has been explored. Further studies reported effects of substitution of the aromatic ring A with heteroaromatic moieties. All studies indicated that various chalcone derivatives inhibit the MAOs, hence the chalcone scaffold has been validated as a promising lead for the discovery of MAO inhibitors. Thus, the current study will contribute with the hypothesis that, cyclic chalcones (restricted analogues) will possess MAO inhibition activities. The restricted analogues are envisaged to at least retain the activity and to have better isoform selectivity compared to the "open-ring" chalcones.

1.3 Rationale

Based on the clinical utility of MAO inhibitors, the discovery of new classes of MAO inhibitors is merited. Therefore, toxicological and adverse effects of MAO inhibitors are an important consideration. Research has identified libraries of MAO inhibitors with excellent inhibitory activity, but drug safety standards often hinder their clinical application. Drug safety issues of MAO inhibitors are mainly associated with irreversible and non-selective inhibitors. These inhibitors are associated with the "cheese reaction" when combined with fermented diets. Other adverse effects such as the serotonin syndrome occur when irreversible especially non-selective MAO inhibitors are used in combination with certain drugs. These include; serotonin re-uptake inhibitors such as venlafaxine, clomipramine and tramadol as well as MAO inhibitors: tranylcypromine, moclobemide and toloxatone (Gillman, 2005).

This study will employ cyclic chalcones (2-benzylidene-1-tetralone derivatives) for the design of novel chalcone-based MAO inhibitors. A series of "open-ring" chalcones have been studied and exhibit excellent potency for MAO-B and moderate inhibition activity for MAO-A. A recent study reported promising MAO-B inhibition activity of chalcones with the most potent compound (**1**) possessing a 50% inhibitory concentration (IC_{50}) of 0.0044 μM and high isoform selectivity (SI > 11364) in favour of MAO-B (Chimenti *et al.*, 2009). In another study, the MAO inhibitory

activities by furanochalcones were examined. The most active compound, 2*E*-3-(5-chlorofuran-2-yl)-1-(3-chlorophenyl)prop-2-en-1-one (**2**), exhibited an IC_{50} value of 0.174 µM for the inhibition of MAO-B and 28.6 µM for the inhibition of MAO-A (Robinson *et al.*, 2013). The data for these studies are summarized in Figure 1.2.



1
MAO-B: IC_{50} = 0.0044 µM

2
MAO-A: IC_{50} = 28.6 µM
MAO-B: IC_{50} = 0.174 µM

Figure 1.2: Examples of chalcones with good MAO inhibitory activities
(Chimenti *et al.*, 2009; Robinson *et al.*, 2013).

It is hypothesised that cyclic chalcones would also act as inhibitors of MAO. The development of analogues with restricted or rigid conformations may result in the selective binding to target sites, which could result in very active drugs with reduced unwanted adverse effects (Gareth, 2007).

1.4 Aims and objectives

The present study aims to synthesise and evaluate a series of 2-benzylidene-1-tetralones as well as 2-heteroarylidene-1-tetralone derivatives as potential new classes of MAO inhibitors. The effect of modification of ring C on MAO inhibition activity and isoform selectivity will also be determined. Furthermore, a variety of substituents will be explored on both rings A and B to determine the optimum substituents for MAO inhibitory activity. This study will thus attempt to design potent and selective inhibitors of MAO.

Objectives:

(a) To synthesise 2-benzylidene-1-tetralone and related 2-heteroarylidene-1-tetralone derivatives with substituents on ring A and/or ring B. This study will also synthesise benzylidene-substituted indanone, benzosuberone, chromone, thiochromone and chomanone derivatives.

(b) To characterise the synthesised compounds using spectroscopic methods such as proton nuclear magnetic resonance (^{1}H NMR), carbon-13 nuclear magnetic

resonance (^{13}C NMR) and high resolution mass spectroscopy (HRMS) as well as to determine purity by high performance liquid chromatography (HPLC) analyses.

(c) To evaluate the synthesised compounds as inhibitors of recombinant human MAO-A and MAO-B.

(d) To analyse the SARs of MAO inhibition by the various derivatives synthesised in this study.

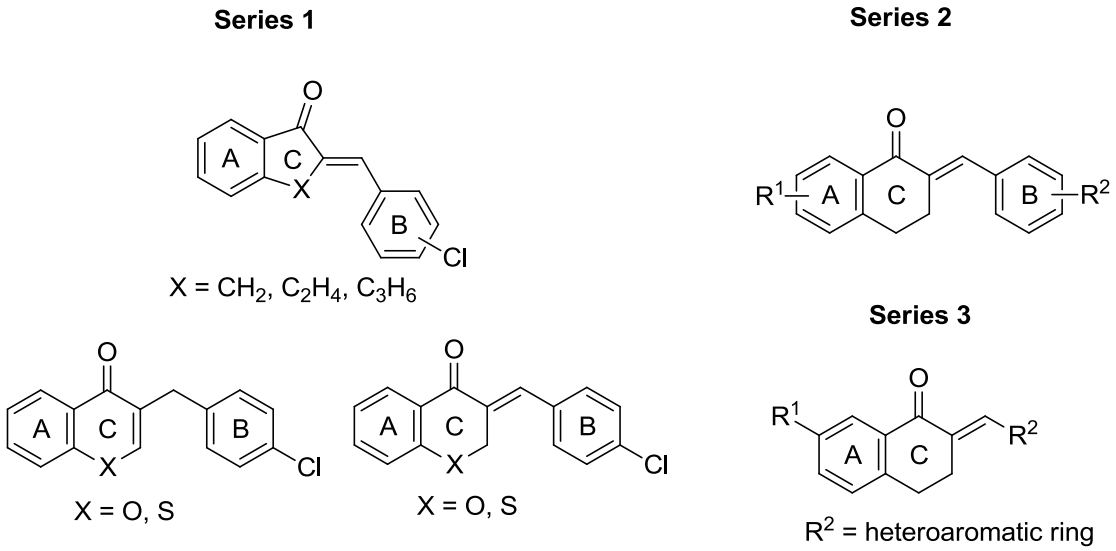


Figure 1.3: General structures of the three series of chalcone classes to be studied.

The following chapter will present the literature background of the study while the experimental section and discussion will be presented in chapters 3 to 5. Chapter 3 will explore the MAO inhibition properties of benzylidene-substituted indanones, tetralones, benzosuberones, chromones, chromanones and thiochromanones. Chapter 4 will explore the MAO inhibition properties of 2-benzylidene-1-tetralones while chapter 5 will focus on the MAO inhibition properties of 2-heteroarylidene-1-tetralone derivatives. Finally chapter 6 will provide a summary of the study.

REFERENCES

Agid, Y., Ahlskog, E., Albanese,A., Calne, D., Chase, T., De Yebenes, J., Factor, S., Fahn,S,, Gershanik,O., Goetz, D., Koller, W., Kurth, M., Lang, A., Lees,A., Lewitt, P., Marsden, D., Melamed, E., Michel, P.P., Mizuno, Y., Obeso, J., Oertel, W., Olanow, W., Poewe, W., Pollak, P., Przedzorski, S., Quinn, N., Raisman-Vozari, R., Rajput, A., Stocchi, F. & Tolosa, E. 1999. Levodopa in the treatment of Parkinson's disease: a consensus meeting. *Movement disorders*, 14(6):911-913.

Chimenti, F., Fioravanti, R., Bolasco, A., Chimenti, P., Secci, D., Rossi, F., Matilde, Y., Orallo, F., Ortuso, F. & Alcaro, S. 2009. Chalcones: a valid scaffold for monoamine oxidases inhibitor. *Journal of medicinal chemistry*, 52(9):2818-2824.

Dauer, W. & Przedborski, S. 2003. Parkinson's disease: mechanisms and models. *Neuron*, 39(6):889-909.

Ferino, G., Cadoni, E., Matos, M.J., Quezada, E., Uriarte, E., Santana, L., Vilar, S., Tatonetti, N.P., Yanez, M., Vina, D., Picciau, C., Serra, S. & Delogu, G. 2013. MAO inhibitory activity of 2-arylbenzofurans versus 3-arylcoumarins: synthesis, in vitro study, and docking calculations. *ChemMedChem*, 8(6):956-966.

Finberg, J.P.M. 2014. Update on the pharmacology of selective inhibitors of MAO-A and MAO-B: focus on modulation of CNS monoamine neurotransmitter release. *Pharmacology & therapeutics*, 143(2):133-152.

Fowler, J.S., Logan, J., Wang, G.J., Volkow, N.D., Zhu, W., Franceschi, D., Pappas, N., Ferrieri, R., Shea, C., Garza, V., Xu, Y., MacGregor, R.R., Schlyer, D., Gatley, S.J., Ding, Y. & Alexoff, D. 2002. PET imaging of monoamine oxidase B in peripheral organs in humans. *Journal of nuclear medicine*, 43(10):1331-1338.

Gareth, T. 2007. Medicinal chemistry: an introduction. Chichester: Wiley. http://www.wiley.com/WileyCDA/WileyTitle/productCd-0470025972.html. Date of access: 31 October 2016.

Gillman, P.K. 2005. Monoamine oxidase inhibitors, opioid analgesics and serotonin toxicity. *British journal of anaesthesia,* 95(4): 434-441.

Hubálek, F., Binda, C., Khalil, A., Li, M., Andrea Mattevi, A., Castagnoli, N. & Edmondson, D.E. 2005. Demonstration of isoleucine 199 as a structural determinant for the selective inhibition of human monoamine oxidase B by specific reversible inhibitors. *Journal of biological chemistry*, 280(16):15761-15766.

Lees, A.J., Hardy, J. & Revesz, T. 2009. Parkinson's disease. *The Lancet*, 373(9680):2055-2066.

Macphee, G.J.A. & Stewart, D.A. 2012. Parkinson's disease - pathology, aetiology and diagnosis. *Reviews in clinical gerontology*, 22(3):165-178.

Muller, T. 2015. Catechol-O-methyltransferase inhibitors in Parkinson's disease. *Drugs,* 75:157-174.

Robinson, S.J., Petzer, J.P., Petzer, A., Bergh, J.J. & Lourens, A.C.U. 2013. Selected furanochalcones as inhibitors of monoamine oxidase. *Bioorganic & medicinal chemistry letters*, 23(17):4985-4989.

Singh, D., Shrimali, S. & Rathore, K.S. 2015. A review on Parkinson's disease: its pathophysiology, treatment and surgery. *Pharmatutor*, 3:25-32.

Stewart, D.A. 2008. Pathology, aetiology and pathogenesis. *(In* Playfer, J.R. & Hindle J.V., *eds*. Parkinson's disease in the older patient. United Kingdom: Radcliffe p. 11-26)

Thanvi, B. & Lo, T. 2004. Long term motor complications of levodopa: clinical features, mechanisms, and management strategies. *Postgraduate medical journal*, 80(946):452-458.

CHAPTER 2: LITERATURE REVIEW

2.1 Parkinson's disease

2.1.1 General Background

PD was first described in 1817 by James Parkinson and is the second most-prevalent age-related neurodegenerative disorder following AD (Dauer & Przedborski, 2003). It is characterised by the depigmentation of the SNpc and the selective and progressive degeneration of dopaminergic neurons. In the affected neurons intraneuronal proteinaceous cytoplasmic inclusions known as Lewy-bodies are found (Wood-Kaczmar *et al.*, 2006). The disease is prevalent in the aged population affecting 2% and 5% of individuals over the age of 65 and 85, respectively (Muller, 2015).

The loss of the SNpc neuronal pathway leads to striatal DA deficiency, which causes the dysregulation of the motor circuits that project throughout the basal ganglia, resulting in the clinical manifestations of PD (Dauer & Przedborski, 2003; Wood-Kaczmar *et al.*, 2006). The classical motor symptoms of PD are bradykinesia, rigidity and tremor, and develop when about 50% of the striatal dopaminergic nigrostriatal neurons and about 80% striatal DA production are lost (Teo & Ho, 2013; Muller, 2015). Non-dopaminergic neurons may also undergo degeneration in PD resulting in a variety of non-motor features such as dementia, psychosis, depression and apathy (Teo & Ho, 2013).

The current drug therapy for PD is symptomatic and primarily aimed at restoring dopaminergic function in the striatum (Brooks, 2000). Whereas, motor symptoms respond well to dopaminergic therapies such as levodopa and DA agonists, non-motor symptoms display little or no response. Thus non-motor symptoms may be more disabling to the patient and may cause a far greater impact on the patient's quality of life than motor symptoms (Teo & Ho, 2013).

2.1.2 Aetiology

Regardless of extensive research done, the cause of PD remains uncertain. However, it is very clear as to which part of the brain is affected and possible mechanisms leading to the pathogenesis of PD have been proposed. It is documented that PD may have genetic and environmental risk factors (Ghavami *et al.*, 2014) (figure 2.2).Thus familial PD accounts for 5 to 10% of PD cases while the common sporadic PD accounts for 90% (Nicoll, 2006).

The role of the environment in the etiology of PD was discovered in 1983 (Dauer & Przedborski, 2003). It was postulated that exposure to environmental toxins such as pesticides (rotenone and dieldrin) as well as herbicides [paraquat (PQ)] may cause PD (Betarbet *et al.*, 2000; Hatano *et al.*, 2009; Berry *et al.*, 2010). Other risks factors associated with PD development are well-water drinking (Singh *et al.*, 2015) and chronic exposure to chemicals such as manganese and carbon disulphide (Berry *et al.*, 2010). Exposure to the synthetic toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been shown to produce permanent Parkinsonism in humans, non-human primates and rodents by exerting an effect primarily on the function of the mitochondrial complex I (Berry *et al.*, 2010). MPTP is metabolised to 1-methyl-4-phenylpyridinium (MPP^+), which is a mitochondrial toxin. The structural similarity between MPP^+ and PQ (Figure 2.1) suggests that PQ may also act as a mitochondrial toxin (Dauer & Przedborski, 2003).

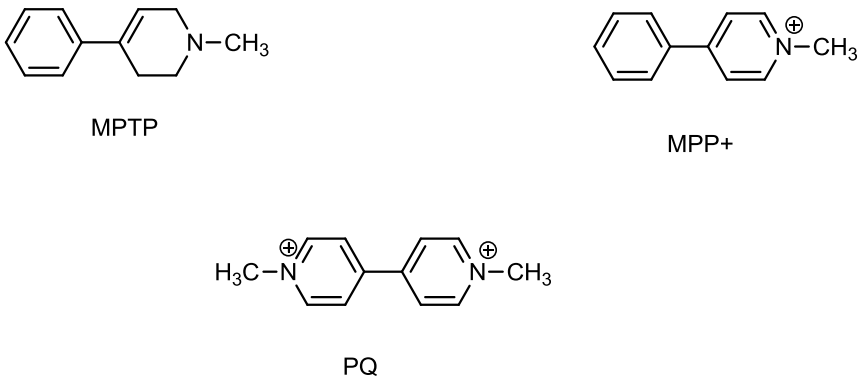


Figure 2.1: Chemical structures for MPTP, MPP^+ and PQ.

It is estimated that approximately 5% of patients with clinical features of PD have a clear familial etiology, which exhibits a classical recessive or dominant Mendelian mode of inheritance (Hatano *et al.*, 2009; Hatano & Hattori, 2011). In recent years, mutations in several genes have been shown to cause PD (Nicoll, 2006). These genes include, amongst others, two autosomal dominant genes α-synuclein (*SNCA*) and leucine-rich repeat kinase 2 (*LRRK2*), and three autosomal recessive genes parkin, *DJ-1* and PTEN-induced putative kinase 1 (*PINK1*) (Wood-Kaczmar *et al.*, 2006). α-Synuclein is an abundant brain protein of 140-amino acid residues that is distributed throughout the brain, especially in presynaptic nerve terminals (Lee & Trojanowski, 2006; Breydo *et al.*, 2012). In 1997, an α-synuclein gene mutation, A53T, was isolated from Italian relatives and three unrelated families of Greek origin with autosomal dominant PD (Hatano *et al.*, 2009). These findings are of much relevance to PD, mainly because of the fact that α-synuclein is a component of Lewy-bodies (Spillantini *et al.*, 1997), which are a

characteristic pathologic hallmark of both familial and sporadic PD (Gasser, 2009). Therefore, similar to other proteins associated with neurodegenerative diseases, aggregation of α-synuclein is considered to be a key event in dopaminergic neuronal cell death in both α-synuclein-linked and sporadic PD (Lee & Trojanowski, 2006). The LRRK2 gene is a very large gene occupying 144 kb of the genomic region and it has been identified to exhibit up to 20 mutations of which six are pathogenic (Gasser, 2009). As opposed to the 140 amino-acid residues of α-synuclein, the LRRK2 protein has 2527 amino acids and LRRK2 mutations account for the most common autosomal dominant familial PD cases identified thus far (Rideout & Stefanis, 2014). It is noteworthy that supporting data on the neuropathological features of these mutations are often conflicting and depend on the type and sensitivity of techniques used in the studies (Wood-Kaczmar *et al.*, 2006). Autopsy of certain patients with LRRK2 mutations have, however, revealed neuronal cell loss accompanied by Lewy-bodies similar to those of sporadic PD (Hatano & Hattori, 2011).

In 1998, Shimura *et al.* found mutations in a gene (Matsumine *et al.*, 1997) that was linked to autosomal recessive familial PD. This gene was designated as PARK2 and encodes for the protein, *parkin*. Parkin is a 465 amino acid ubiquitin-protein ligase that facilitates the degradation of proteins that interact with ubiquitin-conjugating enzyme UbcH7 (Shimura *et al.*, 2012). The second protein implicated with autosomal recessive early-onset PD (EOPD) is DJ-1. The human DJ-1 protein contains 189 amino acid residues and it is localized in the brain as well as extra-cerebral tissues. Although its exact function is unclear, it is reported to be involved in many cellular processes (Bonifati *et al.*, 2003a; Bonifati *et al.*, 2003b) which include the protection of mitochondria against oxidative stress (Canet-Aviles *et al.*, 2004).

Oxidative stress is a central mechanism in DJ-1 linked familial PD. The antioxidant properties of the DJ-1 protein was supported by studies by Taira *et al.* (2004), which tested the *in vitro* and *in vivo* elimination of hydrogen peroxide by recombinant DJ-1 and mutant forms of DJ-1 (transfected into cultured cells), respectively. Other less common autosomal recessive EOPD genes are *PINK1, ATP13A2* and *UCHL1* (Taira *et al*., 2004).

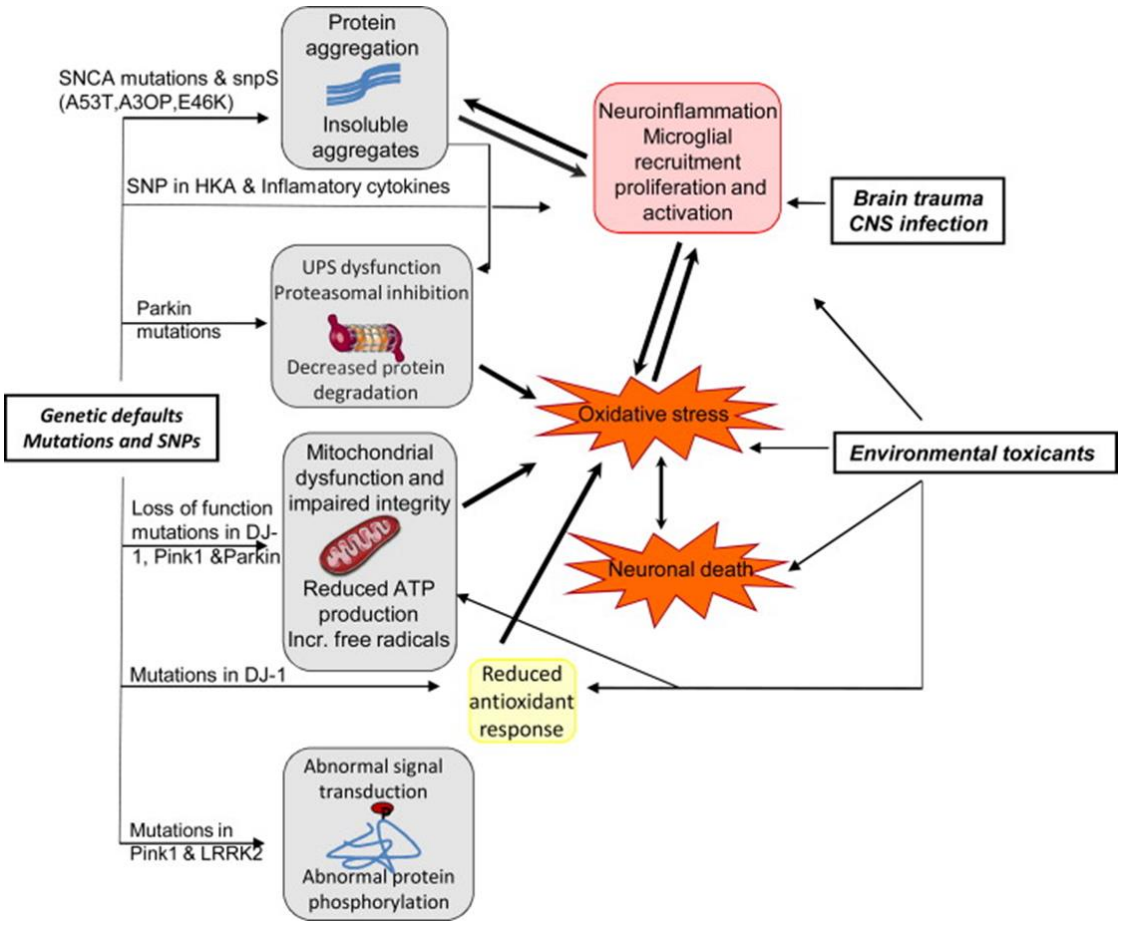


Figure 2.2: The role of genetic factors and their interplay with environmental factors in PD.

Mutations in PINK1 and LRRK2 causes oxidative stress in dopaminergic neurons. Oxidative stress and other cellular-stress stimuli may lead to neuronal cell death by causing mutations in Parkin, DJ-1 or the PINK1 gene which results in mitochondrial dysfunction or inflammatory processes within the neuronal tissues (Ghavami *et al.*, 2014).

2.1.3 Pathophysiology in Parkinson's disease

2.1.3.1 Anatomy and physiology of the basal ganglia

The pathophysiology of PD may be understood from the basal ganglia circuitry point of view. This is because both voluntary and non-voluntary movements are coordinated within the basal ganglia motor circuit. This is in agreement with the understanding that PD is only diagnostic once there is evidence of difficulties in the movement of a living organism. The human basal ganglia is composed of the neostriatum (caudate and putamen), the external and internal pallidal segments (GPe, GPi), the subthalamic nucleus (STN) and the *substantia nigra* which contains the *pars reticulata* (SNpr) *and* SNpc (Mink, 1996). The basal ganglia are located in the subcortical section of the midbrain, where information from the cortex is integrated in order to

coordinate movement (Brown & Williams, 2005). Information from the cortex passes through the basal ganglia to the thalamus, and then returns to the supplementary motor area of the cortex through the dopaminergic pathway (Mink, 1996; Goole & Amighi, 2009).

During normal physiological conditions, the striatum and the subthalamic nucleus receive glutamatergic afferents from specific areas of the cerebral cortex or thalamus and transfer the information to the basal ganglia output nuclei, GPi and SNpr (Goole & Amighi, 2009). Striatonigral dopaminergic projections connect into the circuit via two pathways (direct and an indirect) that are controlled by two DA receptors (D1 and D2) which play a role in the development of PD and in mediating the antiparkinsonian effects of DA substitutes (Deogaonkar & Subramanian, 2005). The direct pathway involves D1 receptors and acts to reduce the inhibitory output from the GPi/SNpr to the motor thalamus that returns it back to the cerebral cortex and then to the striatum. The indirect pathway on the other hand involves the D2 receptors and acts via the GPe (Macphee & Stewart, 2012) (Figure 2.3). Both pathways are mediated by *γ*-aminobutyric acid (GABA).

The two pathways have antagonistic effects. The direct pathway may inhibit the activity of the GPi and SNr causing disinhibition of the thalamocortical interactions, whereas the indirect pathway does the opposite (Mink, 1996). Normal DA release from the SNpc works on both the direct and indirect pathways. As a result, physiological dopaminergic stimulation may increase activity in the thalamocortical projection neurons as it inhibits the GPi and SNpr activity, possibly leading to greater activation of the cerebral cortex that can play a role in the facilitation of movement (Galvan & Wichmann, 2008; Goole & Amighi, 2009). In PD, decreased DA release from the SNpc disrupts the physiological dopaminergic mechanism. Under-stimulation and under-inhibition of the direct and indirect pathways, respectively, result in increased inhibitory output from the GPi/SNpr (Galvan & Wichmann, 2008). Thus, thalamocortical interactions to the motor cortex are diminished and movement is inhibited causing akinesia (Stewart, 2008).

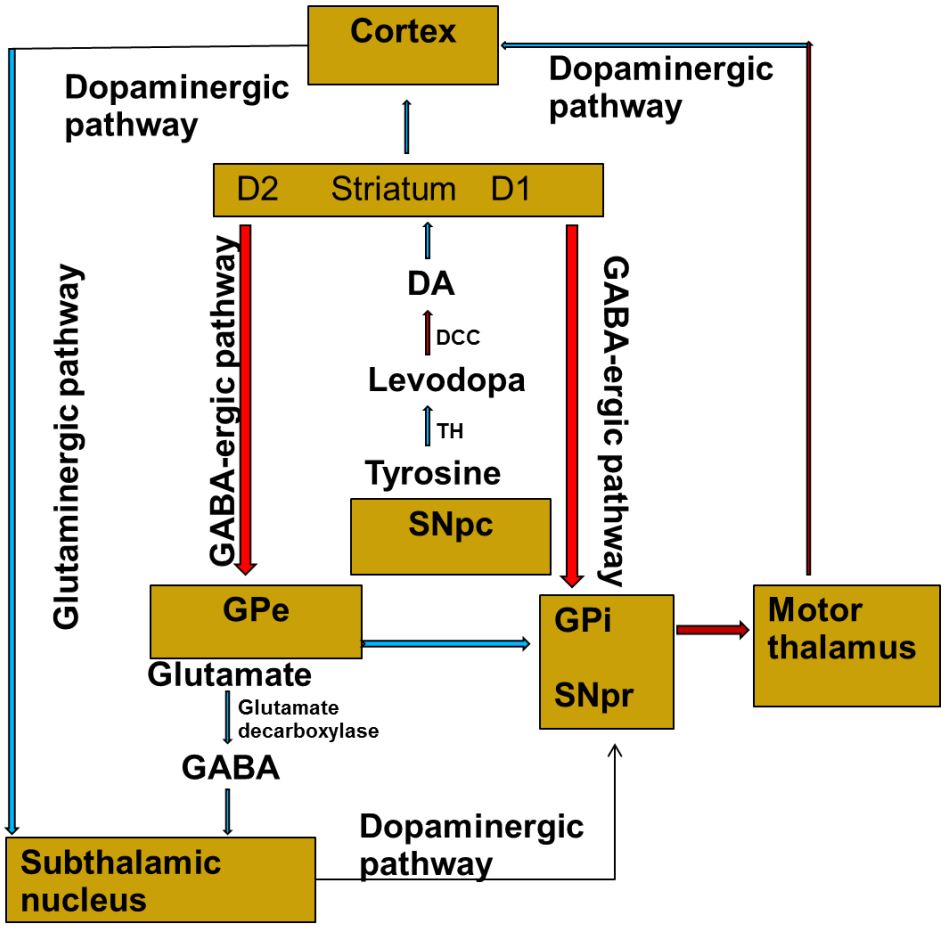


Figure 2.3: Schematic activity in the basal ganglia-thalamocortical motor circuit (Goole & Amighi., 2009).

2.1.3.2 Pathophysiology of PD

2.1.3.2.1 Neurochemical and neuropathological features

As previously stated, the pathological hallmarks of PD are the loss of nigrostriatal dopaminergic neurons and the presence of Lewy-bodies. The main area of neurodegeneration and Lewy-body formation is in the SNpc, which is further divided into the dorsal and ventral tiers. The former tier is known to contain more neuromelanin than the latter, hence DA is more depleted in the dorsal tier in PD (Stewart, 2008). In PD the SNpc neuronal loss occurs mostly in the ventral tier, which is in contrast with normal ageing where the dorsal tier is the most affected (Stewart, 2008). Given these differential patterns of cell loss in PD and normal aging, the hypothesis that aging plays a role in the degenerative process can be nullified. Apart from the SNpc, neurodegeneration and Lewy-body formation affects cells of the locus coeruleus, serotonergic neurons in the raphe, cholinergic neurons in the nucleus basalis of Meynert and the dorsal motor nucleus of the vagus system as well as the cerebral cortex, olfactory bulb and the autonomic nervous system (Dauer & Przedborski, 2003; Stewart, 2008).

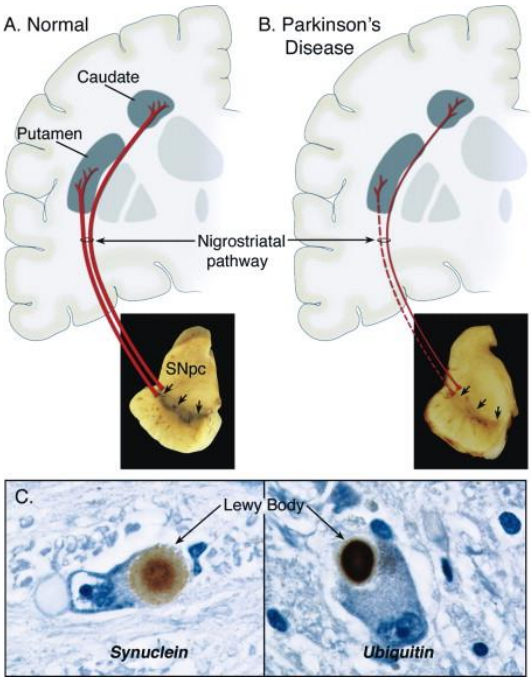


Figure 2.4: Neuropathology of PD.

(A) Schematic representation of the normal nigrostriatal pathway (in red) and a photo of the normal pigmentation of the SNpc (see arrows) due to neuromelanin. (B) A diseased nigrostriatal pathway showing a marked loss of dopaminergic neurons to the putamen (dashed line) and an intense loss of those that project to the caudate (thin red solid line). The photograph illustrates depigmentation of the SNpc (arrows). (C) Immunohistochemical labeling of Lewy-bodies in a SNpc dopaminergic neuron. Immunostaining with an antibody against α-synuclein reveals a Lewy-body (black arrow) with an intensely immunoreactive central zone surrounded by a faintly immunoreactive peripheral zone (left photograph). Conversely immunostaining with an antibody against ubiquitin yields more diffuse immunoreactivity within the Lewy-body (right photograph) (Dauer & Przedborski, 2003).

At the onset of symptoms, approximately 80 % of the putamenal DA and 60 % of dopaminergic neurons in the SNpc have already been lost (Dauer & Przedborski, 2003). The loss of nigrostriatal DA neurons leads to the inhibition of thalamus and motor cortex activity, as well as an increase of striatal cholinergic activity, which contributes to the tremor (Wells *et al.*, 2009). Degeneration of hippocampal structures and cholinergic cortical inputs contribute to dementia (Dauer & Przedborski, 2003) related to PD. Although the diagnosis of PD is made on clinical aspects, it is important to note that the relationship between clinical and pathological features of PD is not clear and a definite diagnosis requires the identification of both Lewy-bodies and SNpc dopaminergic neuron loss. Lewy-bodies can be detected by immunostaining for α-synuclein and even ubiquitin (Nicoll, 2006) (Figure 2.4).

The pathogenesis of PD is still unknown. However, there are important mechanisms that may play major roles and have potential effects in the pathophysiology of PD. These include oxidative stress, mitochondrial dysfunction, inflammation and excitotoxicity (Macphee & Stewart, 2012; Ghavami *et al.*, 2014). These mechanistic events lead to neuronal death in PD via apoptosis (Figure 2.5) (Ali *et al.*, 2011; Macphee & Stewart, 2012). Apoptosis, also termed programmed cell death, is a normal physiological process in the development of the nervous system crucial in the maintenance of its homeostasis (Stewart, 2008).

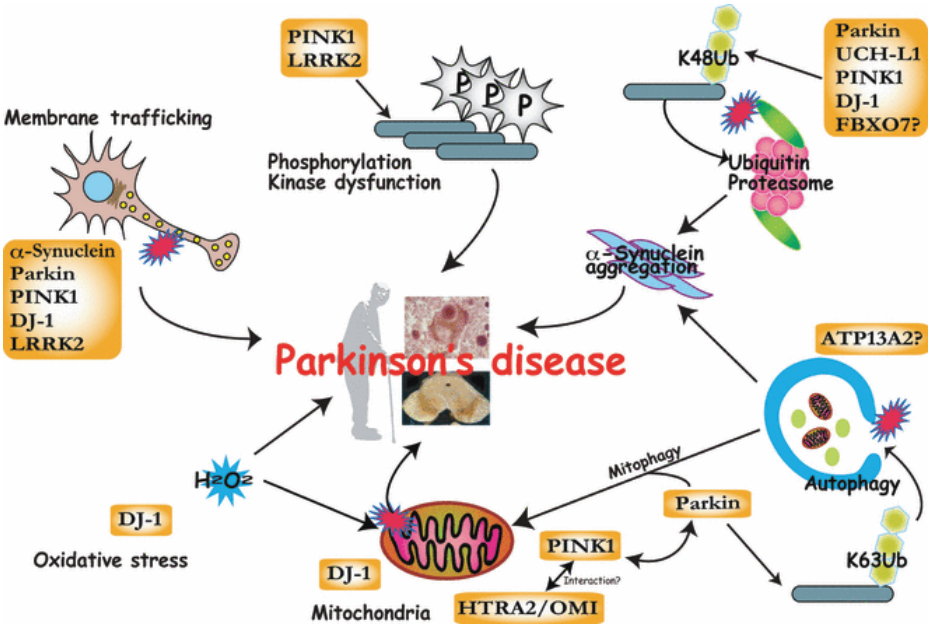


Figure 2.5: Mechanisms of neurodegeneration in PD

(Hatano *et al*., 2009).

2.1.3.2.2 Mechanisms of neurodegeneration

(a) Oxidative stress

Oxidative stress is an imbalance between ROS production and antioxidant activities, leading to potential damage (Sies, 1997; Halliwell, 2007). Several mechanisms including depletion of antioxidants, defects in mitochondrial electron transport, neurotoxin exposure, and excessive oxidation of DA may cause oxidative stress in PD (Alam *et al.*, 1997). The presence of iron, neuromelanin and DA in the SNpc makes these cells vulnerable to oxidative damage. The MAO catalysed metabolism of DA produces hydrogen peroxide, which subsequently, via the Fenton reaction, is converted to highly toxic hydroxyl radicals in the presence of ferrous iron. Neuromelanin can reduce ferric iron to ferrous iron thus increasing hydroxyl radical formation (Macphee & Stewart, 2012). DA can also undergo auto-oxidation that produces hydrogen peroxide and superoxide radicals (Figure 2.6) (Stewart, 2008).

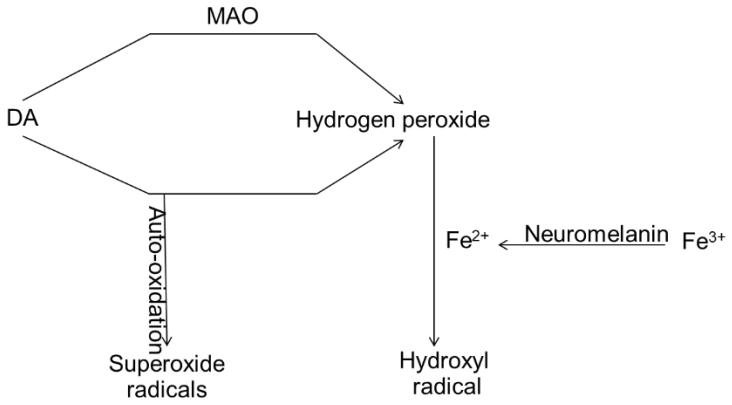


Figure 2.6: Oxidative mechanisms and free radical production in PD

(Adapted from Stewart, 2008).

Although the human body has cellular defense mechanisms against ROS under physiological conditions, it is well documented that ROS lead to the loss of neurons in PD (Dringen, 2000). The enzymes such as catalase and superoxide dismutase (SOD), antioxidants such as ascorbate, α-tocopherol and most importantly glutathione (GSH) represent very important systems for the cellular defense against ROS (Dringen, 2000). However, postmortem and rat studies have shown that levels of GSH are reduced in PD brains (Zhu *et al.*, 2006). Oxidative stress in the SNpc of PD is supported by evidence of increased iron levels, decrease GSH, increased SOD activity, decreased activity of complex I of the mitochondrial respiratory chain, increased levels of malondialhehyde (a product of lipid peroxidation) and of 8-hydroxy-2-deoxyguanosine (positive sign for oxidative damage of DNA) (Dringen, 2000; Stewart, 2008; Macphee & Stewart, 2012).

(b) Mitochondrial dysfunction

Evidence for a role of mitochondrial dysfunction in PD pathology comes from findings that exposure to MPTP causes inhibition of the mitochondrial complex I, essential in the electron transport chain (ETC) (Schapira *et al.*, 1990). This was further supported by PD models of MPTP and other mitochondrial toxins aimed at impairing the ETC in order to observe the impact it has in the SNpc (Dauer & Przedborski, 2003). The studies indicated that normal mitochondrial function is essential in preventing neural death due to oxidative stress as these cells do not have sufficient defense mechanism (Gleave *et al.*, 2014). In addition, postmortem studies on PD patients indicated a deficiency in the function of complex I (Schapira *et al.*, 1990). Deficiency of the mitochondrial complex I is not only present in SNpc but also in platelets and other tissues of PD patients, implying that it can be inherited through mitochondrial DNA (Dauer & Przedborski,

2003). When compared to the activity of normal complex I, the defect in PD is a 35 % reduction in activity (Schapira, 2008). Mitochondrial dysfunction is also attributed to the localisation of both autosomal dominant and recessive gene mutations in the mitochondria (Figure 2.5).

(c) Inflammation

Inflammation is a self-defence reaction against various pathogenic stimuli that helps the organism to respond to pathogens. However, chronically impaired inflammation can become a harmful self-damaging process to host tissues (Ghavami *et al.*, 2014). It is postulated that neuroinflammation can cause neurodegeneration in PD (Macphee & Stewart, 2012). This is evident from the presence of activated microglial cells (Tansey & Goldberg, 2010) and elevated levels of the inflammatory mediators TNF-α, interleukins 1β and 6 (IL-1β and IL-6) and IFN-γ in the cerebrospinal fluids (CSF) and the postmortem brain tissue of PD patients (Hirsch & Hunot, 2009; Glass *et al.*, 2010). The production of ROS and nitric oxide under these conditions is also reported, which causes cellular damage via oxidative stress (Liu & Hong, 2003; Glass *et al.*, 2010). Inflammation alters the permeability of the BBB, allowing entrance of immunocompetent cells to the brain.

(d) Excitotoxicity

In neurological disorders such as PD, excitotoxicity occurs due to an accumulation of certain acidic amino acid neurotransmitters such as glutamate and aspartate. These CNS neurotransmitters, under normal physiological conditions, are involved in the activation of glutamate receptors for neuronal excitation, to render responses such as attention, alertness and learning (Blaylock, 2004). However, excessive concentrations of excitatory amino acids in the synaptic cleft cause over-stimulation of the post-synaptic neurons leading to neurotoxicity. Therefore, regulation of extracellular glutamate levels by glutamate transporters is crucial. Glutamate transporters remove the glutamate for storage in the presynaptic neuron terminal or surrounding glia (Lipton & Rosenberg, 1994).

Glutamate receptors exist as two types, ionotrophic (ion-gated channels) and metabotropic receptors (Simeone *et al.*, 2004). The three ionotrophic receptors are N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainite receptors, which control the influx of sodium, potassium and calcium ions through membrane channels resulting in neuronal excitement (Mattson, 2003). It is noteworthy that glutamate receptor activation can cause accumulation of calcium ions in the cytosol, which contributes to the cytotoxicity process (Blaylock, 2004). An increase in intracellular calcium due to NMDA activation can activate the metabolic activities of enzymes such as protein kinase C,

phospholipases, proteases, protein phosphatases and nitric oxide synthase resulting in the formation of superoxide anions and oxygen free radicals, which in turn may cause lipid peroxidation (Lipton & Rosenberg, 1994). Such conditions favor neuronal death via oxidative stress and dysfunctional mitochondria.

2.1.4 Symptomatic treatment of PD

2.1.4.1 Dopamine

The treatment of PD remains symptomatic. However, with characteristic DA depletion in PD patients , current treatment focuses on DA replacement for restoration of dopaminergic transmission at striatal synapses (Goole & Amighi, 2009). Most motor and some non-motor PD symptoms ameliorate with DA substitution. It is therefore critical to understand the dopaminergic pathway and the mode of action of a particular drug prior to introducing it as drug therapy for PD.

DA is a catecholamine produced in the basal ganglia of the brain and facilitates movement coordination. It is synthesised from the amino-acid tyrosine, which is first converted to levodopa by the enzyme tyrosine hydrolase (TH). Thereafter, levodopa is converted by the enzyme, DCC to DA (Figure 2.7) which is then, transported by vesicular monoamine transporter into storage vesicles (Figure 2.8) (Riederer *et al.*, 2007). DA is metabolised in the postsynaptic cleft by the enzymes, COMT and MAO. COMT adds a methyl group to the catecholamine function of DA, thereby degrading it, whilst MAO catalyses the oxidative deamination of the monoamine group (Goole & Amighi, 2009). DA is also a precursor of epinephrine and norepinephrine.

The disturbances in the homeostasis of DA in the basal ganglia controlled by DA receptors (D1 and D2) forms the basis for DA substitution therapy in PD. DA itself does not pass the BBB, hence its therapeutic delivery mode is via its metabolic precursor, levodopa (Figure 2.9) (Muller, 2015), via the large neutral amino acid transporter type 1 (LAT1) (Pardridge, 2002).

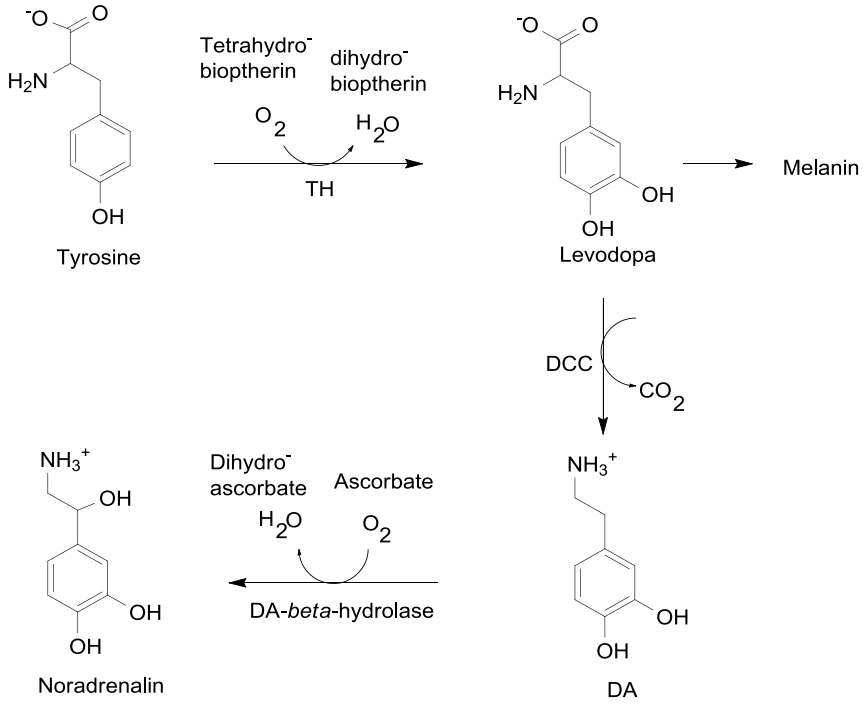


Figure 2.7: The synthesis of noradrenalin in the brain with DA as a precursor

(Fahn, 2008).

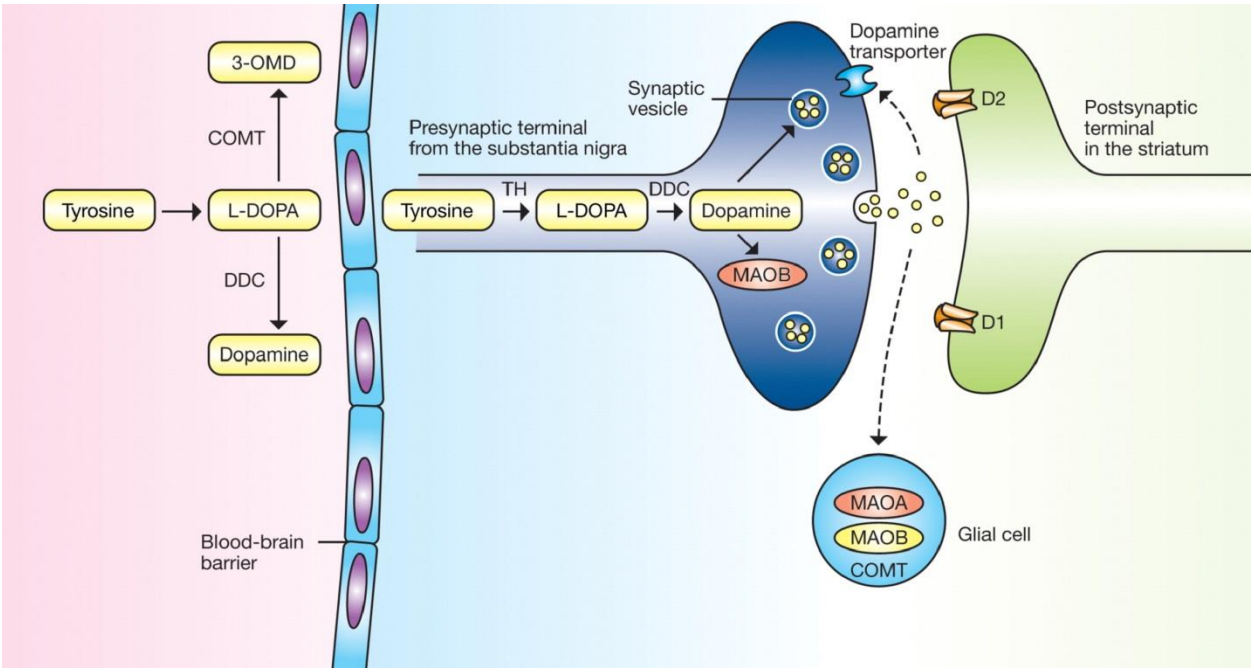


Figure 2.8: DA metabolic pathways in the brain

(Riederer et al., 2007).

2.1.4.2 Levodopa therapy

The use of levodopa to produce DA is deemed more effective at replacing the physiological actions of DA than any other therapeutic compound (Riederer *et al.*, 2007), hence levodopa is considered the gold standard in PD treatment (Olanow *et al.*, 2013). However, any mode of drug application requires plasma transportation to the active site. The delivery of levodopa to the brain in considerable amounts is hampered by the peripheral conversion to DA by the decarboxylase enzyme. This requires the administration of large amounts of levodopa in order to produce clinically relevant antiparkinsonian effects in the early years of clinical experience. These increased dosages are known to trigger the onset of the ON-OFF phenomena (i.e. ON-interval of good levodopa response to adequate dopaminergic neurotransmission and OFF-period of reduced motor performance) and dyskinesia, involuntary movements resulting from over-stimulation of the dopaminergic system (Muller, 2015). Dyskinesia occurs randomly during both the ON- and OFF-periods, leading to severe disability.

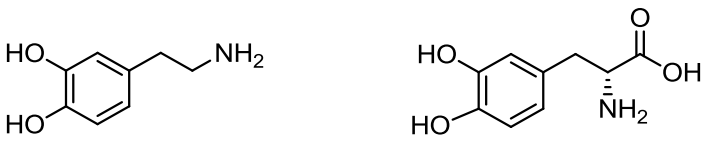
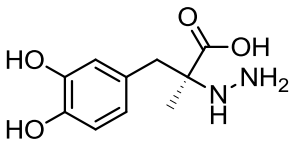


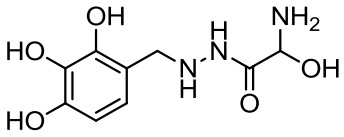
Figure 2.9: The structures of dopamine and levodopa.

The risk of developing dyskinesia is linked to various clinical factors such as the stage of PD, age of the patient and the dosage and dosing strategy of levodopa therapy (Olanow *et al.*, 2013). Levodopa therapy should therefore be strategically implemented to prevent the "ON-OFF" effect. Approaches include the (a) delay of the need for levodopa, (b) reduction of the cumulative dose of levodopa, (c) avoidance of the pulsatile stimulation of DA receptors and (d) implementation of neuroprotection (Goole & Amighi, 2009). All these strategies are aimed at prolonging the plasma half-life of levodopa, which is compromised by the numerous metabolic pathways of levodopa in the periphery.

Therefore, enzymatic inhibition of levodopa degradation in peripheral regions may reduce premature conversions of levodopa to DA. Combination therapy of levodopa with DCC inhibitors such as carbidopa and benserazide (Figure 2.10), which do not cross the BBB, allows for a 4-fold (60-80%) reduction in the levodopa dose with an increase in the levodopa plasma half-life from 60 to 90 minutes (Goole & Amighi, 2009). Additionally, characteristic peripheral side effects (i.e. nausea, vomiting and anorexia) of DA are blocked with this inhibition (Fahn, 2008).



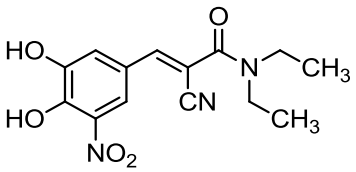
Carbidopa



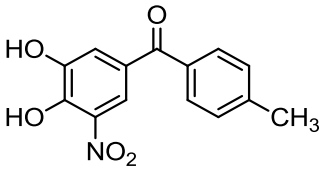
Benserazide

Figure 2.10: The structures of carbidopa and benserazide.

Other enzymatic inhibitors that may be used as adjuncts to levodopa are COMT and MAO inhibitors. The COMT enzymes become highly active as soon as the DCC is inhibited since these enzymes are metabolic competitors. COMT inhibitors such as entacapone and tolcapone (Figure 2.1111) block the metabolism of levodopa to its inactive metabolite, 3-*O*-methyldopa (3-OMD), and thereby increases and stabilises levodopa's plasma concentrations. With COMT inhibitors peripheral DA synthesis is also prevented. It is very important to avoid accumulation of 3-OMD because (a) it competes with levodopa at the large neutral amino acid transport carriers of the gastrointestinal tract (GI) and the BBB, (b) the 3-OMD metabolite has a longer half-life (15-24 hours) compared to levodopa and (c) it has no therapeutic value (Kaakkola, 2000). Therefore, increased peripheral 3-OMD concentrations could reduce the absorption, plasma bioavailability and brain delivery of levodopa (Muller, 2015). It is noteworthy that the inhibition of COMT during PD therapy should only be peripheral as central inhibition could enhance the effect of levodopa by inhibiting its conversion to 3-OMD. COMT inhibition in the CNS may also enhance the effect of DA by blocking the metabolism of DA to 3-methoxytyramine (Kaakkola, 2000). Figure 2. shows the schematic illustration of the metabolism of levodopa (Bonifati & Meco, 1999).



Entacapone



Tolcapone

Figure 2.11: Chemical structures of entacapone and tolcapone.

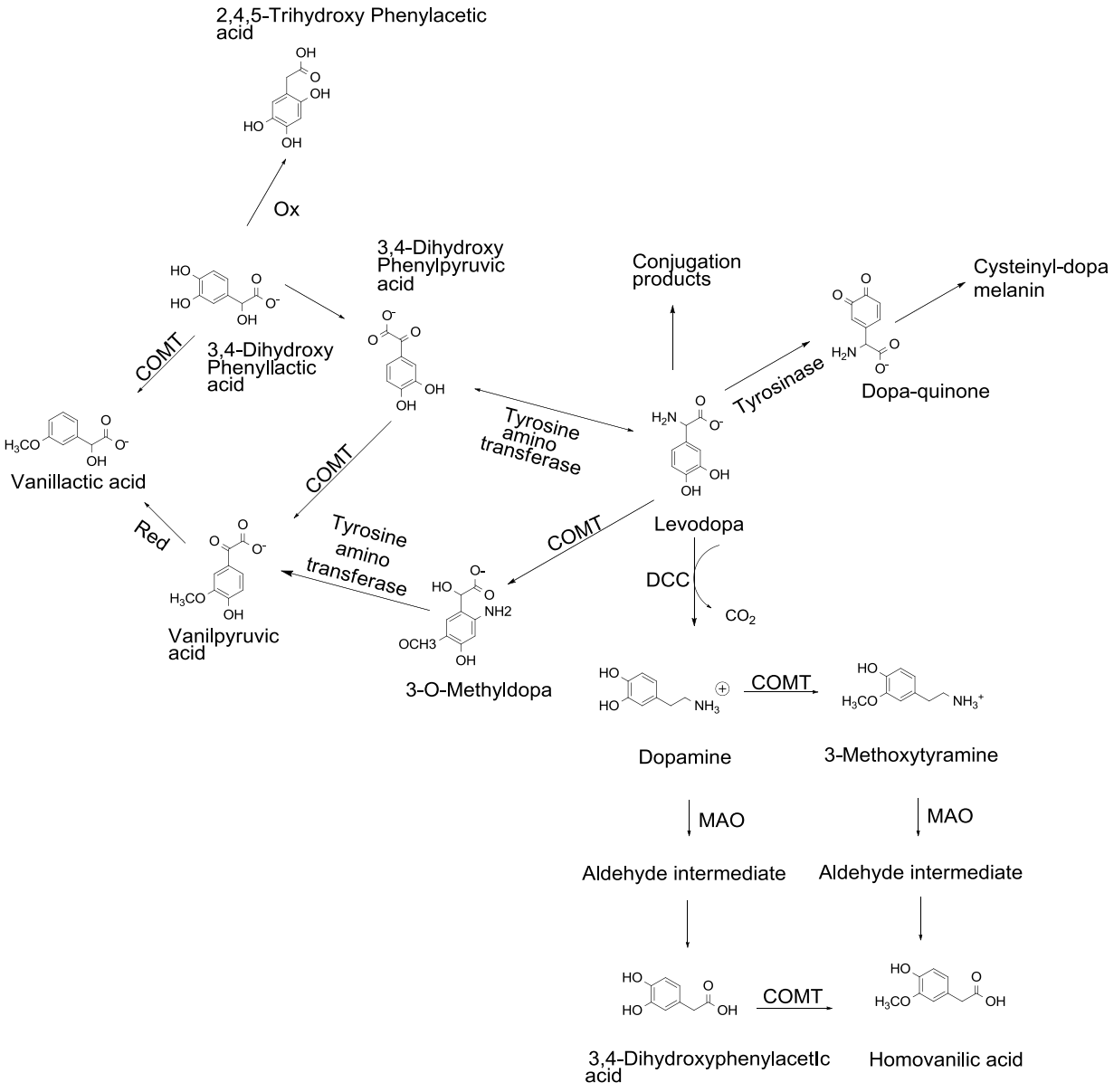


Figure 2.12: Schematic illustration of the metabolism of levodopa

(adapted from Bonifati & Meco, 1999).

The MAO enzymes catalyse the oxidative deamination of monoamines such as DA. Therefore inhibitors of MAO are also used as adjuncts to levodopa in the treatment of PD. MAO exist in two genetically distinct isoforms, known as MAO-A and MAO-B. The two isoforms display different substrate specificities, which is essential for drug development because it allows selectivity for inhibition of a single isoform. Both isoforms inactivate DA. The use of non-selective MAO inhibitors as adjuncts to levodopa results in hyper- and hypotensive episodes, whereas the use MAO-A selective inhibitors causes tyramine-related hypertension (Fahn, 2008). Selective MAO-B inhibitors do not pose such risks and are thus used for the treatment of PD.

Dopaminergic behaviours can also be mimicked by the use of DA agonists, which act directly on striatal DA receptors (Riederer *et al.*, 2007). DA agonists are divided into two subclasses, ergoline and non-ergoline agonists which all target D_2-receptors (Brooks, 2000). Ergoline agonists include bromocriptine, pergolide, lisuride and cabergoline, whereas non-ergoline agonists include ropinirole and pramipexole.

2.2 Monoamine oxidase

The MAOs are FAD-dependent enzymes found in the outer mitochondrial membrane of neuronal, glial and other mammalian cells (Ferino *et al.*, 2013). MAO was discovered by Mary Hare-Bernheim in 1928 as an enzyme that catalyses the oxidative deamination of tyramine. She called the enzyme tyramine oxidase (Youdim *et al.*, 2006). However, a few years later Hugh Blaschko realized that primary, secondary and tertiary amines (MAOs) are metabolised by the same enzyme which was named mitochondrial monoamine oxidase (Youdim *et al.*, 2006). Following these discoveries, it was found that an anti-tuberculosis drug, isoniazid, was also a potent MAO inhibitor and improved moods of patients receiving this drug (Fowler *et al.*, 2002; Youdim & Bakhle, 2006b).

2.2.1 Physiology

As mentioned earlier MAO exists as two subtypes, MAO-A and MAO-B. These are products of different genes and are distinguished by their amino acid sequences (Bach *et al.*, 1988), three-dimensional structures (Ferino *et al.*, 2013), tissue distribution (Grimsby *et al.*, 1990), inhibitor selectivity (Ma *et al.*, 2004) and substrate preferences (Shih *et al.*, 1999; Geha *et al.*, 2001). In humans, MAO-A predominates in the gut, placenta and heart, while MAO-B predominates in platelets and glial cells in the brain. Both enzymes are expressed in the liver (Ramsay, 2012). The MAO-A isoform has higher affinity for serotonin and noradrenaline, whereas the MAO-B isoform preferentially deaminates 2-phenylethylamine and benzylamine (Ma *et al.*, 2004). Both isoforms catalyse the deamination of dopamine and tyramine (Ferino *et al.*, 2013). It is their

affinities for different substrates that provide the basis for different therapeutic applications of inhibitors of these enzymes. For example, MAO-A inhibitors are used for the treatment of anxiety and depression, whereas MAO-B inhibitors are used for the treatment of AD and PD (Fowler *et al.*, 2002; Ferino *et al.*, 2013).

2.2.2 The structural composition of MAO

In order to understand the molecular properties of MAO, Bach *et al*. (1988) isolated and characterised the cloned cDNAs encoding the two MAO isoforms. Convincingly, it was demonstrated that the two enzymes are different, however with various similarities. The enzymes contain an identical pentapeptide fragment, Ser-Gly-Gly-Cys-Tyr, onto which the FAD (Figure 2.) redox cofactor is covalently bound through a thioether linkage between a cysteinyl residue and an 8α-methylene of the isoalloxazine ring (Edmondson *et al.*, 2004). The covalent FAD binding site for the two enzymes is near their carboxyl terminus as opposed to the amino terminus, where the dinucleotide fold of most FAD-depended enzymes is located (Edmondson *et al.*, 2004). DNA sequencing of human placental MAO-A and human liver MAO-B indicated that the two isoforms, respectively, consist of 527 and 520 amino acids. This corresponds to a molecular weight of 59.7 kDa for MAO-A and 58.8 kDa for MAO-B, and hence MAO-A is larger in size by about 2 kDA (Bach *et al.*, 1988).

2.2.2.1 The 3-dimensional structure of MAO-B

Human MAO-B crystalises as a dimer as opposed to the monomeric human MAO-A (Edmondson *et al.*, 2007). The dimer is attached to the mitochondrial membrane via the C-terminal amino acid residues 461-520 (Binda *et al.*, 2002; Edmondson *et al.*, 2004). The insertion onto the membrane is facilitated by the apolar surface of the C-terminal α-helix (Edmondson *et al.*, 2004).

The channel to the active site consists of two hydrophobic cavities through which the substrate passes before reaching the active site. The cavities are termed the “entrance cavity” and the “substrate cavity” (Figure 2.13) and are separated from each other by the side chain of isoleucine199 (Ile-199). This residue serves as a gate between the two cavities (Edmondson *et al.*, 2007). The gate can exist in an open or closed form and this, to a large degree, determines human MAO-B inhibitor specificity (Binda *et al.*, 2003). The substrate firstly enters the smaller entrance cavity (290 $Å^3$) with simultaneous activation of a movement of the four residues, Tyr326, Ile 199, Leu171 and Phe168, that lines the area between the two cavities (Binda *et al.*, 2002). This movement facilitates the substrate diffusion to the larger flat substrate cavity (420 $Å^3$). The entrance cavity is lined by residues Phe103, Pro104, Trp119, Leu164, Leu167,

Phe168, Leu171, Ile199, Ile316 and Tyr326 whereas the substrate cavity is lined by mainly aromatic and aliphatic amino acids, which create a hydrophobic environment for substrates or competitive inhibitors (Binda *et al.*, 2002; Binda *et al.*, 2004; Edmondson *et al.*, 2004).

Since the substrate cavity of MAO-B is larger than the entrance cavity, substrate and inhibitor entry to the cavity is restricted to a certain degree. This conformational restriction is attributed to the presence of the loop 99-112, which acts as a gating switch to the entrance cavity (Edmondson *et al.*, 2007). The side chain of Ile199 may rotate from the cavity in order for the two cavities to fuse, hence forming one larger cavity which accommodates larger substrates and inhibitors (Binda *et al.*, 2002). In contrast, smaller substrates and inhibitors do not require any loop alterations and their entrance to the cavity is relatively unrestricted (Finberg, 2014). The smaller bipartite elongated but compact substrate cavity in MAO-B, as oppose to the rounder and large cavity in MAO-A, gives substrates and inhibitors restricted freedom of rotation, which is known to have an effect on the catalytic properties of the enzyme (Edmondson *et al.*, 2007).

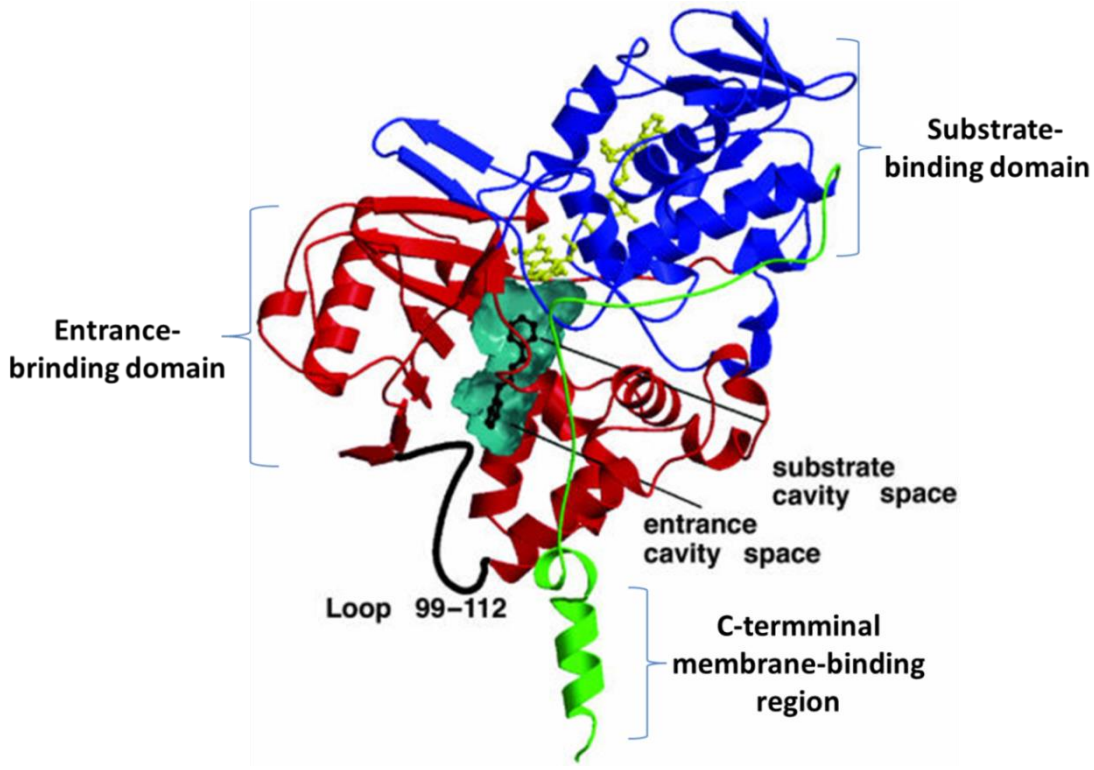


Figure 2.13: The 3-dimensional structure of human MAO-B in complex with rasagiline (black ball and stick), with the FAD-cofactor depicted in yellow ball and stick.

The binding domains are: substrate-binding in red, C-terminal membrane binding in green and the FAD-binding domain in blue (Binda *et al.*, 2003).

The FAD-cofactor in MAO-B is located at the back of the substrate cavity in the substrate binding site domain. The conformation between the amide linkage of the Cys397 (site of the flavin 8α-thioether linkage) and the Tyr398 is *cis* (Kearney *et al.*, 1971). Structural analysis indicates that the flavin ring exists in a bent conformation about the N(5)-N(10) axis as opposed to the normal planar conformation (Edmondson *et al.*, 2009). The substrate amine binds between the phenolic side chains of Tyr398 and Ty435, which forms an aromatic cage with the flavin (Binda *et al.*, 2002).

In a study by Binda *et al.* (2007), a complex between safinamide, a reversible MAO-B inhibitor, and human MAO-B indicated that the polarity of the substrate substituents plays a role in the orientation of inhibitors in the MAO-B active site. The polar substituents orient towards the hydrophilic space in front of the flavin to form H-bond interactions with conserved water molecules and polar protein residues. This behavior is also observed with other reversible inhibitors such as indole-2,3-dione (isatin) (Binda *et al.*, 2003).

2.2.2.2 The 3-dimensional structure MAO-A

The human MAO-A enzyme is monomeric whilst that of the rat is a dimer, similar to MAO-B (DeColibus *et al.*, 2005). The structure of MAO-A is composed of the extra-membrane and membrane binding domains, where the FAD and the substrate/inhibitor binding sites are within the extra-membrane domain (Figure 2.). Similar to MAO-B, attachment to the mitochondrial membrane is facilitated by the 35-40 amino acid residues of the C-terminal trans-membrane helix (Binda *et al.*,2002). The binding of human MAO-A to the membrane is similar to that of the rat MAO-A (Edmondson *et al.*, 2004). However, studies show that there is a difference in the structural binding between the two human isomers. Chimeric enzymes made from fragments of MAO-A and MAO-B indicated that swapping of the respective C-terminal helices causes loss of enzymatic activity and that decreasing the length of the C-terminal helix reduces catalytic activity (Gottowik *et al.*, 1995).

Similar to MAO-B, the FAD cofactor in MAO-A also is covalently bound to the extra-membrane regions through the 8α-thioether linkage at Cys406. In contrast to MAO-B, both rat and human MAO-A have single but larger substrate binding cavities with a protein loop at the entrances. The human and rat MAO-A cavity sizes are 550 $Å^3$ and 450 $Å^3$, respectively (Edmondson *et al.*, 2007). The protein loop composed of Phe208-Ile335 (equivalent to Ile199-Tyr326 in MAO-B) at the entrance of the human MAO-A substrate cavity is essential in determining substrate/inhibitor specificity of the enzyme (Edmondson *et al.*, 2009). When in complex with the reversible inhibitor, harmine, the inhibitor is located in the active site of the enzyme where it interacts with

Tyr69, Asn181, Phe208, Val210, Gln215, Cys323, Ile325, Leu337, Phe352, Tyr407, Tyr444 and the FAD (Son *et al.*, 2008). The space between the inhibitor and these residues is filled with seven water molecules, and the inhibitor and FAD are bridged by hydrogen bonds with two water molecules. The amide group of the Gln215 residue interacts with the inhibitor via π-π interactions (Son *et al.*, 2008).

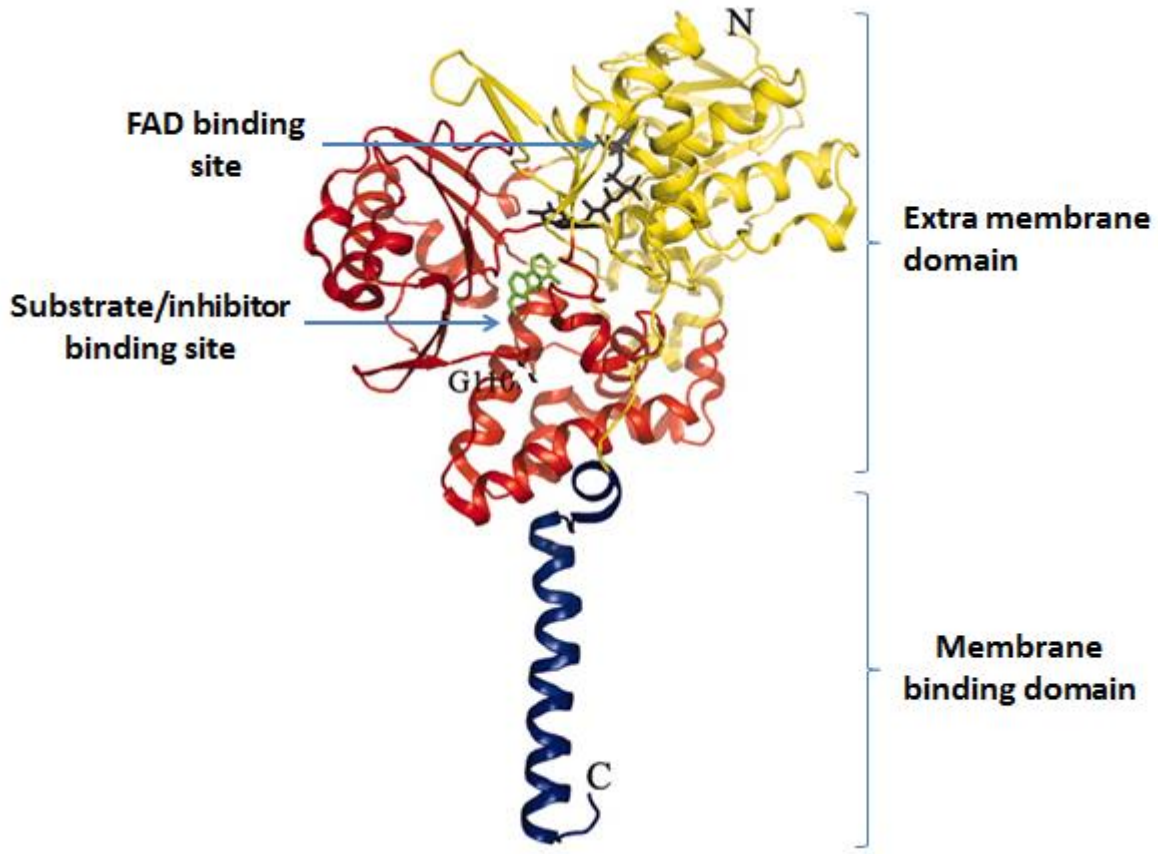


Figure 2.14: The ribbon structure of MAO-A in complex with harmine.

The N-terminus and C-terminus are marked. The FAD and harmine are the black and green stick models, respectively, in the extra-membrane domain (Son et al., 2008).

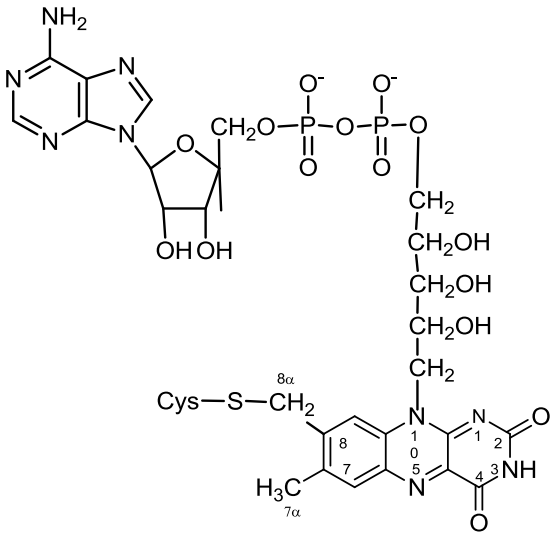


Figure 2.15: Structure of 8α-S-cysteinyl FAD

The conventional numbering scheme of the isoalloxazine ring is included (Edmondson et al., 2004).

2.2.3 The catalytic cycle of MAO

MAO-A and MAO-B catalyse the oxidative deamination of primary, secondary and tertiary amines as shown in the reaction below (Figure 2.6). It is assumed that MAO-A and MAO-B act by the same mechanism and that the differences in substrate and inhibitor specificities stems from structural features of the active sites of the respective isozymes. Figure 2.7 shows a comparison of the active sites of human MAO-A, MAO-B and rat MAO-A in complex with some non-covalent inhibitors. Various kinetic studies showed that the catalytic pathways for these oxidations by MAO-B are dependent on the nature of the substrate (Ramsay & Singer, 1991) while the substrate has no effect on the mechanism of MAO-A oxidation. The oxidative reaction of MAO requires a transfer of hydrogen from the α-CH_2 by cleavage of the C-H bond. Because of the stereochemical relation between the pro-R hydrogen of the substrate and the enzyme acceptor groups during catalysis, the mechanism of cleavage is reported to be strictly stereospecific for the pro-R in both MAO-A and MAO-B (Edmondson *et al.*, 2004).

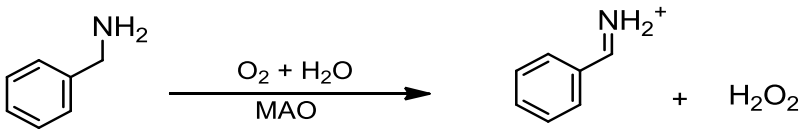


Figure 2.16: MAO-catalysed oxidative deamination reaction.

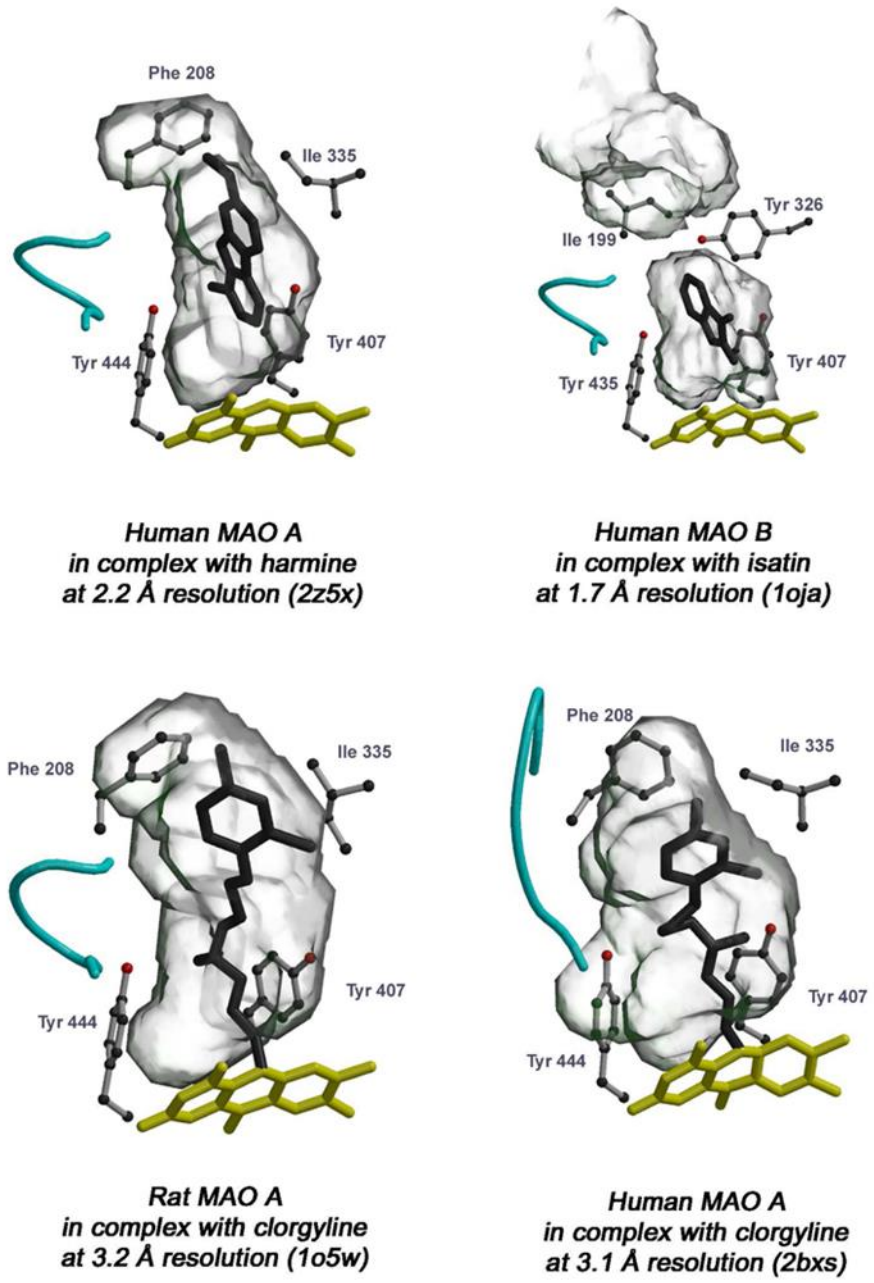


Figure 2.17: Comparison of the active sites of human MAO-A, MAO-B and rat MAO-A in complex with the non-covalent inhibitors, harmine and isatin, and a covalent inhibitor, clorgyline.

Stick models show the FAD in yellow and the inhibitors in black. The cavity shaping loop is indicated in cyan. The amino acid residues around the flavin form the conserved aromatic cage whereas substrate/inhibitor specific residues for MAO-A and MAO-B are also present (Edmondson *et al.*, 2009).

According to the ternary complex mechanism, MAO catalysis occurs via two pathways, either following the top or bottom loop of Figure 2. Most MAO catalysed reactions proceed with the

lower loop. According to the figure, the lower loop shows that oxygen reacts with the enzyme-product complex (the rate limiting step) prior to product (imine) dissociation. In contrast, the top loop indicates the rate limiting step to be product dissociation. Both pathways require a deprotonated form of the amine rather than the protonated form to enter the substrate binding site for interaction with the flavin. This is followed by oxidation of the enzyme-substrate complex to form the imine and the hydroquinone form of the reduced FAD cofactor. Finally the reduced FAD cofactor reacts with oxygen to produce the oxidised form of the FAD cofactor and hydrogen peroxide. After release from the enzyme the protonated imine undergoes hydrolysis to a respective aldehyde and ammonium.

Kinetic studies done on the oxidation of benzylamine and 2-phenethylamine indicated variations in the mechanisms followed for MAO-A and MAO-B, whereby catalysis of benzylamine followed the lower loop for both enzymes, while 2-phenethylamine followed the top for both MAO enzymes (Ramsay & Singer, 1991). A further point of interest is that the K_m values for oxygen differs for MAO-A and MAO-B, with the K_m values at about 6 µM and 250 µM, respectively (Edmondson *et al.*, 2004). The concentration of oxygen in air-saturated solution is equal to the K_m of MAO-B (i.e. 250 µM). Therefore, when the amine substrate is in saturated concentrations, MAO-A operates at maximal velocity while MAO-B is only at about half-maximal velocity under physiological conditions (Edmondson *et al.*, 2004).

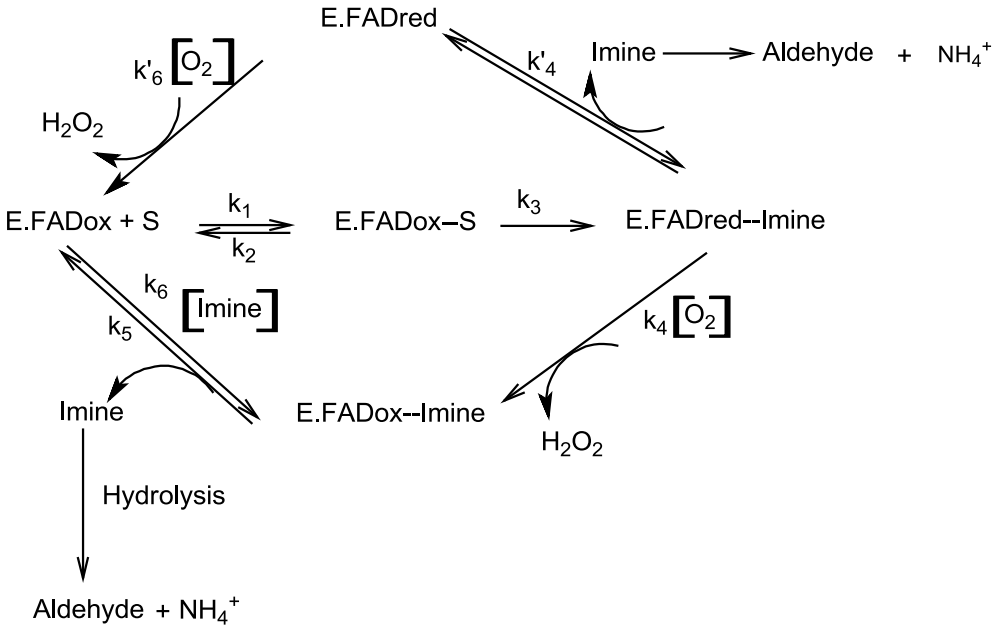


Figure 2.18: Reaction pathway for MAO catalysis

(Edmondson et al., 2004).

2.2.3.1 The mechanisms of C-H bond cleavage

The cleavage of C-H bonds may occur by three different pathways: heterolytic hydride transfer, heterolytic proton abstraction which includes the single electron transfer (SET) (Figure 2.9) and the polar nucleophilic mechanism (Figure 2.20), and the homolytic hydrogen atom abstraction. The hydride mechanism allows for the transfer of the hydrogen with its bonding electrons to the acceptor group. The hydride abstraction is a preferred route for most flavin-dependent amino acid oxidases (Umhau *et al.*, 2000).

Studies on MAO catalysis paid particular attention to the heterolytic proton abstraction mechanisms due to experimental objections to the other two mechanisms. The hydride mechanism for example was deemed impractical due to isotopic effects indicating that the C-H cleavage is not concerted with rehybridisation of the flavin N(5) (MacMillar *et al.*, 2011). The radical mechanism on the other hand was ruled out due to failure to detect semiquinone during turnover (Ramsay, 2012) and other mechanistic probes.

2.2.3.1.1 The single electron transfer mechanism

The SET mechanism begins with a reversible single electron transfer from the unprotonated amine to the flavin N(5) which produces a flavin radical and an aminium cation radical intermediate. The preceding step lowers the pKa of the α-C-H, which triggers proton abstraction by an active site base in the catalytic cycle. However due to the lack of spectral evidence for the flavin radical intermediate, the lack of a magnetic field effect on the rate of enzyme reduction and the low oxidation-reduction potential of the FAD cofactor (Edmondson *et al.*, 2009) the SET mechanism is not supported. In addition to this, structural data on MAO-A and MAO-B indicate that there is no obvious amino acid residue that can serve as a catalytic base in the active sites of these enzymes (Edmondson *et al.*, 2009).

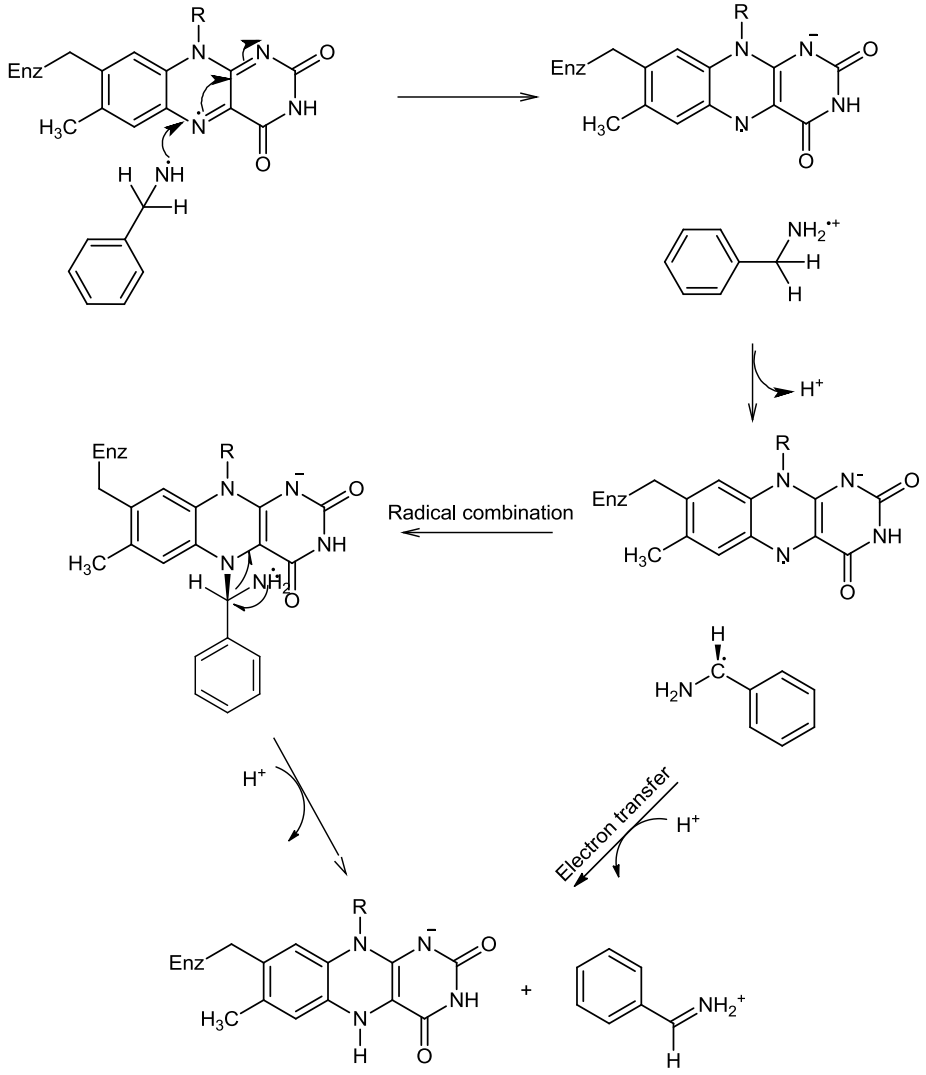


Figure 2.19: The single electron transfer mechanism of MAO oxidation

(MacMillar et al., 2011).

2.2.3.1.2 The polar nucleophilic mechanism

The mechanism which received sufficient support for the appropriate C-H cleavage is the polar nucleophilic mechanism. This mechanism is initiated by a nucleophilic attack of the unprotonated amine on the flavin (C4a) to form a flavin C(4a)-nucleophile adduct, which decomposes into a reduced flavin and protonated imine product (MacMillar *et al.*, 2011). According to computational studies on the isoalloxazine ring system and para-substituted benzylamines undergoing the polar nucleophilic mechanism, the adduct formation step was found to be the rate determining step (Erdem *et al.*, 2006).

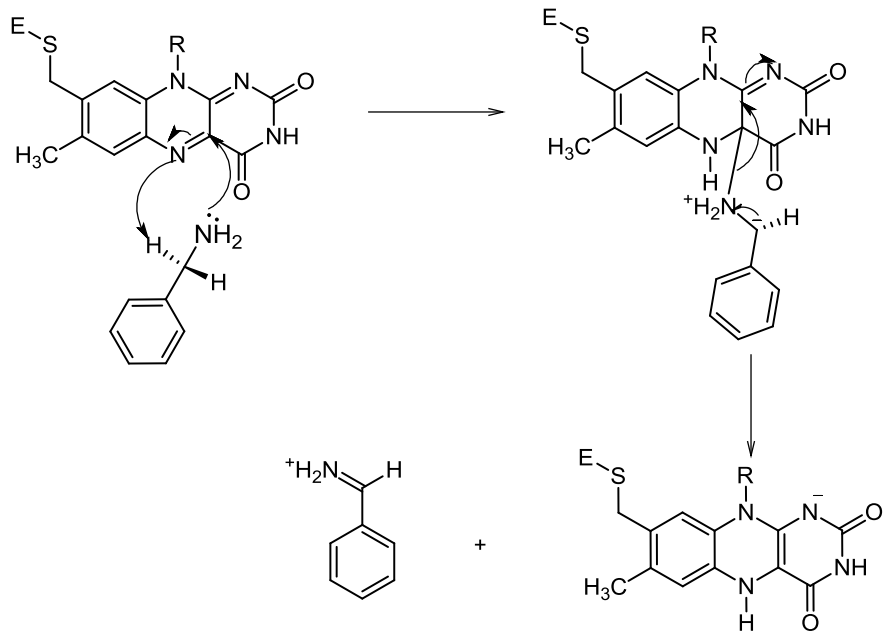


Figure 2.20: The polar nucleophilic mechanism of MAO catalysis

(Edmondson et al., 2004).

2.2.4 MAO inhibitors in PD

As previously stated, the first MAO inhibitor, iproniazid, was discovered by serendipity when an anti-TB drug showed positive effects for MAO inhibition. Iproniazid and other MAO inhibitors, however are associated with certain adverse effects including liver toxicity (iproniazid) and the "cheese effect" (tranylcypromine) (Youdim & Bakhle, 2006).

The "cheese effect" in particular is a very important side effect to consider in MAO inhibitor development. It is caused by tyramine and other indirectly acting sympathomimetic amines in foods such as cheese, and fermented products such as beer and wine (Youdim & Bakhle, 2006). Under normal conditions, metabolism of these exogenous amines occurs in the peripheral organs (mainly the liver) and gut wall, which prevents their access to the circulatory system. However, with MAO inhibition these amines can access the circulatory system and cause a hypertensive crisis.

MAO inhibitors can be categorised as selective or non-selective and irreversible or reversible inhibitors (Table 2-1). Since neurological disorders and diseases such as depression, PD and AD are chronic conditions, it is preferred to use agents with a long-lasting therapeutic action notably found in irreversible inhibitors. However, irreversible inhibitors may possess serious adverse effects such as the serotonin syndrome and the cheese effect (Finberg, 2014). Thus

application of selective reversible MAO inhibitors is recommended for the treatment of disease states.

2.2.4.1 Mechanism of irreversible inhibitors of MAO

The mechanism of irreversible inhibition of MAO involves the formation of a covalent flavin adduct at the N(5) position. The first step is the enzymatic oxidation of the inhibitor to form a reactive electrophile. The electrophile then combines covalently with the flavin. This mechanism is applicable to all of the propargylamine type inhibitors of MAO (e.g. rasagiline and R-deprenyl) (Figure 2.), which are the most successful MAO inhibitors for PD therapy. An example of the proposed mechanism of irreversible MAO-B inhibition is shown with mofegiline in Figure 2.. The mechanism shows that the inhibitor reduces the flavin, which enables nucleophilic reaction with the vinyl fluoride to yield the covalent adduct at N(5) position of the flavin (Edmondson *et al.*, 2009).

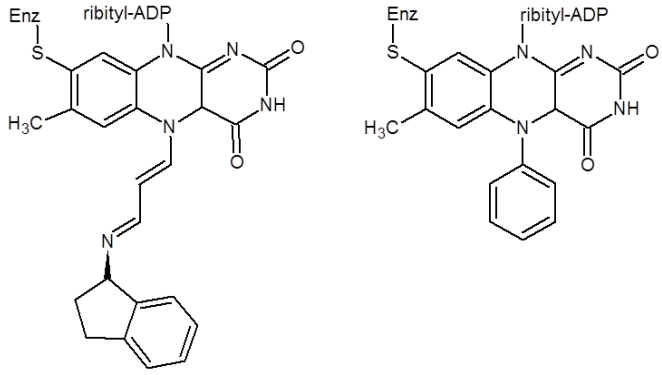


Figure 2.21: The structures of the FAD covalent adducts with rasagiline (a propargilamine) and phenylhydrazine

(Yang et al., 2007; Binda et al., 2013).

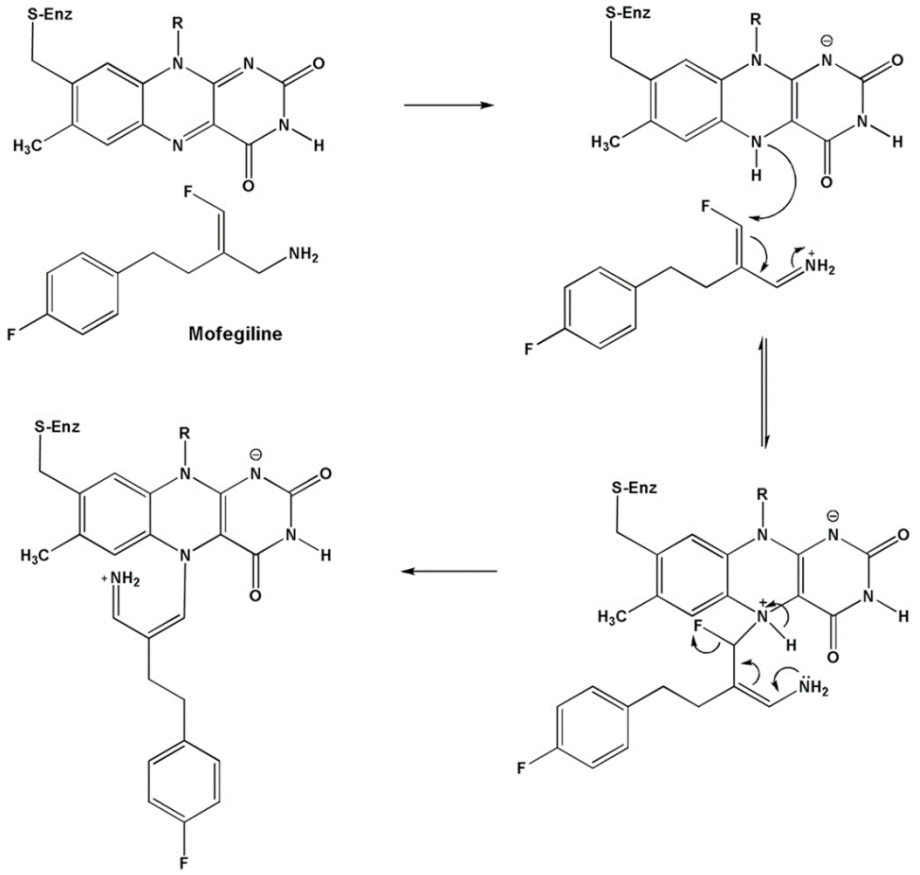


Figure 2.22: The proposed mechanism of MAO-B inhibition by mofegiline

(Edmondson et al., 2009).

Certain inhibitor types such as the cyclopropylamines form adducts at the C(4a) position of the flavin after oxidative ring opening of the cyclopropyl ring (Bonivento *et al.*, 2010). Figure 2.23 shows the ring-opened cyclopropyl moiety linked to the flavin at the C(4a) position (Yang *et al.*, 2007; Binda *et al.*, 2013).

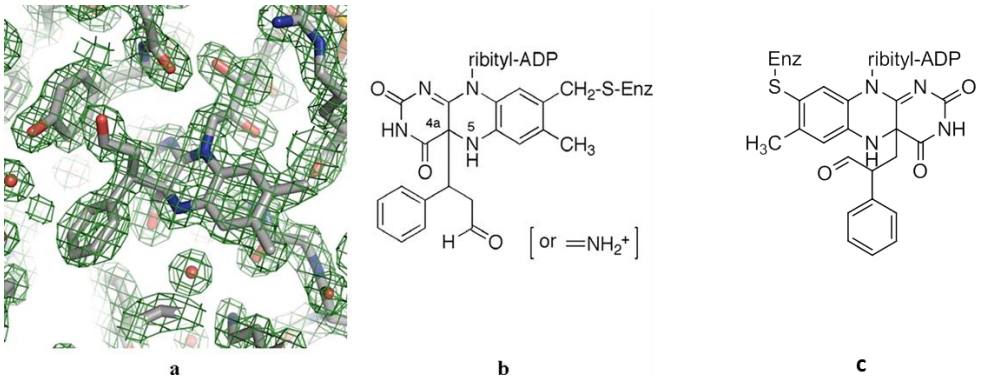
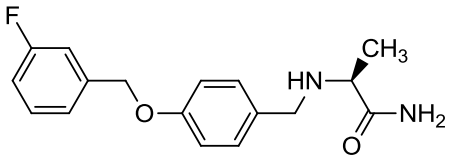


Figure 2.23: The structure of tranylpromine binding to MAO-B

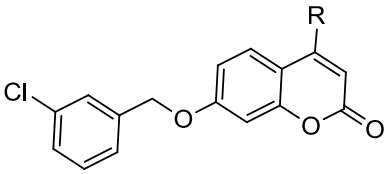
(Edmondson et al., 2004; Bonivento et al., 2010).

2.2.4.2 Reversible MAO inhibitors

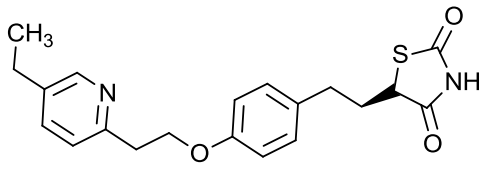
The need to discover MAO inhibitors that are reversible arises from the observation that irreversible MAO inhibitors (especially isoform non-selective) have the risk of inducing hypertensive crisis when combined with certain foods. Selective irreversible MAO-B inhibitors such as selegiline show good inhibitory activity at low doses although isoform selectivity is lost at high doses or repeated administration (Youdim & Bakhle, 2006). Therefore the most pharmacologically desirable MAO-B inhibitor should act reversibly and possess selective inhibition of MAO-B.



Safinamide



R= CH_2NHCH_3: 7-(3-chlorobenzyloxy)4-methylamino)methyl-coumarin
R= CHO: 7-(3-chlorobenzyloxy)4-carboxaldehyde-coumarin



Pioglitazone

Figure 2.24: The structures of safinamide, selected coumarin derivatives and pioglitazone.

Reversible MAO inhibitors that are used for the treatment of PD are limited. Safinamide is a new drug in development for the treatment of PD and is an example of a reversible and selective MAO-B inhibitor (Binda *et al.*, 2007). This drug has entered Phase III clinical trials (Finberg, 2014). Its MAO-B selectivity and high inhibition potency is linked to its linear structure which allows it to bind as a cavity-spanning inhibitor in MAO-B. The 3-fluorobenzyloxy moiety is located in the entrance cavity, while the amide binds in the substrate cavity, oriented towards the FAD (Binda *et al.*, 2007). Similar to other MAO-B complexes there are three ordered water molecules in the active site where safinamide binds. One of the water molecules acts as a bridge for hydrogen bonding between the safinamide amide group and the Gln206 residue (Binda *et al.*, 2007). The binding of safinamide is similar to that of the coumarin derivatives, 7-(3-chlorobenyloxy)-4-(methylamino)methyl-coumarin and 7-(3-chlorobenyloxy)-4-(carboxaldehyde-coumarin), which were examined in the same study (Binda *et al.*, 2007).

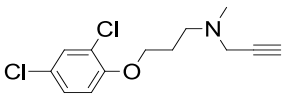
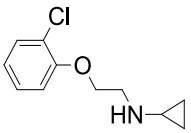
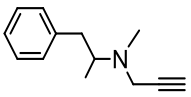
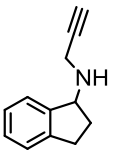
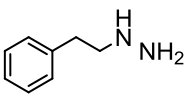
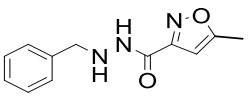
Pioglitazone, which has recently been shown to inhibit MAO-B, exhibits a similar binding mode to MAO-B compared to safinamide (Binda *et al.*, 2011)

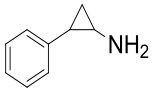
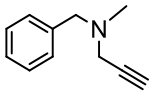
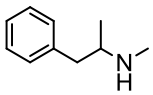
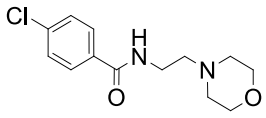
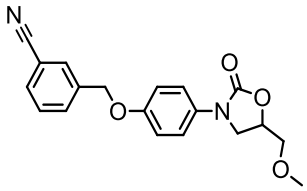
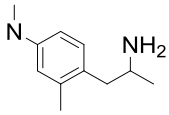
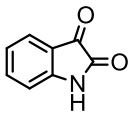
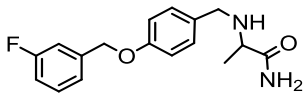
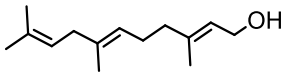
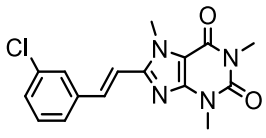
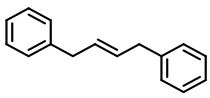
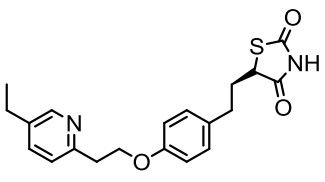
Figure 2.).

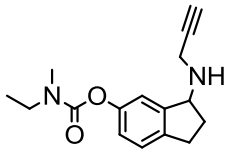
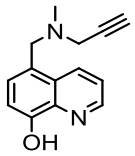
Human MAO-B is known to bind compounds of various sizes due to the versatility of its active site cavity as controlled by the conformation of the Ile199 gate. This control mechanism by the Ile199 gate is beneficial as it provides selectivity between small and large inhibitors. Small inhibitors such as isatin and tranylcypromine bind without any gate alteration and exhibit similar binding affinities for both MAO-A and MAO-B. In contrast, “cavity-filling ligands” (e.g. *trans,trans*-farsenol and 1,4-diphenyl-2-butene) require a compact ellipsoidal cavity for binding and show specificity for the MAO-B enzyme (Edmondson *et al.*, 2004). Medium-sized inhibitors [e.g. rasagiline, selegiline, N-(2-aminoethyl)-*p*-chlorobenzamide], also require an open conformation of the Ile199 gate for access to the active site and like the cavity-filling inhibitors have high affinity for MAO-B.

Table 2-1: Inhibitors of MAO-A and MAO-B

(Finberg, 2014, Hubalék et al., 2005; Binda et al., 2011).

MAO Inhibitors	Selectivity	Reversibility	Structure
Clorgiline	MAO-A	Irreversible	
Ly-51641	MAO-A	Irreversible	
Selegiline	MAO-B	Irreversible	
Rasagiline	MAO-B	Irreversible	
Phenelzine	Non-selective	Irreversible	
Isocarboxazide	Non-selective	Irreversible	

MAO Inhibitors	Selectivity	Reversibility	Structure
Tranylcypromine	Non-selective	Irreversible	
Pargyline	Non-selective	Irreversible	
Methamphetamine	MAO-A	Reversible	
Moclobemide	MAO-A	Reversible	
Cimoxatone	MAO-A	Reversible	
Amiflamine	MAO-A	Reversible	
Isatin	MAO-A	Reversible	
Safinamide	MAO-B	Reversible	
Trans,trans-farnesol	MAO-B	Reversible	
(E)-8-(3-chlorostyryl)caffeine	MAO-B	Reversible	
1,4-Diphenyl-2-butene	MAO-B	Reversible	
Pioglitazone	MAO-B	Reversible	

MAO Inhibitors	Selectivity	Reversibility	Structure
Ladostigil	Non-selective	Reversible	
M30	Non-selective	Irreversible	

2.3 Chalcones as MAO inhibitors

2.3.1 Introduction

Based on the potential adverse effects associated with irreversible MAO-B inhibition, a number of research groups are developing reversible MAO-B inhibitors for the treatment of PD. Besides their potential for potentiating tyramine-induced changes in blood pressure, enzyme activity may require several weeks to recover following irreversible inhibition (Fowler *et al.*, 1994). For the design of reversible MAO-B inhibitors, a number of heteroaromatic chemical classes have been explored, which include caffeine, chalcones, chromones and coumarins. This study will explore SARs of MAO inhibition by chalcone-derived compounds.

2.3.2 General background

Chalcones are compounds from natural or synthetic origin and are members of the flavonoid family, which is a very large and widespread group of plant constituents (Kron *et al.*, 2012). Chalcones are precursors of flavonoids (Chimenti *et al.*, 2009). Due to the complexity of plant components, chalcones from natural products are obtained via numerous extractive techniques. However, because of the increasing growth in the medicinal and biochemical applications of chalcones, scientific discoveries in recent decades led to a number of synthetic routes to chalcones (Devia *et al.*, 1999).

Traditional medicinal usage of chalcone-containing plants led to the discovery of their diverse therapeutic applications (Devia *et al.*, 1999). Chalcones possess a broad spectrum of pharmacological activities including antimalarial, anticancer, antiprotozoal, anti-inflammatory, antibacterial, antifungal, antimicrobial, lavicidal, anticonvulsant, antioxidant, cytotoxic, antitumor and chemopreventive activities (Rahman, 2011; Mousavi *et al.*, 2014; Rozmer & Perjési, 2014). Interestingly, the effectiveness of chalcones and related compounds as therapeutic agents in some of the activities listed are well documented and their applications are of market value.

Physicochemical characteristics such as stereochemistry, acid-base properties, and lipophilicity are principal determinants of the biological actions of natural and synthetic compounds (Kron *et al.*, 2012). Several studies have focused on the effects of substitution on the phenyl rings-A and -B on the activities of chalcones, hence extensive libraries of chalcone analogues exists (Chimenti *et al.*, 2009; Rahman, 2011). Of particular interest is the conformational structure of chalcones, which greatly affects their biological activities. The restricted chalcone scaffold (2-benzylidene-1-tetralone) (Figure 2.25) that will be investigated in this study, was selected based on the idea that restricted or rigid conformations could result in more selective binding of drugs to target sites, which in turn would result in highly active drugs with reduced adverse effects

(Gareth, 2007). This may be supported by rasagiline which is a restricted ring-closed analogue of selegiline with potent MAO-B inhibition activity. Rasagiline is an irreversible MAO-B inhibitor and possesses the propargyl moiety present in selegiline. Rasagiline has high selectivity for human and rat MAO-B, both *in vitro* and *in vivo,* and is 5 to 10-fold more potent than selegiline as a MAO-B inhibitor (Kupsch *et al.*, 2001). Therefore it is expected that cyclic chalcones would be more potent and selective inhibitors than the natural open ring analogues. However, care should be taken because restricted conformations may lead to incorrect conformations for binding to the active site or steric hindrance may occur between the restricting group and the target. These will result in loss of activity.

Chalcones are recognized as *α,β*-unsaturated aromatic ketones and are essentially planar due to the presence of carbon atoms with sp^2 hybridization (de Oliveira *et al.*, 2012). Generally the chalcone chemical structure represents the open-chain form of the flavonoids (Rozmer & Perjési, 2014). The two aromatic rings of this flavonoid moiety are linked by the three-carbon *α,β*-unsaturated carbonyl system, which forms the basis for the many biological activities of these compounds.

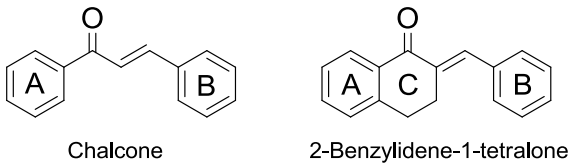


Figure 2.25: The general structure of chalcone and the restricted 2-benzylidene-1-tetralone chalcone analogue.

2.3.3 Stereochemistry

Although in principle chalcones exist as E- or Z-isomers, studies have found that the E-isomer is the most thermodynamically stable form. In cases where there is a possibility of the mixture of the two isomers, recrystallization frequently yields only the E-isomers (Dimmock *et al.*, 2002; Larsen *et al.*, 2005). Steric interactions between the aryl and carbonyl groups makes the Z-conformation unfavourable (Hallgas *et al.*, 2005). The stereochemistry of chalcones can be determined by NMR analysis (Bayer *et al.*, 1991). The vinylic proton is expected to be more deshielded for the (E)-isomer than the (Z)-isomer. It is well established that, in the ^{1}H NMR spectra of 3-benzylidene-4-chromanones the signal of the vinylic β-proton *cis* to the carbonyl group appears at *δ* 7.7 ppm, while the corresponding *trans*-proton appears at *δ* 6.7 ppm (Böhler & Tamm, 1967; Namikoshi *et al.*, 1987). In another study, ^{1}H NMR spectra indicated that the vinylic protons appear at chemical shifts of 7.15-7.95 ppm (Dimmock *et al.*, 1999). The

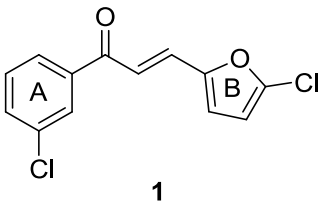
absorptions of the vinylic protons occur at low field due to the proximity of the carbonyl group which exerts an anisotropic effect, and frequently overlapped with the complex multiplet of the aromatic protons.

2.3.4 MAO inhibition

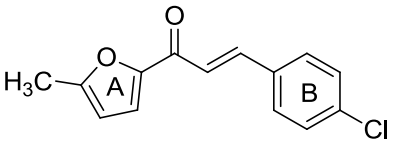
The potential of chalcones as MAO inhibitors has been documented. A study by Chimenti and colleagues (2009) reported promising activities of chalcones as inhibitors of MAO-B, with the most potent compound **(3)** showing an IC_{50} of 0.0044 µM and high isoform selectivity (SI > 11364) in favour of MAO-B (Figure 2.6). In addition, Robinson, *et al.* (2013) examined the MAO inhibition activities of furanochalcone derivatives. The most active compound **(1)**, 2*E*-3-(5-chlorofuran-2-yl)-1-(3-chlorophenyl)prop-2-en-1-one, exhibited an IC_{50} value of 0.174 µM for the inhibition of MAO-B and 28.6 µM for the inhibition of MAO-A. The results demonstrate that these furan substituted chalcone derivatives exhibit moderate to good inhibitory activities towards MAO-B, but showed weak or no inhibition of the MAO-A enzyme.

Further analysis of these studies suggests that chalcones are in general more potent inhibitors of MAO-B than furanochalcone derivatives. Furthermore, it is clear from these reports that both furanochalcones and chalcones are more potent inhibitors of MAO-B than of MAO-A, and that substitution with appropriate groups on both ring-A and -B could lead to better MAO inhibition activity. This suggests that chalcones and their derivatives are suitable scaffolds for the design of MAO-B selective inhibitors. The studies also indicate that chalcones act as reversible MAO inhibitors (Robinson *et al.*, 2013). This is of interest since adverse effects of MAO inhibition are associated with the irreversible inhibition of particularly MAO-A (Youdim & Weinstock, 2004).

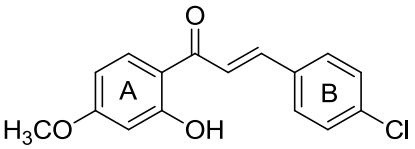
In order to orient optimally in the human MAO active sites, the appropriate substituents are required. Ring-A of the chalcone scaffold binds in close proximity to the FAD cofactor. Polar functional groups on the A-ring would facilitate hydrogen bonding with the FAD, waters and polar residues of the aromatic cage (Chimenti *et al.*, 2009). Inhibitor interaction with residue Tyr326 plays a major role in activity and selectivity for human MAO-B. Halogen substitution on ring-B is also expected to increase MAO-B inhibition potency via hydrophobic interaction at the entrance cavity of the enzyme (Chimenti *et al.*, 2009).



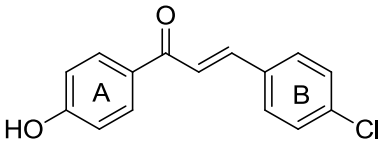
1
MAO-A: IC_{50} = 28.6 µM
MAO-B: IC_{50} = 0.174 µM



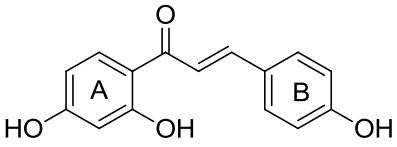
2
MAO-A IC_{50} = 40.9 mM
MAO-B IC_{50} = 0.616 mM



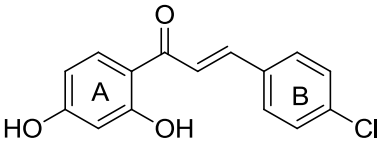
3
MAO-B: IC_{50} = 0.0044 µM



4
MAO-B: IC_{50} = 0.031 µM



5
MAO-A: IC_{50} = 13.9 µM
MAO-B: IC_{50} = 47.2 µM



6
MAO-B: IC_{50} = 0.0051 µM

Figure 2.26: Examples of chalcone derivatives with MAO inhibitory activity

(Chimenti et al., 2009; Robinson et al., 2013).

2.4 Conclusion

PD is a neurodegenerative disorder which is characterised by dopamine deficiency in the CNS. Current therapy for PD remains symptomatic with levodopa being the mainstay. However, due to extensive peripheral metabolism of levodopa, levodopa monotherapy is not efficient in ameliorating PD symptoms. Therefore, MAO-inhibitors particularly MAO-B selective inhibitors are used as adjuncts to levodopa. MAO-A inhibitors on the other hand are associated with serious side effects, most notably the potentiation of tyramine-induced changes in blood pressure. An understanding of the MAO catalytic cycle and the mechanisms of action has been instrumental in developing irreversible inhibitors. Chalcones have recently emerged as having potent MAO-B selective inhibition properties. Of particular interest is the finding that chalcones are reversible MAO inhibitors, which makes them attractive candidates for the development of PD therapy. This study will explore new chalcone derivatives as potential MAO inhibitors. In this regard, this study will focus on restricted ring-closed derivatives of chalcones.

REFERENCES

Alam, Z.I., Jenner, A., Daniel, S.E., Lees, A.J., Cairns, N., Marsden, C.D., Jenner, P. & Halliwell, B. 1997. Oxidative DNA damage in the parkinsonian brain: an apparent selective increase in 8-hydroxyguanine levels in subtantia nigra. *Journal of neurochemistry*, 69(3):1196-1203.

Ali, S.F., Binienda, Z.K. & Imam, S.Z. 2011. Molecular aspects of dopaminergic neurodegeneration: gene-environment interaction in parkin dysfunction. *International journal of environmental research and public health*, 8(12):4702-7413.

Bach, A.W.J., Lan, N.C., Johnson, D.L., Abell, C.W., Bembenek, M.E., Kwa, S., Seeburg, P.H. & Shih, J.C. 1988. cDNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymatic properties. *Proceedings of the national academy of sciences of the United States of America*, 85(13):4934-4938.

Bayer, H., Batzl, C., Hartman, R.W. & Mannschreck, A. 1991. New aromatase inhibitors. synthesis and biological activity of pyridyl-substituted tetralone derivatives. *Journal of medicinal chemistry*, 34(9):2685-2691.

Berry, C., La Vecchia, C. & Nicotera, P. 2010. Paraquat and Parkinson's disease. *Cell death and differentiation*, 17(7):1115-1125.

Betarbet, R., Sherer, T.B., MacKenzie, G., Garcia-Osuna, M., Panov, A.V. & Greenamyre, J.T. 2000. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nature neuroscience*, 3(12):1301-1306.

Binda, C., Aldeco, M., Geldenhuys, W.J., Tortorici, M., Mattevi, A. & Edmondson, D.E. 2011. Molecular insights into human monoamine oxidase B inhibition by the glitazone anti-diabetes drugs. *ACS medicinal chemistry letters*, 3(1):39-42.

Binda, C., Edmondson, D.E. & Mattevi, A. 2013. Monoamine oxidase inhibitors: diverse and surprising chemistry with expanding pharmacological potential. *(In* Read, R., Urzhumtsev A.G. & Lunin V.Y., *eds*. Advancing methods for biomolecular crystallography. Netherlands: Springer p. 309-312).

Binda, C., Hubálek, F., Li, M., Edmondson, D.E. & Mattevi, A. 2004. Crystal structure of human monoamine oxidase B, a drug target enzyme monotopically inserted into the mitochondrial outer membrane. *Federation of European biochemical societies letters*, 564(3):225-228.

Binda, C., Li, M., Hubálek, F., Restelli, N., Edmondson, D.E. & Mattevi, A. 2003. Insights into the mode of inhibition of human mitochondrial monoamine oxidase B from high-resolution crystal structures. *Proceedings of the national academy of sciences of the United States of America*, 100(17):9750-9755.

Binda, C., Newton-Vinson, P., Hubalek, F., Edmondson, D.E. & Mattevi, A. 2002. Structure of human monoamine oxidase B, a drug target for the treatment of neurological disorders. *Nature structural biology*, 9(1):22-26.

Binda, C., Wang, J., Pisani, L., Caccia, C., Carotti, A., Salvati, P., Edmondson, D.E. & Mattevi, A. 2007. Structures of human monoamine oxidase B complexes with selective noncovalent inhibitors: safinamide and coumarin analogs. *Journal of medicinal chemistry*, 50(23):5848-5852.

Blaylock, R. 2004. Excitotoxicity: a possible central mechanism in fluorine neurotocxicity. *Fluoride*, 37(4):301-314.

Böhler, P. & Tamm, C. 1967. The homo-isoflavones, a new class of natural product. Isolation and structure of eucomin and eucomol. *Tetrahedron letters*, 8(36):3479-3483.

Bonifati, V. & Meco, G. 1999. New, selective catechol-O-methyltransferase inhibitors as therapeutic agents in Parkinson's disease. *Pharmacology & therapeutics*, 81(1):1-36

Bonifati, V., Rizzu, P., Squitieri, F., Krieger, E., Vanacore, N., van Swieten, J.C., Brice, A., van Duijn, C.M., Oostra, B., Meco, G. & Heutink, P. 2003a. DJ-1 (park7), a novel gene for autosomal recessive, early onset parkinsonism. *Neurological science*, 24(3):159-160.

Bonifati, V., Rizzu, P., van Baren, M.J., Schaap, O., Breedveld, G.J., Krieger, E., Dekker, M.C., Squitieri, F., Ibanez, P., Joosse, M., van Dongen, J.W., Vanacore, N., van Swieten, J.C., Brice, A., Meco, G., van Duijn, C.M., Oostra, B.A. & Heutink, P. 2003b. Mutations in DJ-1 gene associated with autosomal recessive early-onset parkinsonism. *Science*, 299(5604):256-259.

Bonivento, D., Milczek, E.M., McDonald, G.R., Binda, C., Holt, A., Edmondson, D.E. & Mattevi, A. 2010. Potentiation of ligand binding through cooperative effects in monoamine oxidase B. *Journal of medicinal chemistry*, 285(47):36849-36856.

Breydo, L., Wu, J.W. & Uversky, V.N. 2012. a-Synuclein misfolding and Parkinson's disease. *Biochimica et biophysica acta,* 1822(2):216-285.

Brooks, D.J. 2000. Dopamine agonists: their role in the treatment of Parkinson's disease. *Journal of neurology, neurosurgery & psychiatry*, 68(6):685-689.

Brown, P. & Williams, D. 2005. Basal ganglia local field potential activity: character and functional significance in the human. *Clinical neurophysiology*, 116(11):2510-2519.

Canet-Aviles, R.M., Wilson, M.A., Miller, D.W., Ahmad, R., McLendon, C., Bandyopadhyay, S., Baptista, M.J., Ringe, D., Petsko, G.A. & Cookson, M.R. 2004. The Parkinson's disease protein DJ-1 is neuroprotective due to cystein-sulfinic acid-driven mitochondrial localization. *Proceedings of the national academy of sciences of the United States of America*, 101(24):9103-9108.

Chimenti, F., Fioravanti, R., Bolasco, A., Chimenti, P., Secci, D., Rossi, F., Matilde, Y., Orallo, F., Ortuso, F. & Alcaro, S. 2009. Chalcones: a valid scaffold for monoamine oxidases inhibitor. *Journal of medicinal chemistry*, 52(9):2818-2824.

Dauer, W. & Przedborski, S. 2003. Parkinson's disease: mechanisms and models. *Neuron*, 39(6):889-909.

de Oliveira, G.R., de Oliveira, H.C.B., Silva, W.A., da Silva, V.H.C., Sabino, J.R. & Martins, F.T. 2012. Structure and theoretical approaches to a chalcone deriative. *Structural chemistry journal*, 2012(23):1667-1676.

DeColibus, L., Li, M., Binda, C., Lustig, A., Edmondson, D.E. & Mattevi, A. 2005. Three-dimensional structure of human monoamine oxidase A (MAO A): relation to the structures of rat MAO A and human MAO B. *Proceedings of the national academy of sciences of the United States of America*, 102(36):12684-12689.

Deogaonkar, M. & Subramanian, T. 2005. Pathophysiological basis of drug-induced dyskinesias in Parkinson's disease. *Brain research reviews*, 50(1):156-168.

Devia, A.C., Ferretti, F.H., Ponce, C.A. & Tomás, F. 1999. Conformational equilibrium and intramolecular hydrogen bond of 4′X and 4X substituted 2′(OH) chalcones. *Journal of molecular structure: THEOCHEM*, 493(1–3):187-197.

Dimmock, J.R., Kandepu, N.M., Nazarali, A.J., Kowalchuk, T.P., Motaganahalli, N., Quail, J.W., Mykytiuk, P.A., Audette, G.F., Prasad, L., Perjesi, P., Allen, T.M., Santos, C.L., Szydlowski, J., De Clercq, E. & Balzarini, J. 1999. Conformational and quantitative structure-activity

relationship study of cytotoxic 2-arylidenebenzocycloalkanones. *Journal of medicinal chemistry*, 42(8):1358-1366.

Dimmock, J.R., Zello, G.A., Oloo, E.O., Quail, J.W., Kraatz, H.B., Perjesi, P., Aradi, F., Takacs-Novak, K., Allen, T.M., Santos, C.L., Balzarini, J., De Clercq, E. & Stables, J.P. 2002. Correlations between cytotoxicity and topography of some 2-arylidenebenzocycloalkanones determined by X-ray crystallography. *Journal of medicinal chemistry*, 45(14):3103-3111.

Dringen, R. 2000. Metabolism and functions of glutathione in brain. *Progress in neurobiology*, 62(6):649-671.

Edmondson, D.E., Binda, C. & Mattevi, A. 2004. The FAD binding sites of human monoamine oxidases A and B. *Neurotoxicology*, 25(1–2):63-72.

Edmondson, D.E., Binda, C. & Mattevi, A. 2007. Structural insights into the mechanism of amine oxidation by monoamine oxidases A and B. *Archives of biochemistry & biophysics*, 464(2):269-276.

Edmondson, D.E., Binda, C., Wang, J., Upadhyay, A.K. & Mattevi, A. 2009. Molecular and mechanistic properties of the membrane-bound mitochondrial monoamine oxidases. *Proceedings of the national academy of sciences of the United States of America*, 48(20):4220-4230.

Erdem, S.S., Karahan, Ö., Yıldıza, İ. & Yelekçib, k. 2006. A computational study on the amine-oxidation mechanism of monoamine oxidase: insight into the polar nucleophilic mechanism. *Organic & biomolecular chemistry*, 4(4):646-658.

Fahn, S. 2008. The history of dopamine and levodopa in the treatment of Parkinson's disease. *Movement disorders*, 23(3):497-508.

Ferino, G., Cadoni, E., Matos, M.J., Quezada, E., Uriarte, E., Santana, L., Vilar, S., Tatonetti, N.P., Yanez, M., Vina, D., Picciau, C., Serra, S. & Delogu, G. 2013. MAO inhibitory activity of 2-arylbenzofurans versus 3-arylcoumarins: synthesis, in vitro study, and docking calculations. *ChemMedChem*, 8(6):956-966.

Finberg, J.P.M. 2014. Update on the pharmacology of selective inhibitors of MAO-A and MAO-B: focus on modulation of CNS monoamine neurotransmitter release. *Pharmacology & therapeutics*, 143(2):133-152.

Fowler, J.S., Logan, J., Wang, G.J., Volkow, N.D., Zhu, W., Franceschi, D., Pappas, N., Ferrieri, R., Shea, C., Garza, V., Xu, Y., MacGregor, R.R., Schlyer, D., Gatley, S.J., Ding, Y. & Alexoff, D. 2002. PET imaging of monoamine oxidase B in peripheral organs in humans. *Journal of nuclear medicine*, 43(10):1331-1338.

Fowler, J.S., Volkow, N.D., Logan, J., Wang, G.-J., Mac Gregor, R.R., Schlyer, D., Wolf, A.P., Pappas, N., Alexoff, D., Shea, C., Dorflinger, E., Kruchowy, L., Yoo, K., Fazzini, E. & Patlak, C. 1994. Slow recovery of human brain MAO B after l-deprenyl (selegeline) withdrawal. *Synapse*, 18(2):86-93.

Galvan, A. & Wichmann, T. 2008. Pathophysiology of parkinsonism. *Clinical neurophysiology*, 119(7):1459-1474.

Gareth, T. 2007. Medicinal chemistry: an introduction. Chichester: Wiley. http://www.wiley.com/WileyCDA/WileyTitle/productCd-0470025972.html. Date of access: 31 October 2016.

Gasser, T. 2009. Mendelian forms of Parkinson's disease. *Biochimica et biophysica acta* 1792(7):587-596.

Geha, R.M., Rebrin, I., Chen, K. & Shih, J.C. 2001. Substrate and inhibitor specificities for human monoamine oxidase A and B are influenced by a single amino acid. *The journal of biological chemistry*, 276(13):9877-9882.

Ghavami, S., Shojaei, S., Yeganeh, B., Ande, S.R., Jangamreddy, J.R., Mehrpour, M., Christoffersson, J., Chaabane, W., Moghadam, A.R., Kashani, H.H., Hashemi, M., Owji, A.A. & Łos, M.J. 2014. Autophagy and apoptosis dysfunction in neurodegenerative disorders. *Progress in neurobiology*, 112(0):24-49.

Glass, K.C., Saijo, K., Winner, Marchetto, M.C. & Gage, F.H. 2010. Mechanisms underlying inflammation in neurodegeneration. *Journal of cell*, 140(6):918-934.

Gleave, J.A., Perri, P.D. & Nash, J.E. 2014. Mitochondrial dysfunction in Parkinson's disease: a possible target for neuroprotection. *Frontiers in biology*, 9(6):498-503.

Goole, J. & Amighi, K. 2009. Levodopa delivery systems for the treatment of Parkinson's disease: an overview. *International journal of pharmaceutics*, 380(1–2):1-15.

Gottowik, J., Malherbe, P., Lang, G., Prada, M.D. & Cesura, A.M. 1995. Structure/function relationships of mitochondrial monoamine oxidase A and B chimeric forms. *European journal of biochemistry*, 230(3):934-942.

Grimsby, J., Lan, N.C., Neve, R., Chen, K. & Shih, J.C. 1990. Tissue distribution of human monoamine oxidase A and B mRNA. *Journal of neurochemistry*, 55(4):1166-1169.

Hallgas, B., Dobos, Z., Ősz, E., Hollósy, F., Schwab, R.E., Szabó, E.Z., Erős, D., Idei, M., Kéri, G. & Lóránd, T. 2005. Characterization of lipophilicity and antiproliferative activity of E-2-arylmethylene-1-tetralones and their heteroanalogues. *Journal of chromatography B*, 819(2):283-291.

Halliwell, B. 2007. Biochemistry of oxidative stress. *Biochemical society transactions*, 35(5):1147-1150.

Hatano, T. & Hattori, N. 2011. Etiology and pathogenesis of Parkinson's disease. Croatia: InTech. http://www.intechopen.com/books/etiology-and-phathophysiology-of-parkinson-s-disease/etiology-and-pathogenesis-of-parkinson-s-disease. Date of access: 20 September 2015.

Hatano, T., Kubo, S.I., Sato, S. & Hattori, N. 2009. Pathogenesis of familial Parkinson's disease: new insights based on monogenic forms of Parkinson's disease. *Journal of neurochemistry*, 111(5):1075-1093.

Hirsch, E.C. & Hunot, S. 2009. Neuroinflammation in Parkinson's disease: a target for neuroprotection? *The lancet neurology*, 8(4):382-397.

Hubálek, F., Binda, C., Khalil, A., Li, M., Mattevi, A., Castagnoli, N. & Edmondson, D.E. 2005. Demonstration of isoleucine 199 as a structural determinant for the selective inhibition of human monoamine oxidase B by specific reversible inhibitors. *Journal of biological chemistry*, 280(16):15761-15766.

Kaakkola, S. 2000. Clinical pharmacology, therapeutic use and potential of COMT inhibitors in Parkinson's disease. *Drugs*, 59(6):1233-1250.

Kearney, E.B., Salach, J.I., Walker, W.H., Seng, R.L., Kenney, W., Zeszotek, E. & Singer, T.P. 1971. The covalently-bound flavin of hepatic monoamine oxidase. *European journal of biochemistry*, 24(2):321-327.

Kron, I., Pudychova-Chovanova, Z., Velika, b., Guzy, J. & perjesi, P. 2012. (E)-2-Benyzylidenebenzocyclanones, part VIII: spectrophotometric determination of pKa values of some natural and synthetic chalcones and their cyclic analogues. *Monatshefte fur chemie*, 2012(143):13-17.

Kupsch, A., Sautter, J., Gots, M.E., Breithaupt, W., Schwarz, J., Youdim, M.B.H., Riederer, P., Gerlach, M. & Oertel, W.H. 2001. Monoamine oxidase-inhibition and MPTP-induced neurotoxicity in the non-human primate: comparison of rasagiline (TVP 1012) with selegiline. *Journal of neural transmission*, 108:985-1009.

Larsen, M., Kromann, H., Kharazmi, A. & Nielsen, S.F. 2005. Conformationally restricted anti-plasmodial chalcones. *Bioorganic & medicinal chemistry letters*, 15(21):4858-4861.

Lee, V.M. & Trojanowski, J.Q. 2006. Mechanisms of Parkinson's disease linked to pathological a-synuclein: new targets for drug discovery. *Neuron*, 52:33-38.

Lipton, S.A. & Rosenberg, P.A. 1994. Excitatory amino acids as a final common pathway for neurologic disorders. *New England journal of medicine*, 330(9):613-622.

Liu, B. & Hong, J.-S. 2003. Role of microglia in inflammation-mediated neurodegenerative diseases: mechanisms and strategies for therapeutic intervention. *Journal of pharmacology & experimental therapeutics*, 304(1):1-7.

Ma, J., Yoshimura, M., Yamashita, E., Nakagawa, A., Ito, A. & Tsukihara, T. 2004. Structure of rat monoamine oxidase A and its specific recognitions for substrates and inhibitors. *Journal of molecular biology*, 338(1):103-114.

MacMillar, S., Edmondson, D.E. & Matsson, O. 2011. Nitrogen kinetic isotope effects for the monoamine oxidase B-catalyzed oxidation of benzylamine and (1,1-2H2)benzylamine: nitrogen rehybridization and CH bond cleavage are not concerted. *Journal of the american chemical society*, 133(32):12319-12321.

Macphee, G.J.A. & Stewart, D.A. 2012. Parkinson's disease - pathology, aetiology and diagnosis. *Reviews in clinical gerontology*, 22(3):165-178.

Matsumine, H., Saito, M., Shimoda-Matsubayashi, S., Tanaka, H., Ishikawa, A., Nakagawo-Hattori, Y., Yokochi, M., Kobayashi, T., Igarashi, S., Takano, H., Sanpei, K., Koike, R., Mori, H., Kondo, T., Mizutani, Y., Schaffer, A.A., Yamamura, Y., Nakamura, S., Kuzuhara, S., Tsuji, S. &

Mizumo, Y. 1997. Localization of a gene for an autosomal recessive form of juvenile Parkinsonism to chromosome 6q25.2-27. *The American journal of human genetics*, 60(3):588-596.

Mattson, M.P. 2003. Excitotoxic and excitoprotective mechanisms. *Neuromolecular medicine*, 3(2):65-95.

Mink, J.W. 1996. The basal ganglia: focused selection and inhibition of competing motor programs. *Progress in neurobiology*, 50(4):381-425.

Mousavi, M.R., Maghsoodlou, M.T. & Habibi-khorassani, S.M. 2014. One-pot diastereoselective synthesis of highly functionalized cyclohexenones: 2-oxo-N,4,6-triarylycyclohex-3-ene carboxamides. *Molecular diversity*, 2014(18):821-828.

Muller, T. 2015. Catechol-O-methyltransferase inhibitors in parkinson's disease. *Drugs*, 75(2):157-174.

Namikoshi, M., Nakata, H. & Saitoh, T. 1987. Homoisofiavonoids and related compounds. IV.[i)] configurations of homoisoflavonoids from *Caesaipinia sappan* L. *Chemical and pharmaceutical bulletin*, 35(9):3597-3602.

Nicoll, J.A.R. 2006. Neurodegenerative diseases, movement disorders and system degenerations. *(In* Nicoll, J.a.R., Bone I. & Graham G., *eds*. Adams and Graham's introduction to neurophathology. 3rd ed. London: Hodder Arnold. p. 338-339).

Olanow, C.W., Kieburtz, K., Rascol, O., Poewe, W., Schapira, A.H., Emre, M., Nissinen, H., Leinonen, M. & Stocchi, F. 2013. Factors predictive of the development of levodopa-induced dyskinesia and wearing-off in Parkinson's disease. *Movement disorders*, 28(8):1064-1071.

Pardridge, W.M. 2002. Drug and gene delivery to the brain: the vascular route. *Neuron*, 36(4):555-558.

Rahman, M.A. 2011. Chalcone: a valuable insight into the recent advances and potential pharmacological activities. *Chemical sciences journal*, 2011(29):1-16.

Ramsay, R.R. 2012. Monoamine oxidases: the biochemistry of the proteins as targets in medicinal chemistry and drug discovery. *Current topics in medicinal chemistry*, 12(20):2189-2209.

Ramsay, R.R. & Singer, P.T. 1991. The kinetic mechanisms of monoamine oxidases A and B. *Biochemical society transactions*, 19(1):219-223.

Rideout, H.J. & Stefanis, L. 2014. The neurobiology of LRRK2 and its role in the pathogenesis of Parkinson's disease. *Neurochemical research*, 39(3):576-592.

Riederer, P., Gerlach, M., Müller, T. & Reichmann, H. 2007. Relating mode of action to clinical practice: dopaminergic agents in Parkinson's disease. *Parkinsonism & related disorders*, 13(8):466-479.

Robinson, S.J., Petzer, J.P., Petzer, A., Bergh, J.J. & Lourens, A.C.U. 2013. Selected furanochalcones as inhibitors of monoamine oxidase. *Bioorganic & medicinal chemistry letters*, 23(17):4985-4989.

Rozmer, Z. & Perjési, P. 2014. Naturally occurring chalcones and their biological activities. *Phytochemistry reviews*, 15(1):87-120.

Schapira, A.H.V. 2008. Mitochondria in the aetiology and pathogenesis of Parkinson's disease. *The lancet neurology*, 7(1):97-109.

Schapira, A.H.V., Cooper, J.M., Dexter, D., Jenner, P., Clark, J.B. & Marsden, C.D. 1990. Mitochondrial complex I deficiency in Parkinson's disease. *Journal of neurochemistry*, 54(3):823-857.

Shih, J.C., Chen, K. & Ridd, M.J. 1999. Monoaminde oxidase: from genes to behavior. *Annual reviews neuroscience*, 22(1):197-217.

Shimura, H., Mizuno, Y. & Hattori, N. 2012. Parkin and Parkinson's disease. *Clinical chemistry*, 58(8):1260-1261.

Sies, H. 1997. Oxidative stress: oxidants and antioxidants. *Experimental physiology*, 85(2):291-295.

Simeone, T.A., Sanchez, R.M. & Rho, J.M. 2004. Molecular biology and ontogeny of glutamate receptors in the mammalian central nervous system. *Journal of child neurology*, 19(5):343-360.

Singh, D., Shrimali, S. & Rathore, K.S. 2015. A review on Parkinson's disease: its pathophysiology, treatment and surgery. *Pharmatutor*, 3:25-32.

Son, S.-Y., Ma, J., Kondou, Y., Yoshimura, M., Yamashita, E. & Tsukihara, T. 2008. Structure of human monoamine oxidase A at 2.2-Å resolution: the control of opening the entry for substrates/inhibitors. *Proceedings of the national academy of sciences of the United States of America*, 105(15):5739-5744.

Spillantini, M.G., Schmidt, M.l., Lee, V.M., Trojanowski, J.Q., Jakes, R. & Goedert, M. 1997. alpha-Synuclein in lewy bodies. *Nature*, 388:839-840.

Stewart, D.A. 2008. Pathology, aetiology and pathogenesis. *(In* Playfer, J.R. & Hindle J.V., *eds*. Parkinson's disease in the older patient. United Kingdom: Radcliffe p. 11-26).

Taira, T., Saito, J., Niki, T., Iguchi-Agriga, S.M., Takahashi, K. & Ariga, H. 2004. DJ-1 has a role in antioxidative stress to prevent cell death. *EMBO reports*, 5(2):213-5218.

Tansey, M.G. & Goldberg, M.S. 2010. Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention. *Inflammation in neuropsychiatric disease*, 37(3):510-518.

Teo, K.C. & Ho, S. 2013. Monoamine oxidase-B (MAO-B) inhibitors: implications for disease-modification in Parkinson's disease. *Translational neurodegeneration*, 2(19):1-10.

Umhau, S., Pollegioni, L., Molla, G., Diederichs, K., Welte, W., Pilone, M.S. & Ghisla, S. 2000. The x-ray structure of d-amino acid oxidase at very high resolution identifies the chemical mechanism of flavin-dependent substrate dehydrogenation. *Proceedings of the national academy of sciences of the United States of America*, 94(23):12463-12468.

Wells, B.G., Dipiro, J.T., Schwinghammer, T.L. & Dipiro, C.V. 2009. Pharmacotherapy handbook. New York: McGraw-Hill

Wood-Kaczmar, A., Gandhi, S. & Wood, N.W. 2006. Understanding the molecular causes of Parkinson's disease. *Trends in molecular medicine*, 12(11):521-528.

Yang, M., Culhane, J.C., Szewczuk, L.M., Jalili, P., Ball, H.L., Machius, M., Cole, P.S. & Yu, H. 2007. Structural basis for the inhibition of the LSD1 histone demethylase by the antidepressant trans -2-phenylcyclopropylamine. *Journal of biochemistry*, 46(27):8048-8065.

Youdim, M.B.H. & Bakhle, Y.S. 2006. Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness. *British journal of pharmacology*, 147(1):287-296.

Youdim, M.B.H., Edmondson, D. & Tipton, K.F. 2006. The therapeutic potential of monoamine oxidase inhibitors. *Nature reviews neuroscience*, 7:295-309.

Youdim, M.B.H. & Weinstock, M. 2004. Therapeutic applications of selective and non-selective inhibitors of monoamine oxidase A and B that do not cause significant tyramine potentiation. *Neurotoxicology*, 25(1–2):243-250.

Zhu, Y., Carvey, P.M. & Ling, Z. 2006. Age-related changes in glutathione and glutathione-related enzymes in rat brain. *Brain research*, 1090(1):35-44.

CHAPTER 3: ARTICLE 1

Synthesis and evaluation of selected cyclic chalcones for monoamine oxidase inhibitory activity

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ABSTRACT

Chalcone was identified as a promising lead for the design of monoamine oxidase (MAO) A and B inhibitors. This study attempted to discover potent and selective chalcone-derived MAO inhibitors by synthesising a series consisting of eight cyclic chalcone derivatives. The cyclic chalcones were selected based on the possibility that their restricted structures would confer a higher degree of MAO isoform selectivity, and included the following chemical classes: 1-indanone, 1-tetralone, 1-benzosuberone, chromone, thiochromone, 4-chromanone and 4-thiochromanone. The results obtained indicated that the cyclic chalcones are in most instances selective inhibitors of the human MAO-B isoform. The most active MAO-B inhibitor was compound **1b**, a 4-chromanone, with an IC_{50} value of 0.156 μM. This compound did not inhibit human MAO-A at a maximal tested concentration of 100 μM. This study thus shows that certain cyclic chalcones are human MAO-B inhibitors, compounds that could be suitable for the treatment of neurodegenerative disorders such as Parkinson's disease.

Degeneration of the dopaminergic neurons of the substantia nigra pars compacta (SNpc) leads to the striatal dopamine (DA) deficiency and is the main pathological hallmark of Parkinson's disease (PD) (Wood-Kaczmar *et al.*, 2006). The subsequent dysregulation of the motor circuits that project throughout the basal ganglia is responsible for the clinical manifestations of PD (Dauer & Przedborski, 2003). Symptomatic PD therapy relies on DA replacement with its metabolic precursor, levodopa. Levodopa therapy is frequently combined with drugs such as aromatic amino acid decarboxylase (AADC) inhibitors, catechol-*O*-methyltransferase (COMT) inhibitors and monoamine oxidase (MAO) inhibitors, which prevent the metabolism of DA and/or levodopa leading to enhanced dopaminergic neurotransmission. MAO exists as two distinct isoforms, MAO-A and MAO-B (Bach *et al.*, 1988). In the brain MAO-B predominates and represents a key metabolic pathway for DA. Thus, MAO-B inhibitors are currently used as adjuncts to levodopa in PD therapy (Fernandez & Chen, 2007). Furthermore, MAO-B inhibitors may also act as neuroprotectants in PD by reducing the formation of potentially injurious metabolic by-products (such as hydrogen peroxide and aldehydes) of the MAO-B catalytic cycle (Fowler *et al.*, 1994).

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Due to the role of MAO-B inhibitors in the treatment of PD, several research groups are attempting to discover new MAO-B inhibitors with good selectivity over the MAO-A isoform. Chalcone (1,3-diphenyl-2-propen-1-one) derivatives have recently been shown to act as potent and selective inhibitors of MAO-B. Chalcones evolve naturally in edible plants and are the precursors of flavonoids and isoflavonoids (Patil *et al.*, 2009; Prashar *et al.*, 2012). Chalcones possess a variety of biological activities including anti-malaria, anti-inflammatory and antioxidant activity (Patil *et al.*, 2009; Prashar *et al.*, 2012). Recent studies have indicated that chalcones are a general class of MAO-B specific inhibitors. Chimenti and coworkers (2009) used open chain chalcones to identify suitable substituents for MAO inhibition while other researchers substituted the phenyl ring-A for furan. Although the natural form of chalcone displayed better MAO inhibition than the furan-based chalcones, both classes possess specificity and highest potency for the MAO-B isoform over the MAO-A isoform (Chimenti *et al*., 2009; Robinson *et al*., 2013). The current study further explores the SARs for chalcones as MAO inhibitors by introducing conformational restriction. This will be done by cyclising the structure of chalcone to yield 2-benzylidene-1-tetralone (Figure 3.1). The development of analogues with restricted or rigid conformations may result in the selective binding to target sites, which could result in very active drugs with reduced unwanted adverse effects (Gareth, 2007). The effect of modification of ring-C on MAO inhibitory activity and isoform selectivity will be determined in this study. In this respect, this study will vary the size of ring C (n = 5–7) and introduce heteroatoms (O and S) on position 1. In this manner chalcone derivatives of the following chemical classes were investigated: *1-indanone, 1-tetralone, 1-benzosuberone, chromone, thiochromone, 4-chromanone and 4-thiochromanone*. It is important to note that for PD therapy MAO inhibitors should be MAO-B selective since MAO-A inhibition may potentiate tyramine-induced changes in blood pressure, an adverse effect that may be fatal (Finberg, 2014).

The proposal that cyclic chalcones may inhibit MAO-B is supported by recent findings that 1-tetralones (Legoabe *et al.*, 2014) and chromones (Legoabe *et al.*, 2012) are good potency MAO-B specific inhibitors. Based on the structural similarities of these moieties with cyclic chalcones, it may thus be anticipated that cyclic chalcones may act as MAO inhibitors. In this study, the cyclic chalcones illustrated in Table 1 will be syntesised and evaluated as inhibitors of recombinant human MAO-A and MAO-B. The cyclic chalcone derivatives were synthesised via a one-pot Claisen-Schmidt condensation reaction. 1-Tetralone, 1-indanone, 1-benzosuberone, chromone, thiochromone, 4-chromanone and 4-thiochromanone were thus reacted with a slight excess of an aldehyde (1.1 equiv.). The reaction solvent was methanol or ethanol for the acid catalysed reactions (hydrochloric acid), methanol for base catalyse reactions (NaOH or KOH) or solventless conditions for the piperidine catalysed reactions (Figure 3.2). The crude products were recrystallised from appropriate solvents yielding **1a–i** in moderate to high yields (49–84%).

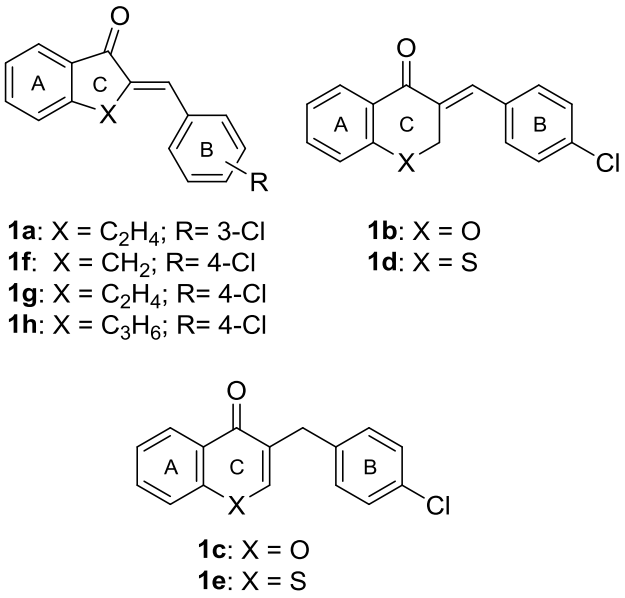


Figure 3.1: The general structure of the cyclic chalcones investigated in this study.

The MAO inhibitory properties of the synthesised compounds were evaluated by employing the recombinant human MAO-A and MAO-B enzymes. To determine the catalytic activities of both enzymes, the non-fluorescent MAO-A/B substrate (kynuramine) was used (Novaroli *et al.*, 2005). Kynuramine is oxidised to yield a fluorescent product, 4-hydroxyquinoline. The concentrations of 4-hydroxyquinoline formed were measured by fluorescence spectrophotometry at excitation and emission wavelengths of 310 nm and 400 nm, respectively. None of the test inhibitors affected the fluorescence of 4-hydrozyquinoline under the conditions of this study. By measuring MAO activities in the presence of different concentrations of the test inhibitors, sigmoidal activity-concentration curves were constructed, from which IC_{50} values were estimated. All experiments were carried out in triplicate and the IC_{50} values (Table 3.) were express as mean ± standard deviation (SD).

The data obtained indicate that the compounds are moderate inhibitors of MAO with specificity for the MAO-B isoform. With the exception of compound **1f**, the cyclic chalcones are weak MAO-A inhibitors**.** Compound **1f**, a 1-indanone derivative, exhibited good MAO inhibitory activities with IC_{50} values of 0.346 µM and 0.420 µM for the inhibition of MAO-A and MAO-B, respectively. However, compound **1f** shows poor isoform selectivity (SI = 0.826).

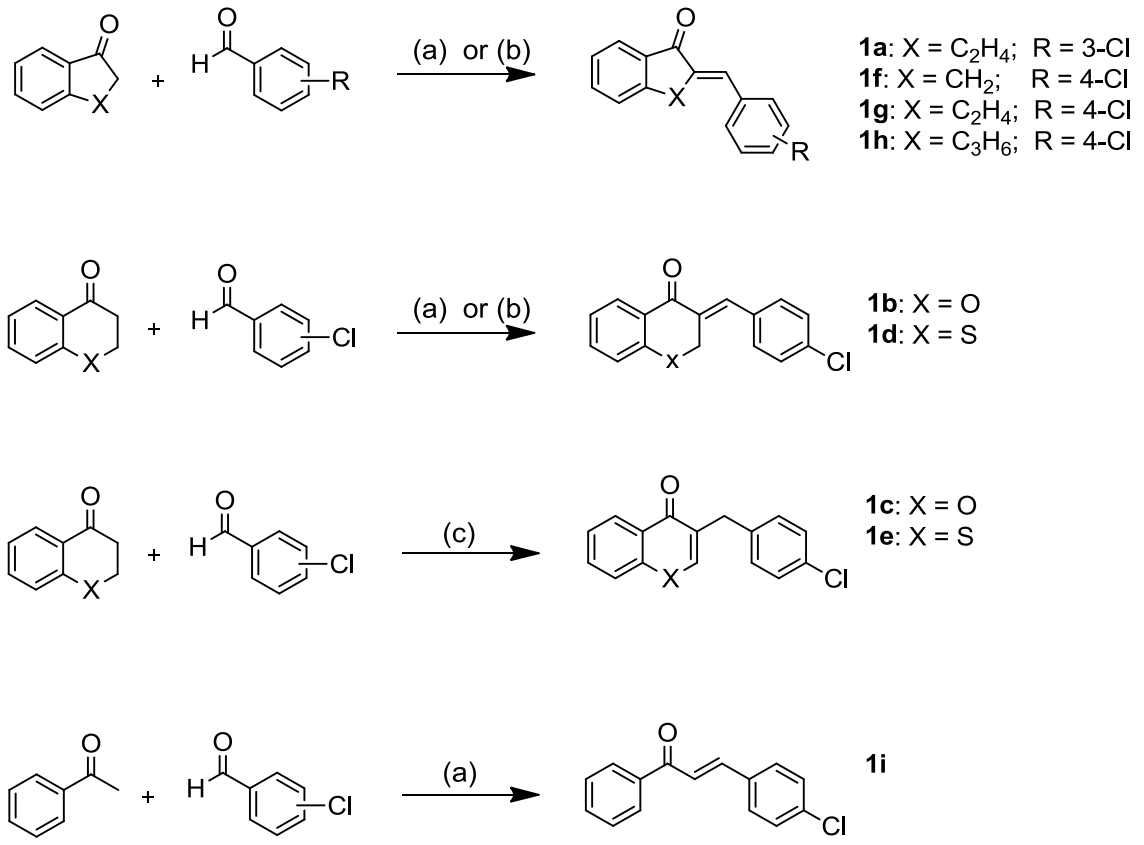


Figure 3.2: Synthetic routes to cyclic chalcone derivatives

1a–i. Reagents and conditions (a) NaOH/KOH, rt, 2–12 h (1a, g & h) (b) HCl, reflux, 4 h (1f); (c) piperidine, rt. 2 h.

The analysis of the IC_{50} values for MAO-A inhibition suggests that: (a) since the 1-indanone derivative is the most potent MAO-A inhibitor, ring expansion is not desirable for MAO-A inhibition by cyclic chalcones; (b) the position of the lipophilic substituent (Cl) on ring-B does not affect MAO-A inhibition (compare **1a** versus **1g**); (c) while the 1-tetralone (**1a**) and 4-chromanone (**1b**) derivatives do not inhibit MAO-A, the thiochromanone (**1d**) yields slight inhibition (IC_{50} = 78.8 µM). This shows that hydrogen bonding of the 4-chromanone oxygen does not contribute to inhibitor binding; (d) both the chromone (**1c**) and thiochromone (**1e**) derivatives exhibit poor MAO-A inhibition; (e) the open chain chalcone (**1i**), also is a weak MAO-A inhibitor.

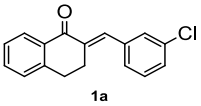
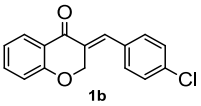
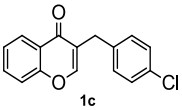
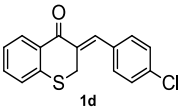
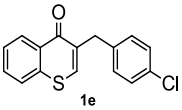
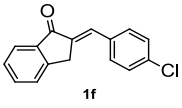
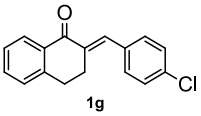
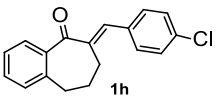
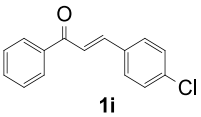
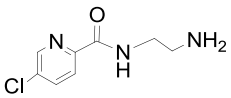
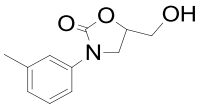
The IC_{50} values for the inhibition of MAO-B, shows that the cyclic chalcone derivatives are moderately potent MAO-B inhibitors. Compound **1b**, the 4-chromanone derivative, proved to be the most potent MAO-B inhibitor of the series with an IC_{50} value of 0.156 µM**.** It may thus be concluded that the 4-chromanone derivative is best suited for potent MAO-B inhibition. This compound is also a very selective MAO-B inhibitor since it does not inhibit MAO-A at a maximal tested concentration of 100 µM. Among the cyclic chalcones evaluated, **1b** is thus the most

appropriate lead for the discovery of PD therapy. It is noteworthy that *meta* chlorine substitution (**1a**) yields more potent MAO-B inhibition compared to *para* substitution (**1g**). Interestingly, the 1-benzosuberone derivative, (**1h**), is not a MAO-B inhibitor, which shows that expansion of ring-C beyond n = 6 is not favourable for MAO-B inhibition. The chromone (**1c**) and thiochromone (**1e**) derivatives also are weak MAO-B inhibitors. In this respect, the 4-chromanone (**1b**) and 4-thiochromanone (**1d**) derivatives are significantly more potent MAO-B inhibitors.

None of the cyclic chalcones evaluated are more potent MAO-B inhibitors than the reference inhibitor, lazabemide (IC_{50} = 0.091 μM). Compared to the open-chain chalcone (**1i**), 4-chromanone derivative (**1b**), is 6-fold more potent as a MAO-B inhibitor. This further supports the proposal that **1b** is a suitable lead for the design of specific MAO-B inhibitors.

This study therefore concludes that cyclic chalcones, such as 4-chromanone derivative (**1b**) represent suitable scaffolds for the development of MAO-B specific inhibitors. Such compounds may be useful as adjuvants to levodopa in the treatment of PD.

Table 3.1: The IC_{50} values for the inhibition of recombinant human MAO-A and MAO-B by compounds 1a–h.

Compound	MAO-A IC50 (μM)[a]	MAO-B IC50 (μM)[a]	Selectivity Index (SI)[b]
 1a	No Inhibition	0.593 ± 0.105	-
 1b	No Inhibition	0.156 ± 0.053	-
 1c	19.5 ± 3.40	12.1 ± 0.409	1.6
 1d	78.8± 5.54	0.883 ± 0.166	89
 1e	64.7 ± 3.71	75.3 ± 13.9	0.86
 1f	0.346 ± 0.041	0.420 ± 0.138	0.826
 1g	No Inhibition	0.995 ± 0.146	-
 1h	No Inhibition	No Inhibition	-
 1i	54.31 ± 7.83	0.964 ± 0.139	56
 lazabemide	202 ± 26.0	0.091 ± 0.015[c]	2219
 toloxatone	3.92 ± 0.015[c]	-	-

All values are reported as mean ± standard deviation (SD) of triplicate experiments. [b]The selectivity index (SI) is the selectivity for the MAO-B isoform and is given as the ratio of IC_{50}(MAO-A)/IC_{50}(MAO-B. [c]Inhibition values for the reference inhibitors were obtained from literature (Petzer *et al.*, 2013).

3.1 Acknowledgements

The NMR and MS spectra were recorded by André Joubert and Johan Jordaan of the SASOL Centre for Chemistry, North-West University. This work is based on the research supported in part by the Medical Research Council and National Research Foundation of South Africa (Grant specific unique reference numbers (UID) 85642, 96180, 96135). The Grantholders acknowledge that opinions, findings and conclusions or recommendations expressed in any publication generated by the NRF supported research are that of the authors, and that the NRF accepts no liability whatsoever in this regard.

3.2 Supplementary data

3.2.1 Materials and methods

All reagents were obtained from Sigma-Aldrich and were used without purification. Column chromatography was carried out with silica gel 60, while thin-layer chromatography was performed on 0.20 mm thick aluminium silica gel sheets. Developed sheets were visualised under UV light (254 and 366 nm) or by staining with iodine vapour. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III 600 spectrometer at 600 MHz and 151 MHz, respectively, in deuterated chloroform ($CDCl_3$) or deuterated dimethyl sulfoxide (DMSO-d_6). The chemical shifts are reported in parts per million (δ) downfield from the signal of tetramethylsilane (TMS) added to the deuterated solvent. Spin multiplicities are given as s (singlet), d (doublet), t (triplet), dd (doublet of doublets) dt (doublet of triplets), tt (triplet of triplets), q (quartet), qn (quintet), m (multiplet) or brs (broad singlet). Melting points (mp) were determined with a Buchi B-545 melting point apparatus and are not corrected. Fluorescence measurements were conducted with a Varian Cary Eclipse fluorescence spectrophotometer. Microsomes from insect cells containing recombinant human MAO-A and MAO-B (5 mg/mL), kynuramine dihydrobromide, lazabemide and toloxatone were obtained from Sigma-Aldrich.

Chemical purity was determined by high performance liquid chromatography (HPLC): HPLC analyses were performed using an Agilent 1200 series HPLC system equipped with a quaternary gradient pump, auto sampler, diode array detector and Chemstation data acquisition

and analysis software. A Venusil XBP C18 column (4.60 × 150 mm, 5 µm) was used and elution was effected by means of a linear gradient starting at 30% acetonitrile and 70% water, increasing to 85% acetonitrile after 5 min and holding at 85% acetonitrile until the end of the HPLC run at 10 min. The instrument was allowed to equilibrate to the starting conditions for 5 min. Standard solutions (1 mM) of each of the eight test compounds were prepared in analytical grade acetonitrile and analysed at wavelengths of 210, 254 and 300 nm. The flow rate was set to 1 mL/min and the injection volume was 20 µL.

3.2.2 Chemical synthesis

3.2.2.1 Synthesis and characterisation of compounds 1a–i

The cyclic chalcone analogues **1b** and **1f** were synthesised via the acid catalysed Claisen-Schmidt condensation reaction of 1-indanone and 4-chromanone (1.850 mmol) and the appropriate aldehyde (2.035 mmol), dissolved in 15 mL methanol. Compounds **1a, c**–**e, g**–**h** and open chain chalcone **1i** on the other hand, were synthesised by base catalysis, reacting 1-tetralone, 1-benzosuberone, chromone, thiochromone, 4-thiochromanone and acetophenone (2.052 mmol) with an appropriate aldehyde (2.257 mmol) in 5 mL methanol or solventless. For the acid catalysed reaction, hydrochloric acid (22.5ml) was added to the reaction whereas potassium and sodium hydroxide (4.10 mmol) or piperidine (2 drops) were added to the base catalysed reactions. The acid catalysed reactions were stirred under reflux for 1–6 h while the base catalysed reactions were stirred for 10–18 h or 2–3 h for the solventless reactions at room temperature. With the exceptions of **1c** and **1e**, which were solventless, the products were precipitated by addition of a minimum of 20 mL water before they were collected by filtration and recrystallised from the appropriate solvents. The analytical data for the cyclic chalcone derivatives and the open–chain chalcone are given below.

3.2.3 MAO inhibition studies

The protocol for the measurement of IC_{50} values for the inhibition of the MAOs has been reported in literature (Mostert *et al*., 2015). In short, the enzyme reactions were carried out to a volume of 200 µL in 96-well microtiter plates and contained kynuramine (50 µM), the test inhibitors (0.003–100 µM), and potassium phosphate buffer (pH 7.4, 100 mM). Each reaction also contained 4% DMSO as co-solvent, and control reactions, performed in the absence of inhibitor, were included for each inhibitor evaluated. The enzyme reactions were initiated with the addition of recombinant human MAO-A (0.0075 mg protein/mL) or MAO-B (0.015 mg protein/mL) and incubated for 20 min at 37 °C. The reactions were subsequently terminated with the addition of 80 µL sodium hydroxide (2 N) and the oxidation product of kynuramine, 4-hydroxyquinoline, was quantified by fluorescence spectrophotometry (λ_{ex} = 310; λ_{em} = 400 nm)

(Novaroli *et al*., 2005). A linear calibration curve was prepared with authentic 4-hydroxyquinoline (0.047–1.56 µM). The rate data were fitted to the one site competition model of the Prism 5 software package (GraphPad, San Diego, CA, USA) to obtain sigmoidal plots, from which the IC_{50} values were estimated. All measurements were carried out in triplicate and IC_{50} values are given as the mean ± standard deviation (SD).

3.2.4 Spectral data

(2*E*)-2-(3-Chlorobenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (1a)

The title compound is a product of 3, 4-dihydronaphthalen-1(2*H*)-one and 3-chlorobenzaldehyde in a yield of 76.41%: yellow crystals, mp 112.1–113.2 ^{o}C (ethanol). ^{1}H NMR ($CDCl_3$) δ ppm 8.11 (d, *J* = 7.8 Hz, 1H), 7.76 (s, 1H), 7.48 (td, *J* = 7.5, 0.9 Hz, 1H), 7.41 – 7.21 (m, 6H), 3.08 (dd, *J* = 9.1, 3.8 Hz, 2H), 2.94 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 187.57, 143.17, 137.62, 136.61, 134.91, 134.35, 133.45, 133.25, 129.71, 129.48, 128.47, 128.26, 128.23, 127.96, 127.10, 28.76, 27.15. APCI-HRMS *m/z*: calcd for $C_{17}H_{13}O$ $[M+H]^+$, 269.0728, found: 269.0751. Purity (HPLC): 100%.

(3*E*)-3-(4-Chlorobenzylidene)-2,3-dihydro-4*H*-chromen-4-one (1b)

The title compound is a product of chroman-4-one and 4-chlorobenzaldehyde in a yield of 49.2%: white crystals, mp 173.2–174.0 ^{o}C (ethanol). ^{1}H NMR ($CDCl_3$) δ ppm 8.00 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.78 (s, 1H), 7.48 (ddd, *J* = 8.7, 7.3, 1.7 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.23 (t, *J* = 7.8 Hz, 2H), 7.09 – 7.02 (m, 1H), 6.97 – 6.92 (m, 1H), 5.29 (d, *J* = 1.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 181.93, 161.05, 135.99, 135.55, 132.75, 131.35, 131.16, 129.02, 127.93, 122.00, 121.86, 117.91, 67.40. APCI-HRMS *m/z* calcd for $C_{16}H_{11}ClO_2$ $[M+H]^+$, 271.0520, found 271.0538. Purity (HPLC): 98.2%.

3-(4-Chlorobenzyl)-4*H*-chromen-4-one (1c)

The title compound is a product of chroman-4-one and 4-chlorobenzaldehyde in a yield of 54.8%: brown crystals, mp 140.2–140.8 ^{o}C (ethanol). ^{1}H NMR ($CDCl_3$) δ ppm 8.19 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.70 – 7.58 (m, 2H), 7.44 – 7.31 (m, 2H), 7.30 – 7.12 (m, 4H), 3.72 (d, *J* = 23.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 177.31, 156.41, 152.96, 137.17, 133.54, 132.27, 130.27, 128.66, 125.90, 125.03, 124.11, 123.79, 118.03, 31.16. APCI-HRMS (*m/z*) calcd for $C_{16}H_{11}ClO_2$ $[M+H]^+$, 271.0520, found 271.0510. Purity (HPLC): 97.0%.

(3*Z*)-3-(4-Chlorobenzylidene)-2,3-dihydro-4*H*-thiochromen-4-one (1d)

The title compound is a product of thiochroman-4-one and 4-chlorobenzaldehyde in a yield of 84%: yellow crystals, mp 140.2–143.2 ^{o}C (ethanol). ^{1}H NMR ($CDCl_3$) δ ppm 8.17 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.68 (s, 1H), 7.47 – 7.35 (m, 3H), 7.34 – 7.16 (m, 4H), 4.46 – 3.81 (m, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 185.64, 140.94, 136.18, 134.92, 133.38, 133.13, 132.18, 130.75, 130.43, 129.03, 127.88, 125.85, 29.11. APCI-HRMS *m/z* calcd for $C_{16}H_{11}ClOS$ $[M+H]^+$, 287.0292, found 287.0291. Purity (HPLC): 89.4%.

3-(4-Chlorobenzyl)-4*H*-thiochromen-4-one (1e)

The title compound is a product of thiochroman-4-one and 4-chlorobenzaldehyde in a yield of 80.2%: brown crystals, mp 104.4–105.3 ^{o}C (ethanol).^{1}H NMR ($CDCl_3$) δ ppm 8.55 (d, *J* = 7.9 Hz, 1H), 7.61 – 7.47 (m, 3H), 7.42 (s, 1H), 7.32 – 7.12 (m, 4H), 3.94 (s, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 178.90, 137.30, 137.16, 136.18, 134.28, 132.32, 131.55, 131.11, 130.69, 129.05, 128.74, 127.57, 126.46, 37.28. APCI-HRMS *m/z* calcd for $C_{16}H_{11}ClOS$ $[M+H]^+$, 287.0292, found 287.0300. Purity (HPLC): 100%.

(2*E*)-2-(4-Chlorobenzylidene)-2,3-dihydro-1*H*-inden-1-one (1f)

The title compound is a product of 2,3-dihydro-1-inden-1-one and 4-chlorobenzaldehyde in a yield of 84.3%: white crystals, mp 180.3–184 ^{o}C (dichloromethane).^{1}H NMR ($CDCl_3$) δ ppm 7.87 (d, *J* = 7.6 Hz, 1H), 7.66 – 7.49 (m, 5H), 7.39 (d, *J* = 7.2 Hz, 3H), 3.97 (s, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 194.04, 149.37, 137.81, 135.61, 135.10, 134.73, 133.80, 132.40, 131.74, 129.17, 127.74, 126.14, 124.44, 32.29. APCI-HRMS *m/z* calcd for $C_{16}H_{11}ClO$ $[M+H]^+$, 255.0571, found 255.0581. Purity (HPLC): 99.8%.

(2*E*)-2-(4-Chlorobenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (1g)

The title compound is a product of 3,4-dihydronaphthalen-1(2H)-one and 3-chlorobenzaldehyde in a yield of 76.41%: yellow crystals, mp 138.2–139.9 ^{o}C (petroleum ether/DCM).^{1}H NMR ($CDCl_3$) δ ppm 8.11 (d, *J* = 7.7 Hz, 1H), 7.78 (s, 1H), 7.54 – 7.09 (m, 7H), 3.07 (dd, *J* = 9.1, 3.7 Hz, 2H), 2.93 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 187.60, 143.10, 135.93, 135.22, 134.42, 134.22, 133.37, 133.30, 131.10, 128.68, 128.22, 128.18, 127.07, 28.73, 27.14. APCI-HRMS *m/z*: calcd for $C_{17}H_{13}ClO_2$ $[M+H]^+$, 269.0728, found 269.0719. Purity (HPLC): 100%.

(6*E*)-6-(4-Chlorobenzylidene)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one (1h)

The title compound is a product of 3,4-dihydronaphthalen-1(2H)-one and 3-chlorobenzaldehyde in a yield of 50.9%: yellow crystals, mp 91.1–107.7 ^{o}C (petroleum ether).^{1}H NMR ($CDCl_3$) δ ppm 7.78 – 7.72 (m, 2H), 7.45 (td, *J* = 7.4, 1.3 Hz, 1H), 7.43 – 7.31 (m, 5H), 7.18 (d, *J* = 7.4 Hz, 1H), 2.87 (t, *J* = 6.9 Hz, 2H), 2.55 (t, *J* = 6.8 Hz, 2H), 2.04 (p, *J* = 6.9 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 197.79, 139.55, 138.48, 138.35, 136.56, 134.53, 134.19, 132.53, 130.69, 129.17, 128.97, 128.84, 127.07, 31.60, 26.45, 24.77. APCI-HRMS *m/z* calcd for $C_{18}H_{15}ClO$ $[M+H]^+$, 283.0884, found 283.0896. Purity (HPLC): 99.4%.

(2*E*)-3-(4-Chlorophenyl)-1-phenylprop-2-en-1-one (1l)

The title compound is a product of acetophenone and 4-chlorobenzaldehyde in a yield of 30.7%: yellow crystals, mp 114.8–115.9 ^{o}C (ethanol).^{1}H NMR ($CDCl_3$) δ ppm 7.37 (d, *J* = 8.28 Hz, 2 H) 7.46–7.52 (m, 3 H) 7.53–7.60 (m, 3 H) 7.74 (d, *J* = 15.81 Hz, 1 H) 7.98–8.02 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ ppm 190.19, 143.27, 137.98, 136.40, 133.34, 132.91, 129.56, 129.22, 128.65, 128.47, 122.41. APCI-HRMS *m/z* calcd for $C_{15}H_{11}ClO$ $[M+H]^+$, 243.0571, found 243.0555. Purity (HPLC): 96.1%.

REFERENCES

Bach, A.W.J., Lan, N.C., Johnson, D.L., Abell, C.W., Bembenek, M.E., Kwa, S., Seeburg, P.H. & Shih, J.C. 1988. cDNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymatic properties. *Proceedings of the national academy of sciences of the United States of America*, 85(13):4934-4938.

Chimenti, F., Fioravanti, R., Bolasco, A., Chimenti, P., Secci, D., Rossi, F., Matilde, Y.n.e., Orallo, F., Ortuso, F. & Alcaro, S. 2009. Chalcones: a valid scaffold for monoamine oxidases inhibitor. *Journal of medicinal chemistry*, 52(9):2818-2824.

Dauer, W. & Przedborski, S. 2003. Parkinson's disease: mechanisms and models. *Neuron*, 39(6):889-909.

Fernandez, H.H. & Chen, J.J. 2007. Monoamine oxidase-B inhibition in the treatment of Parkinson's disease. *Pharmacotherapy*, 27(12 pt 2):174-185.

Finberg, J.P.M. 2014. Update on the pharmacology of selective inhibitors of MAO-A and MAO-B: focus on modulation of CNS monoamine neurotransmitter release. *Pharmacology & therapeutics*, 143(2):133-152.

Fowler, J.S., Volkow, N.D., Logan, J., Wang, G.-J., Mac Gregor, R.R., Schlyer, D., Wolf, A.P., Pappas, N., Alexoff, D., Shea, C., Dorflinger, E., Kruchowy, L., Yoo, K., Fazzini, E. & Patlak, C. 1994. Slow recovery of human brain MAO B after l-deprenyl (selegeline) withdrawal. *Synapse*, 18(2):86-93.

Gareth, T. 2007. Medicinal chemistry: an introduction. Chichester: Wiley. http://www.wiley.com/WileyCDA/WileyTitle/productCd-0470025972.html. Date of access: 31 October 2016.

Legoabe, L.J., Petzer, A. & Petzer, J.P. 2012. Inhibition of monoamine oxidase by selected C6-substituted chromone derivatives. *European journal of medicinal chemistry*, 49:343-353.

Legoabe, L.J., Petzer, A. & Petzer, J.P. 2014. α-Tetralone derivatives as inhibitors of monoamine oxidase. *Bioorganic & medicinal chemistry letters*, 24(12):2758-2763.

Mostert, S., Petzer, A. & Petzer, J.P. 2015. Indanones as high-potency reversible inhibitors of monoamine oxidase. *ChemMedChem*, 10(5):862-873.

Novaroli, L., Reist, M., Favre, E., Carotti, A., Catto, M. & Carrupt, P.-A. 2005. Human recombinant monoamine oxidase B as reliable and efficient enzyme source for inhibitor screening. *Bioorganic & medicinal chemistry*, 13(22):6212-6217.

Patil, C.B., Mahajan, S.K. & Katti, S.A. 2009. Chalcone: a versatile Molecule. *Journal of pharmaceutical sciences and research*, 1(3):11-22.

Prashar, H., Chawla, A., Sharma, A.K. & Kharb, R. 2012. Chalcone as a versatile moiety for diverse pharmacological activities. *International journal of pharmaceutical sciences and research*, 3(7):1000-1014.

Robinson, S.J., Petzer, J.P., Petzer, A., Bergh, J.J. & Lourens, A.C.U. 2013. Selected furanochalcones as inhibitors of monoamine oxidase. *Bioorganic & medicinal chemistry letters*, 23(17):4985-4989.

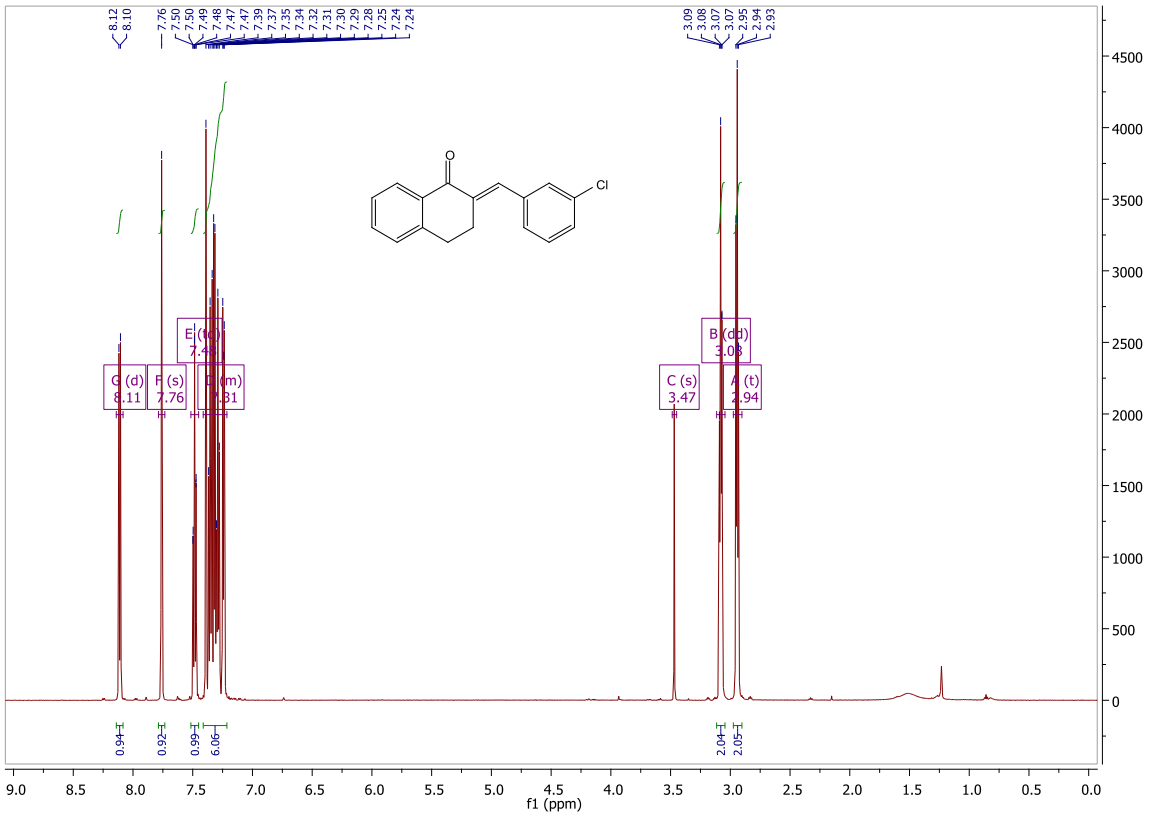
Wood-Kaczmar, A., Gandhi, S. & Wood, N.W. 2006. Understanding the molecular causes of Parkinson's disease. *Trends in molecular medicine*, 12(11):521-528.

APPENDIX A: SPECTRA

^{1}H NMR, ^{13}C NMR, HRMS and HPLC

(2*E*)-2-(3-Chlorobenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (1a)

^{1}H NMR



HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
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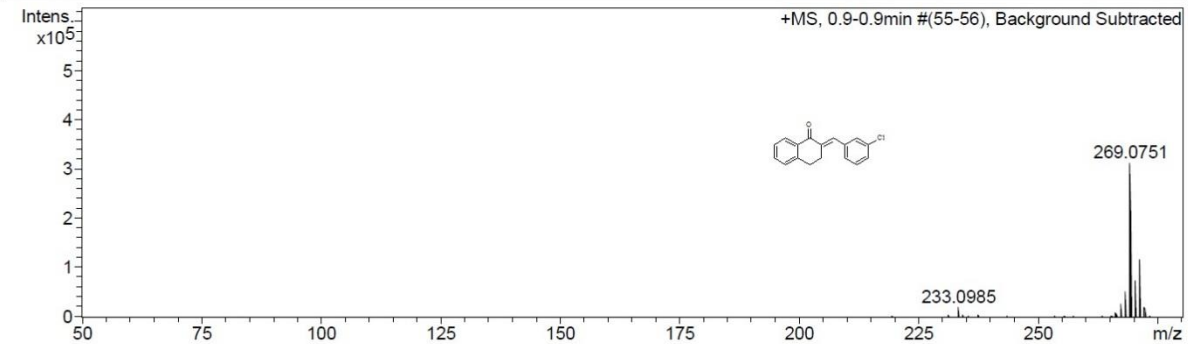
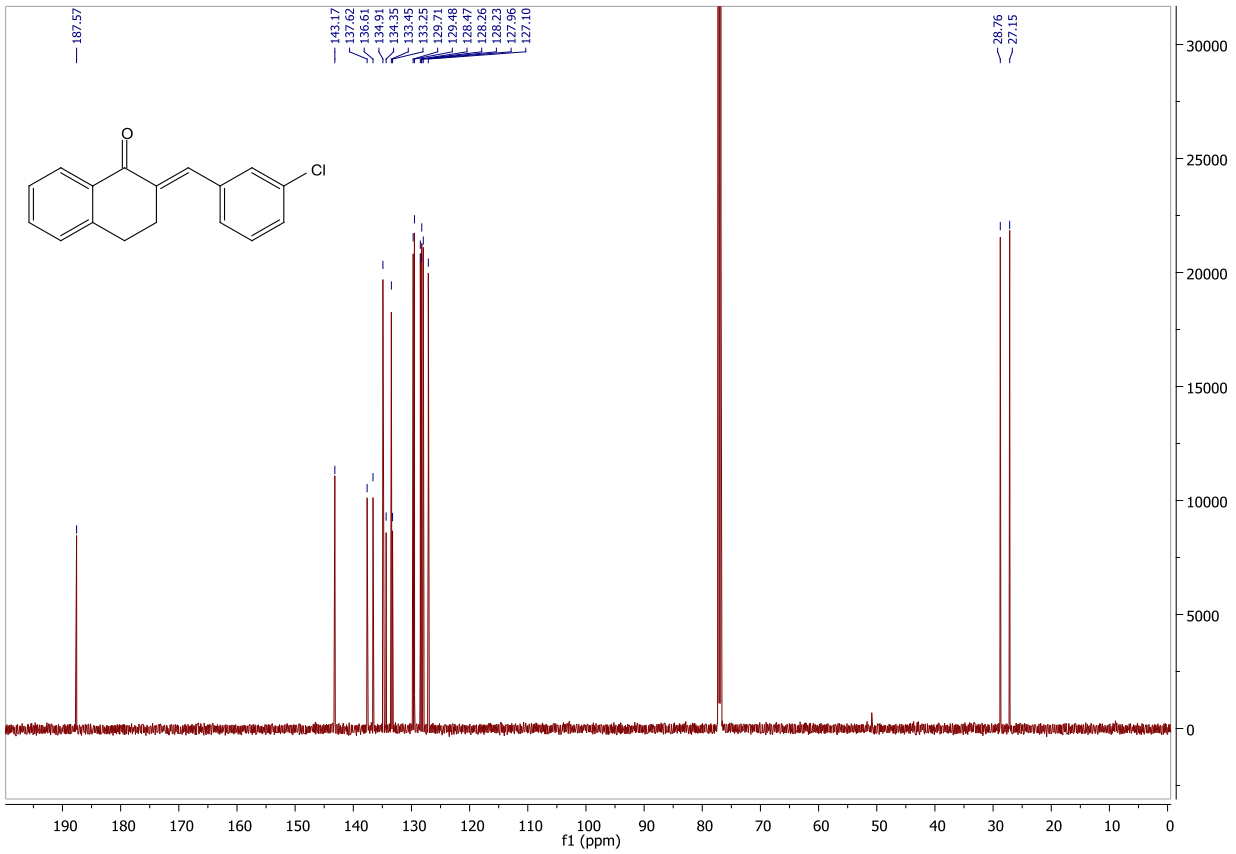
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste



^{13}C NMR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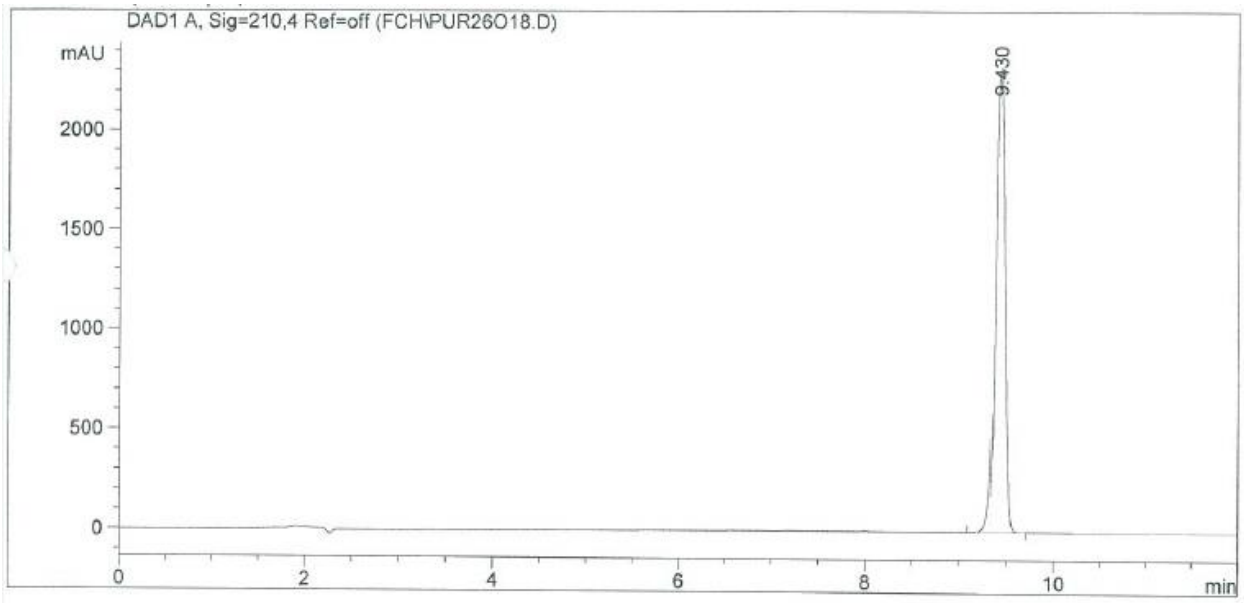
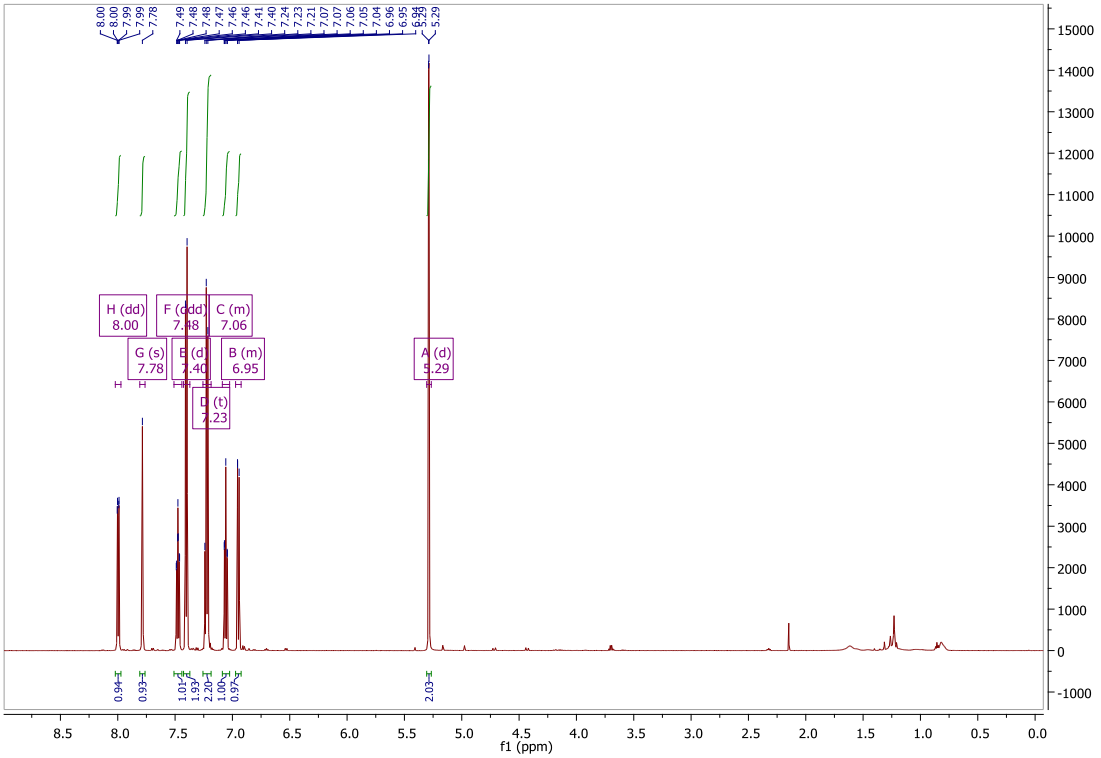
143.17
137.62
136.61
134.91
134.35
133.45
133.25
129.71
129.48
128.47
128.26
128.23
127.96
127.10
28.76
27.15
O
Cl
30000
25000
20000
15000
10000
5000
0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

HPLC

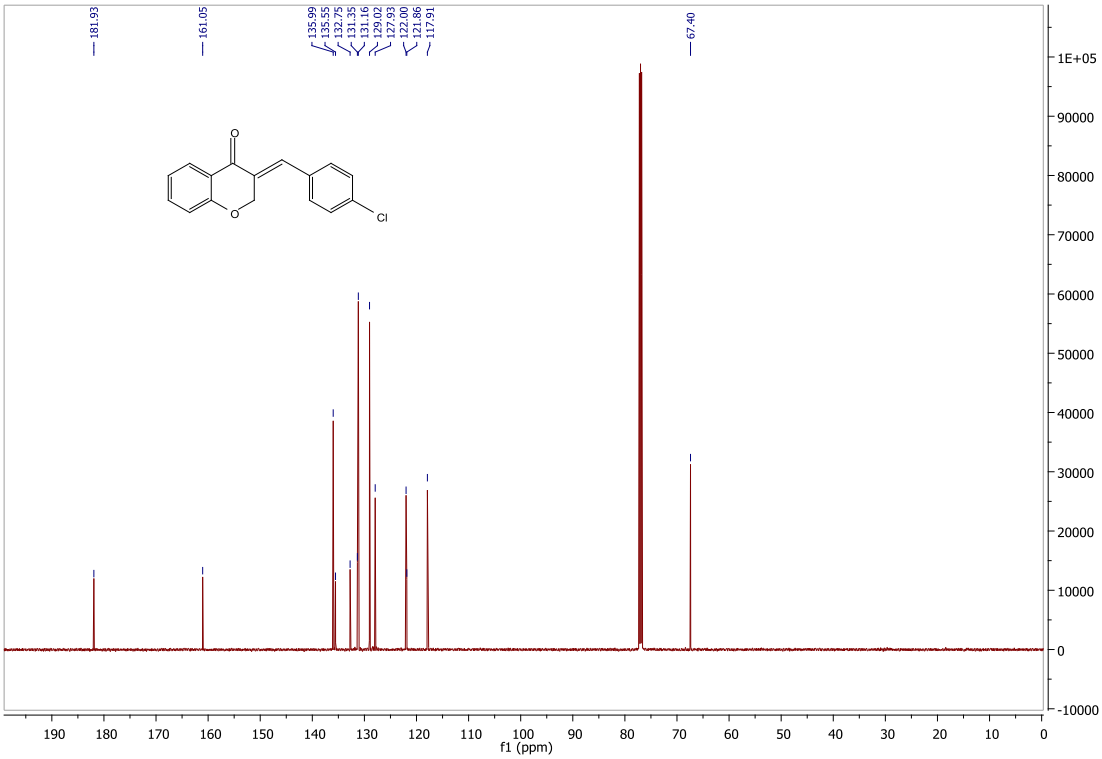

DAD1 A, Sig=210,4 Ref=off (FCH\PUR26O18.D)
mAU
2000
1500
1000
500
0
9.430
0
2
4
6
8
10
min

(3*E*)-3-(4-Chlorobenzylidene)-2,3-dihydro-4*H*-chromen-4-one (1b)

^{1}H NMR



^{13}C NMR



HRMS

Acquisition Parameter

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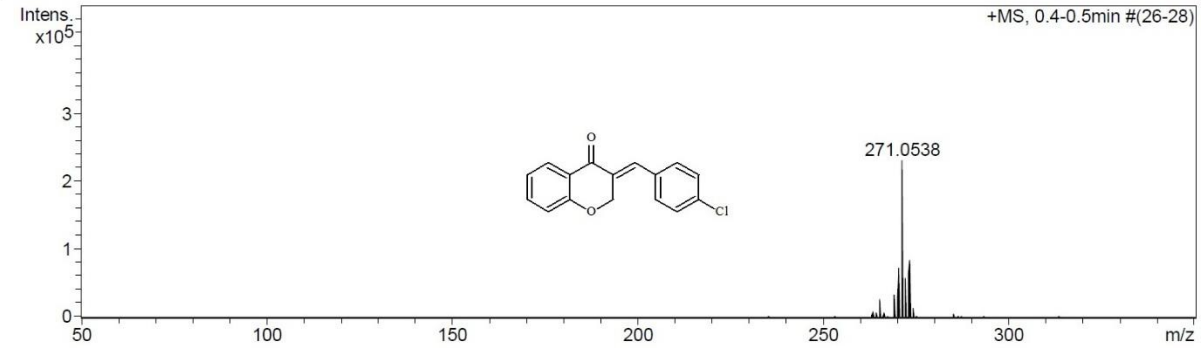
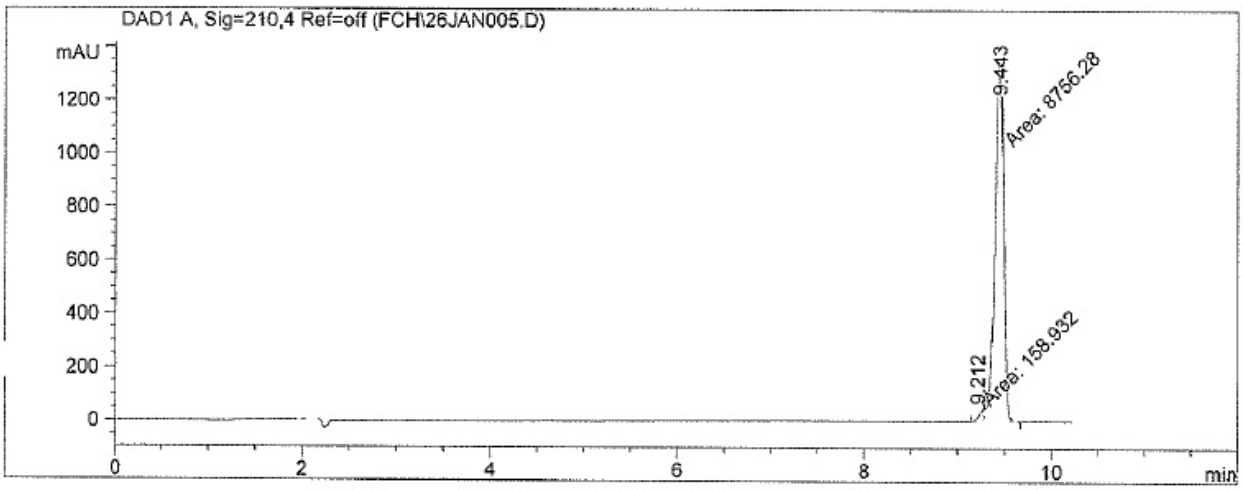
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste

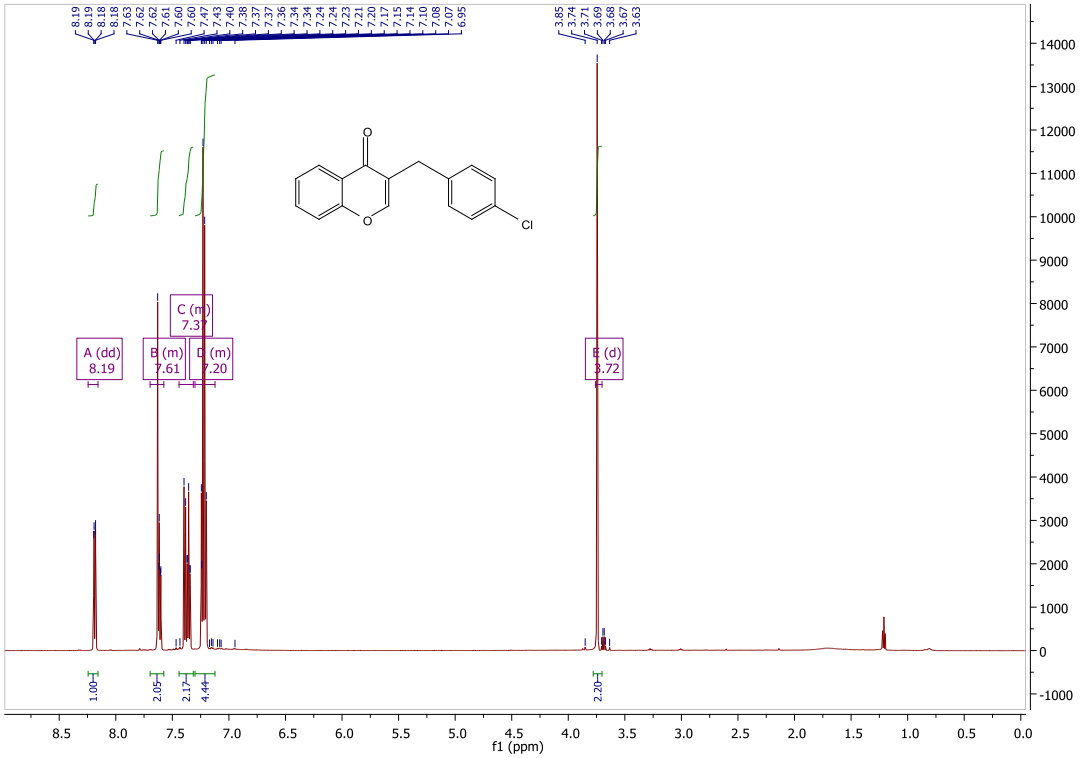


HPLC

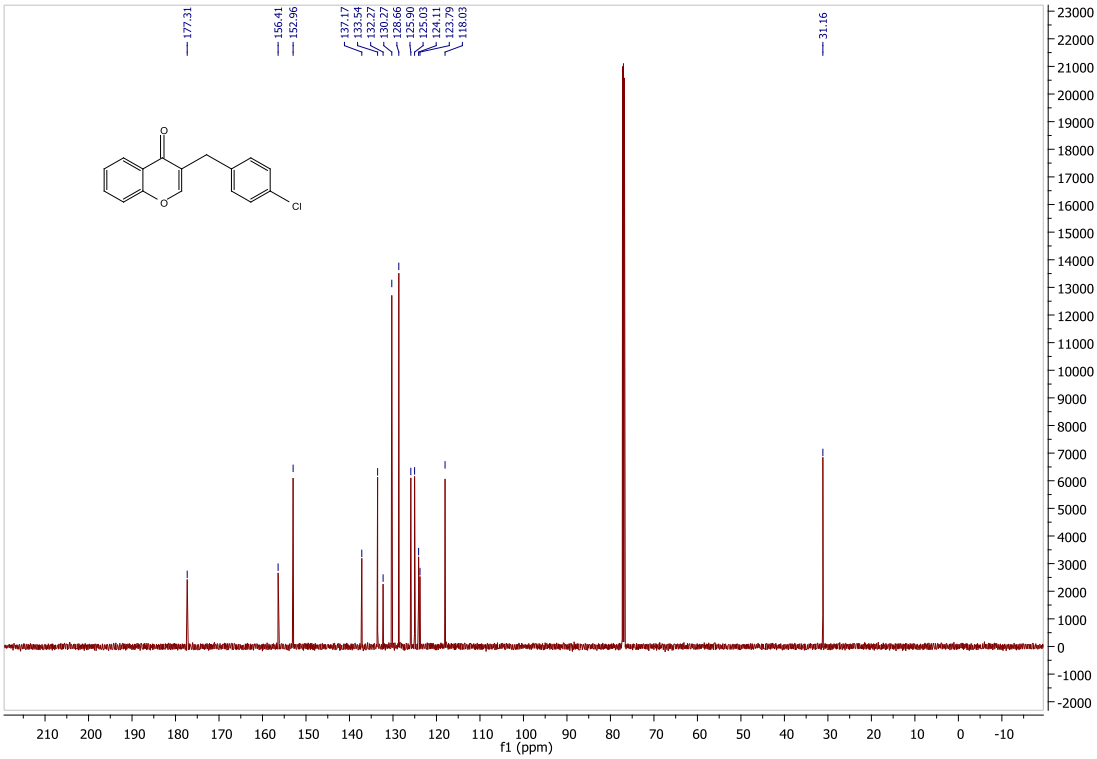


3-(4-Chlorobenzyl)-4*H*-chromen-4-one (1c)

^{1}H NMR



^{13}C NMR



HRMS

Acquisition Parameter

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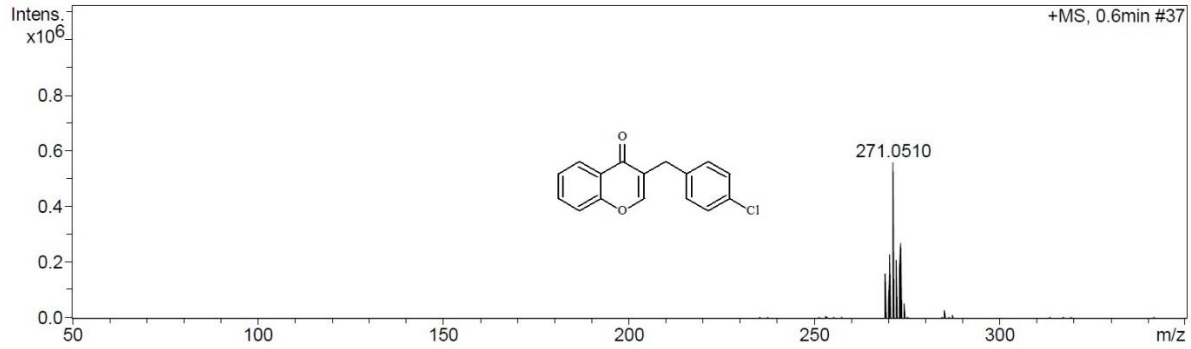
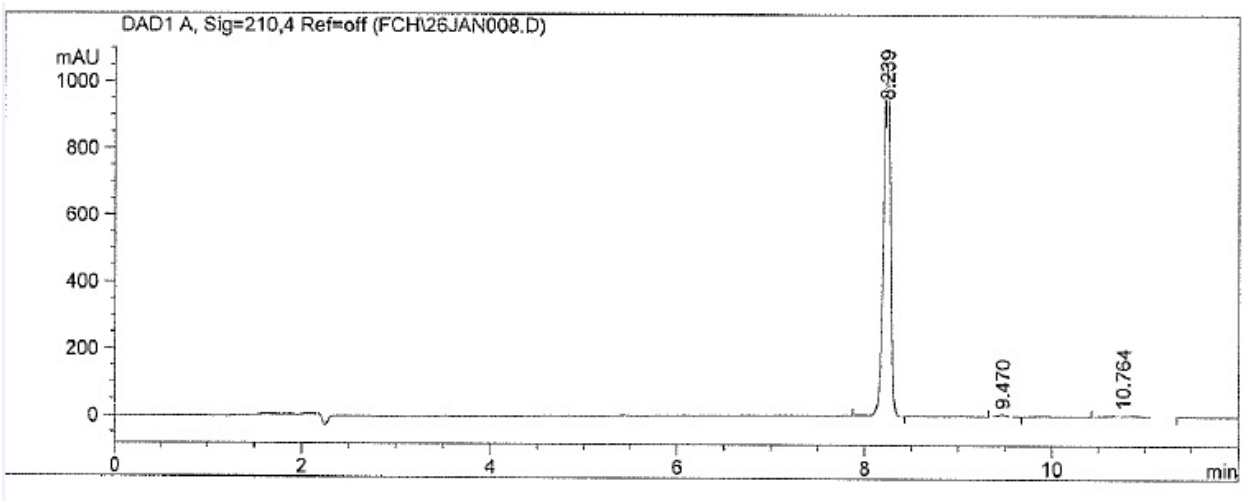
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste

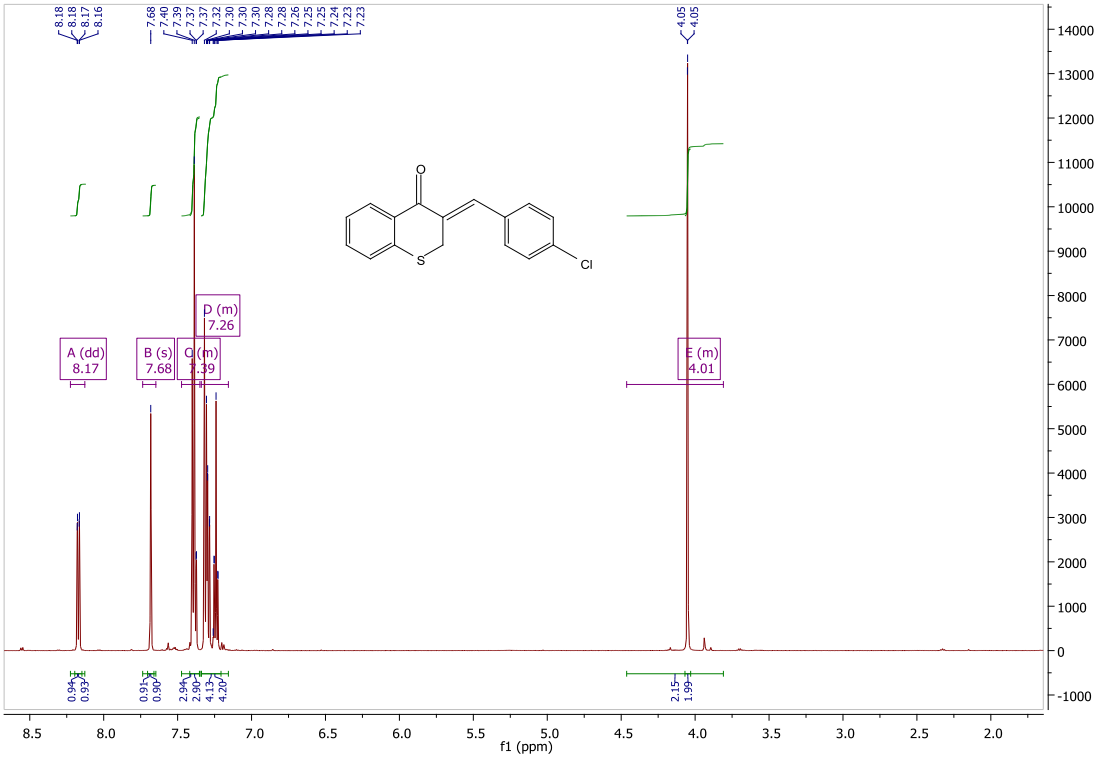


HPLC

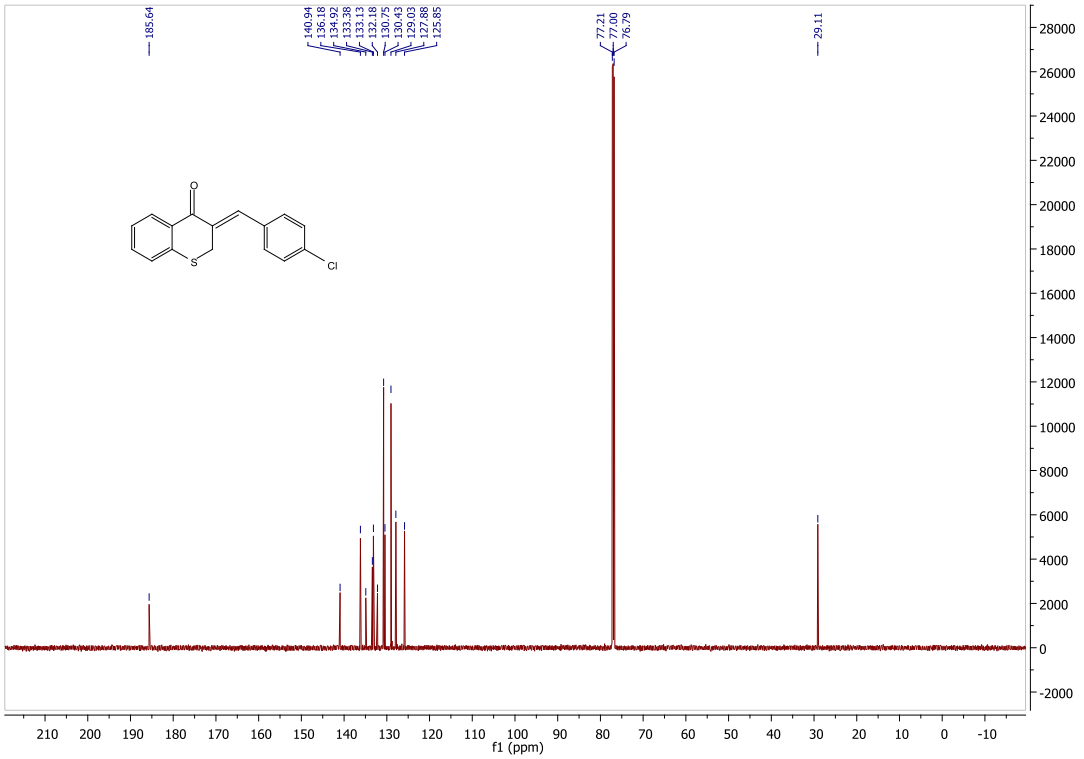


(3*Z*)-3-(4-Chlorobenzylidene)-2,3-dihydro-4*H*-thiochromen-4-one (1d)

^{1}H NMR



^{13}C NMR



HRMS

Acquisition Parameter

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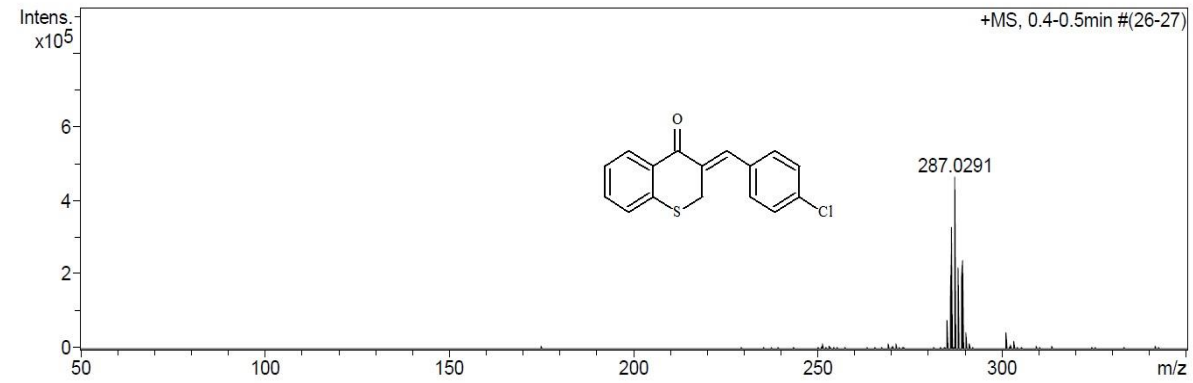
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste



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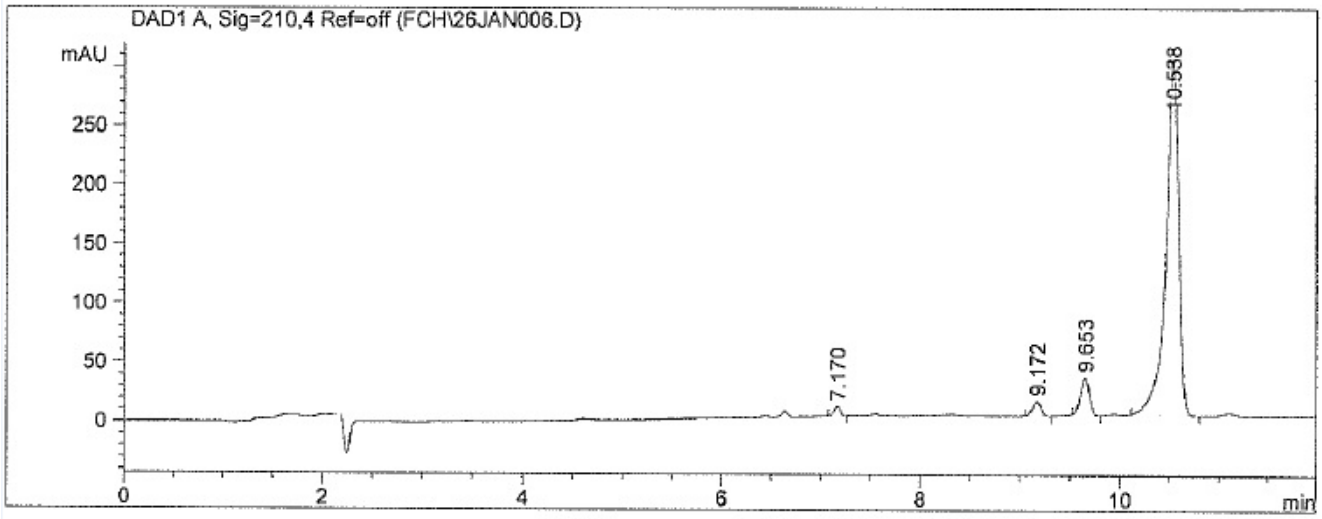
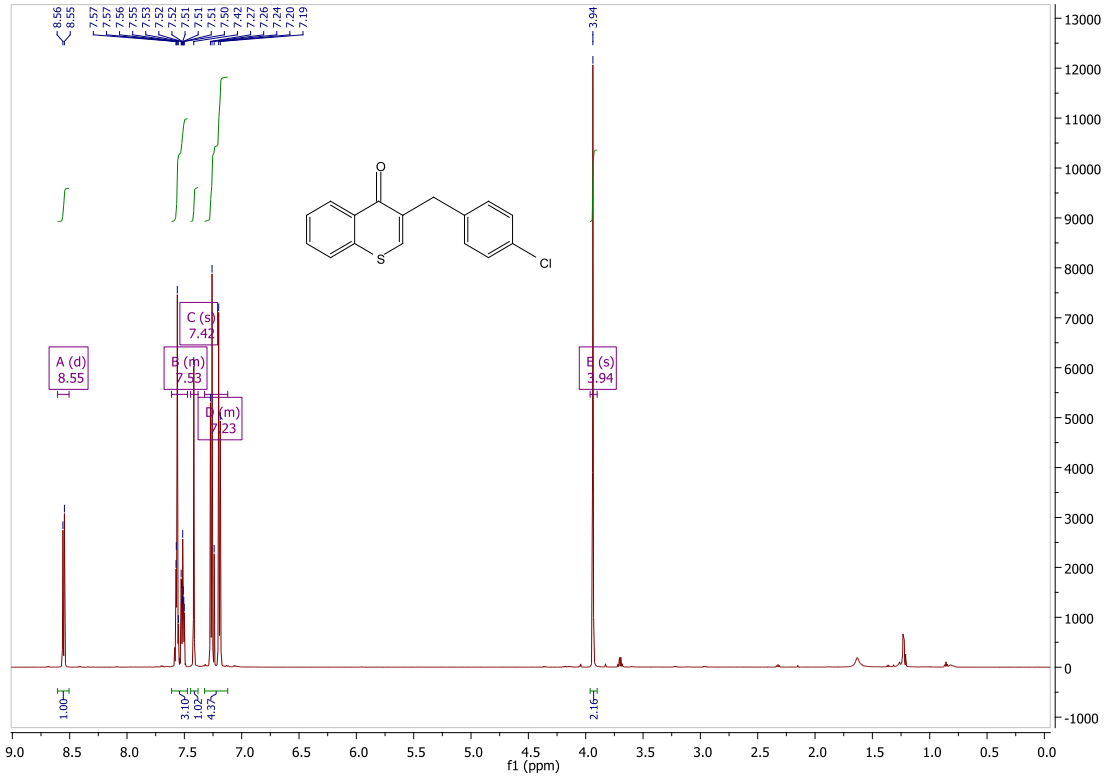
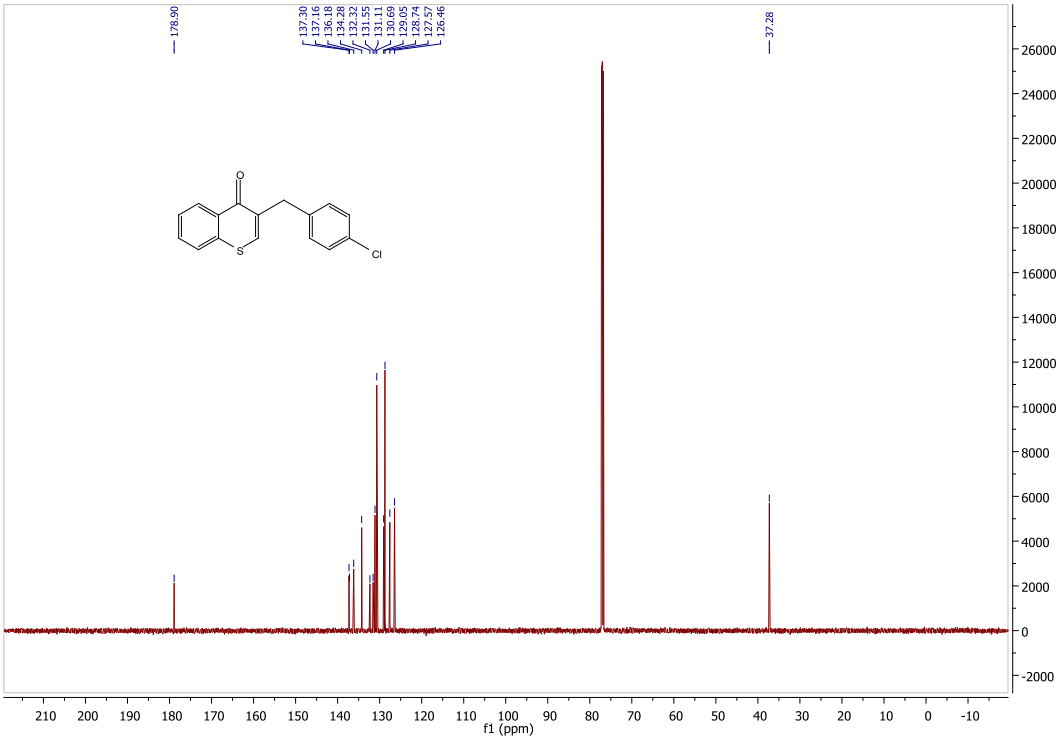


3-(4-Chlorobenzyl)-4*H*-thiochromen-4-one (1e)

^{1}H NMR



^{13}C NMR



HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
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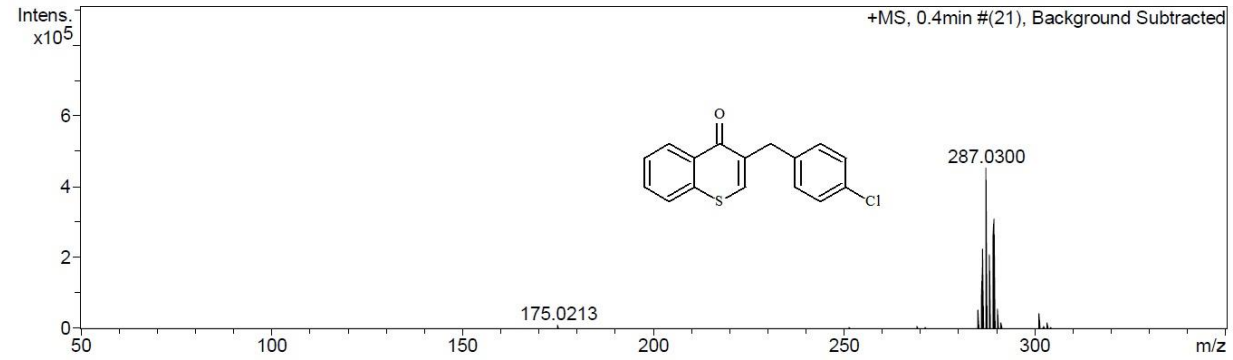
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste



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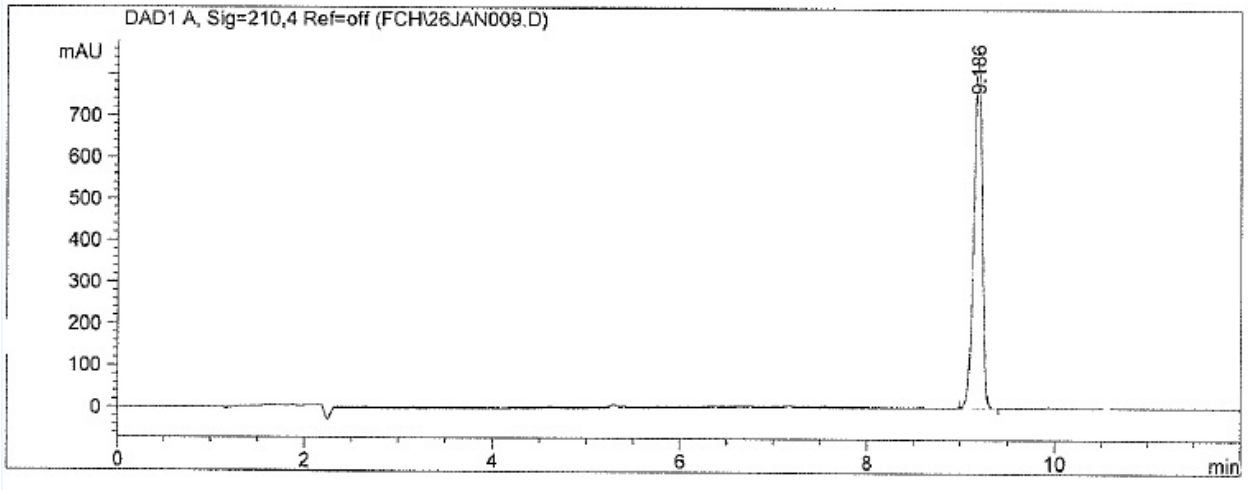
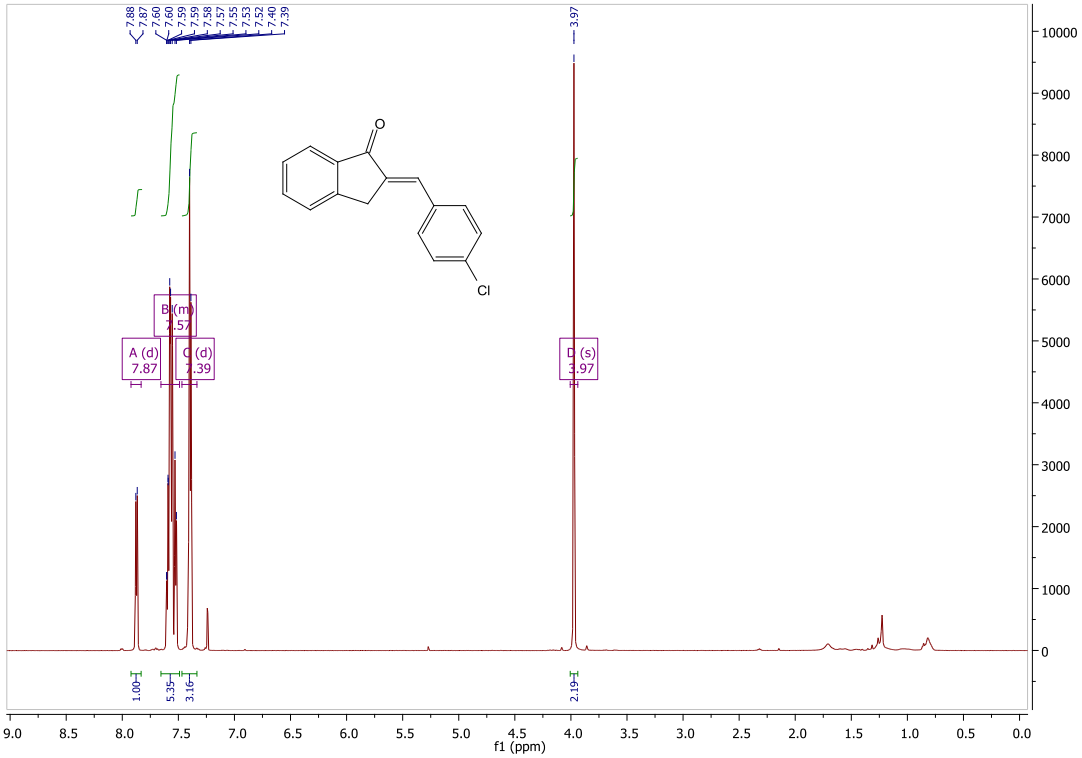
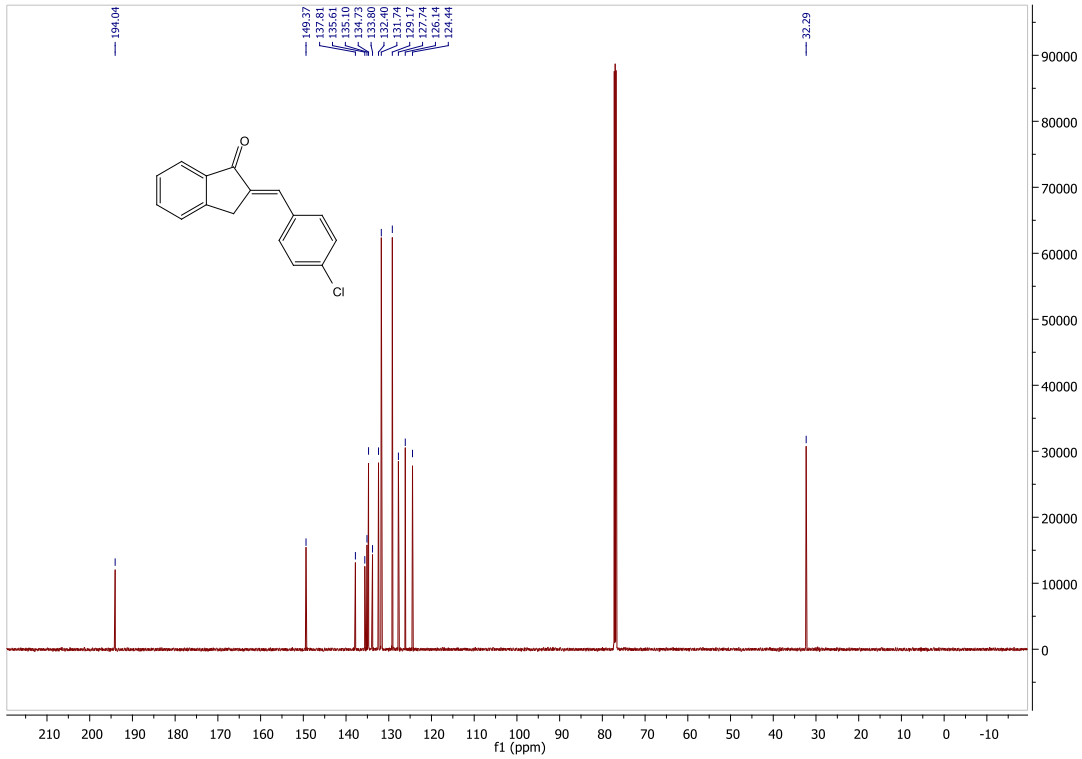


(2*E*)-2-(4-Chlorobenzylidene)-2,3-dihydro-1*H*-inden-1-one (1f)

^{1}H NMR



^{13}C NMR



HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
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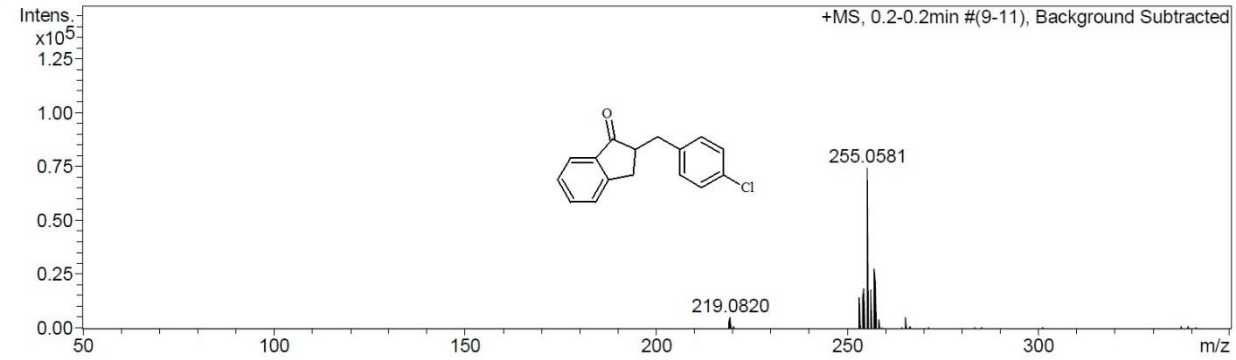
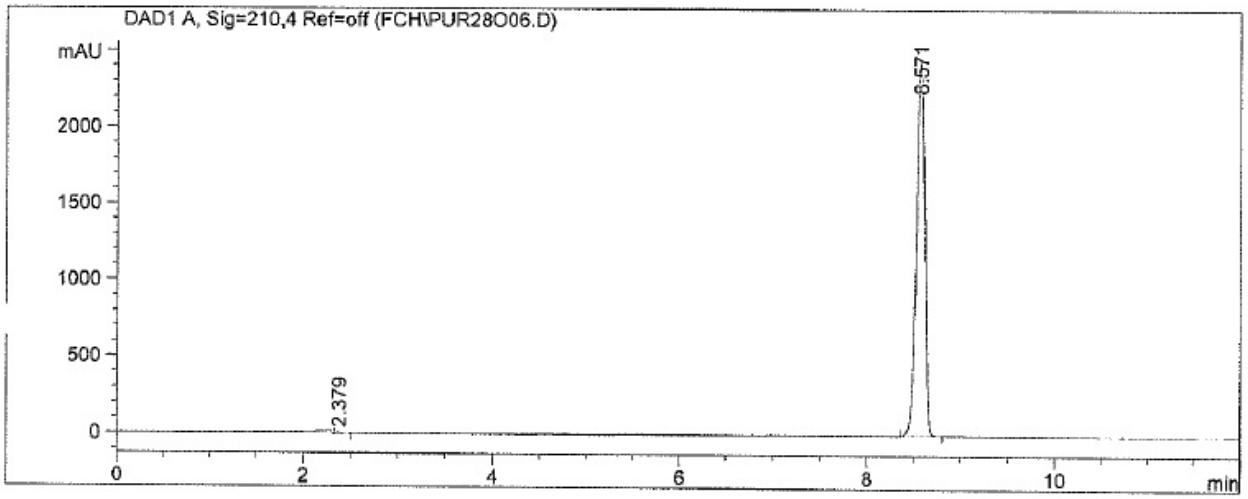
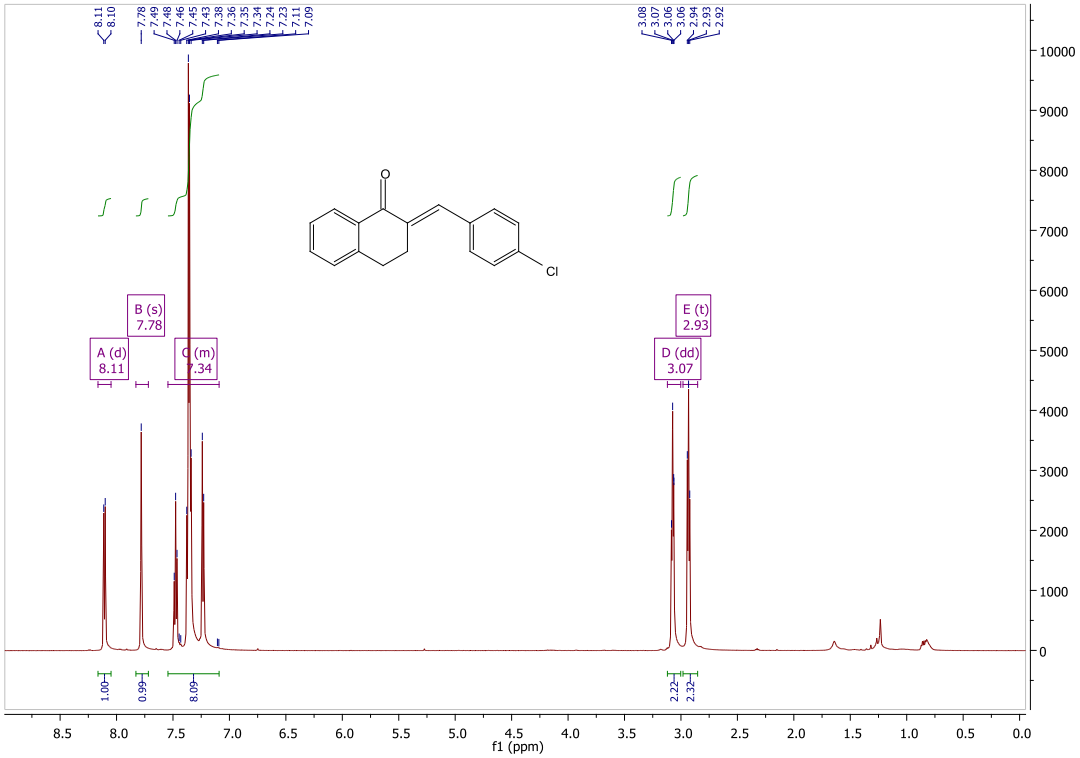


HPLC

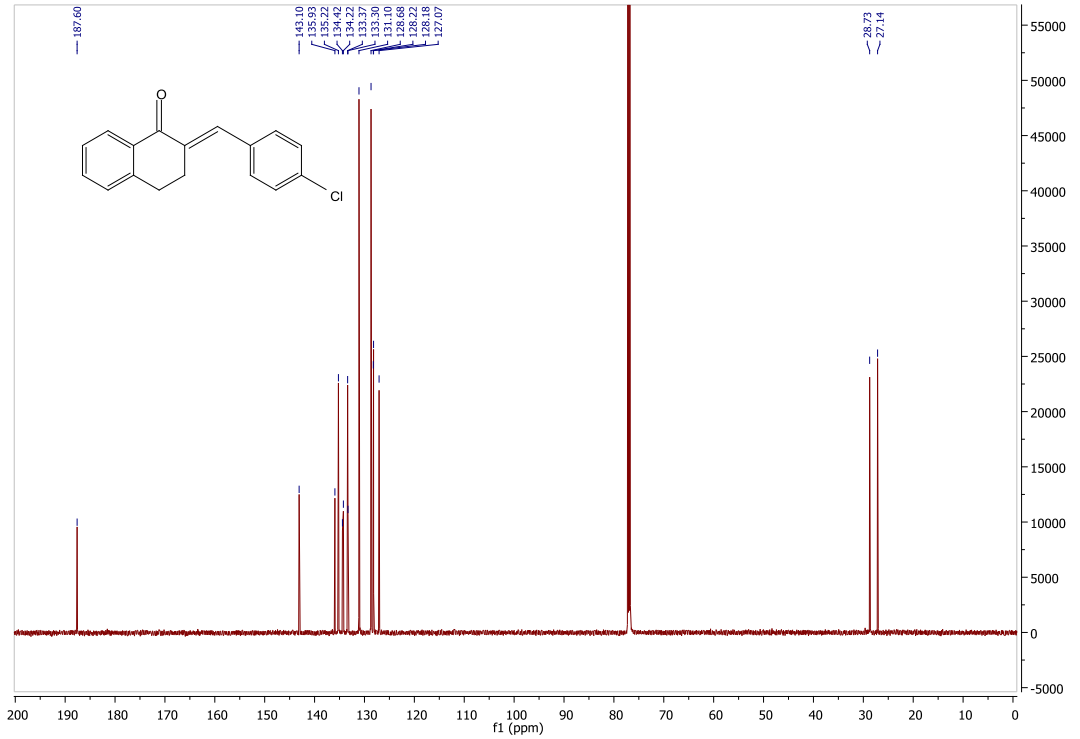


(2*E*)-2-(4-Chlorobenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (1g)

^{1}H NMR



^{13}C NMR



HRMS

Acquisition Parameter

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Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
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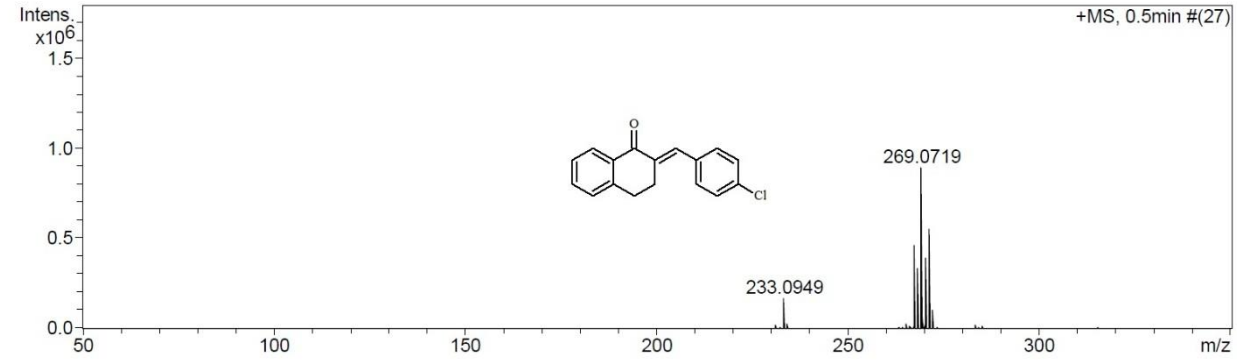
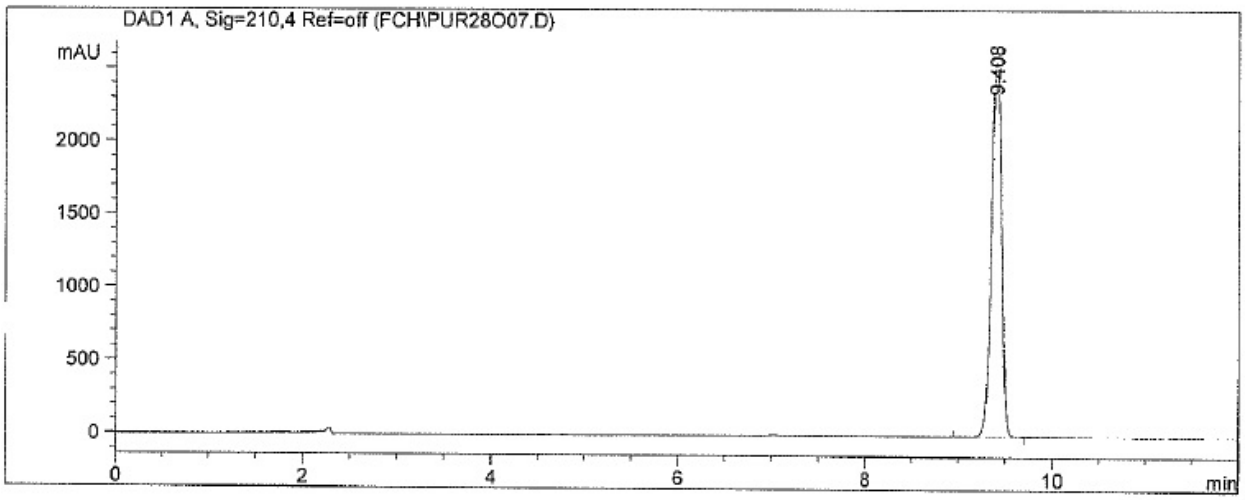
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste

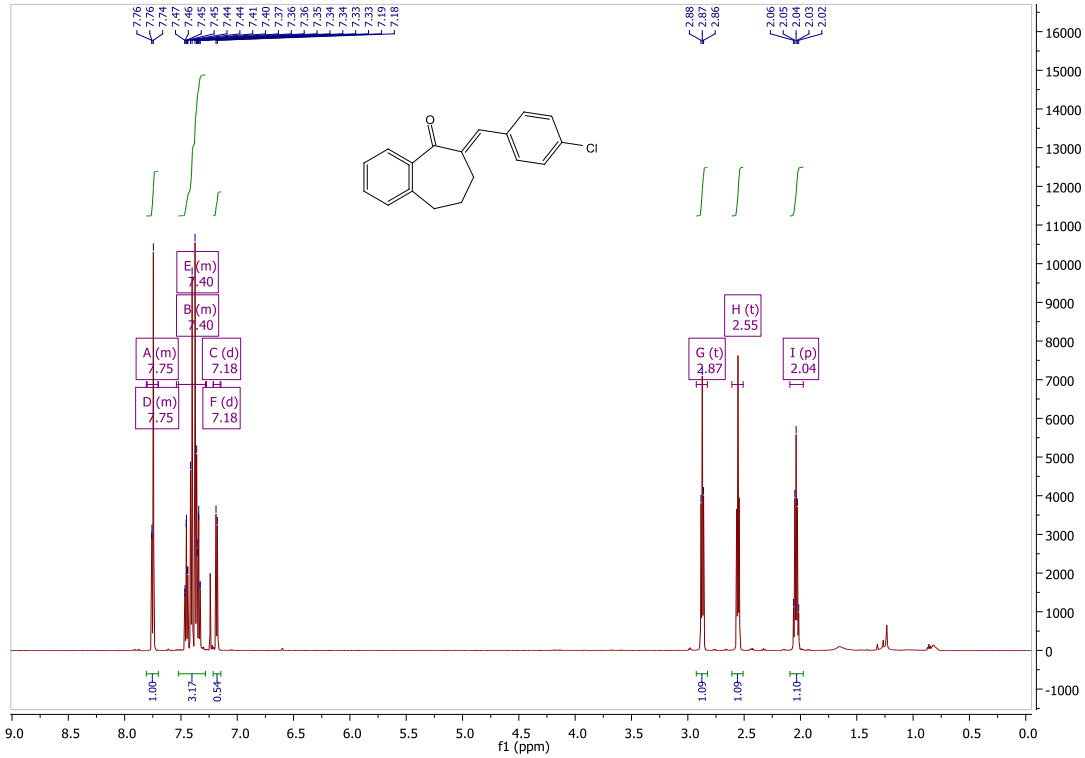


HPLC



(6*E*)-6-(4-Chlorobenzylidene)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one (1h)

^{1}H NMR



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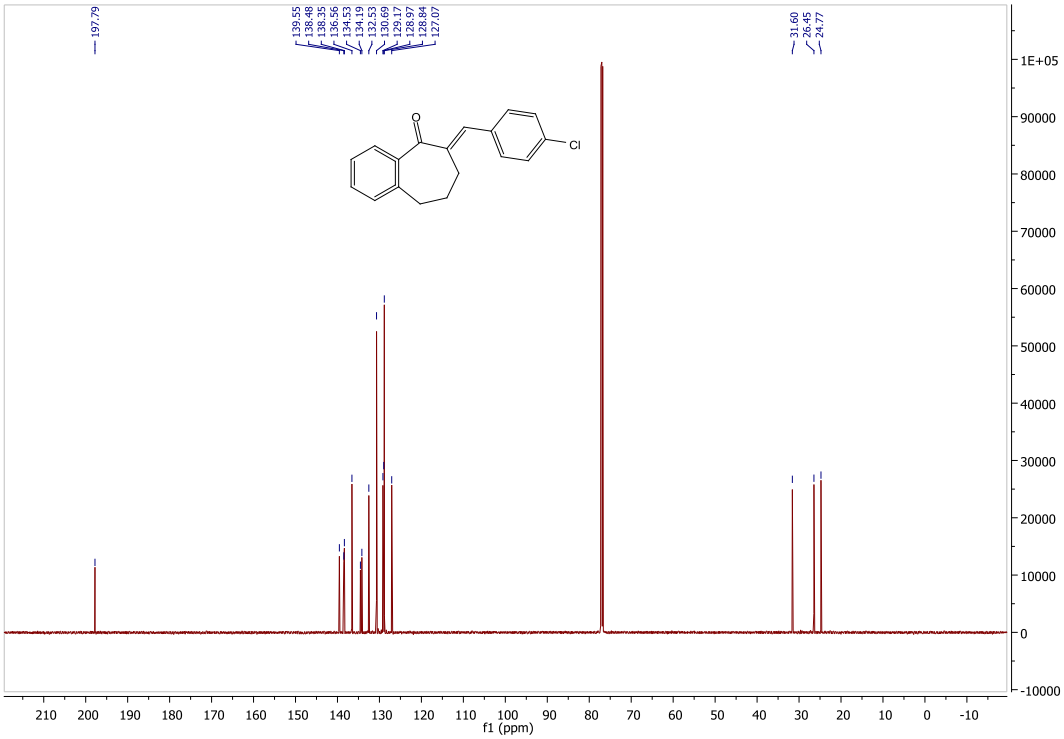

HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
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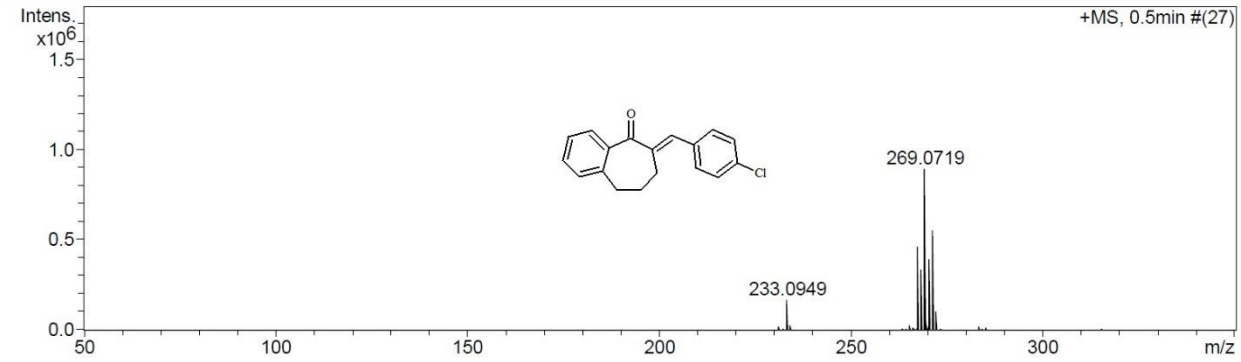
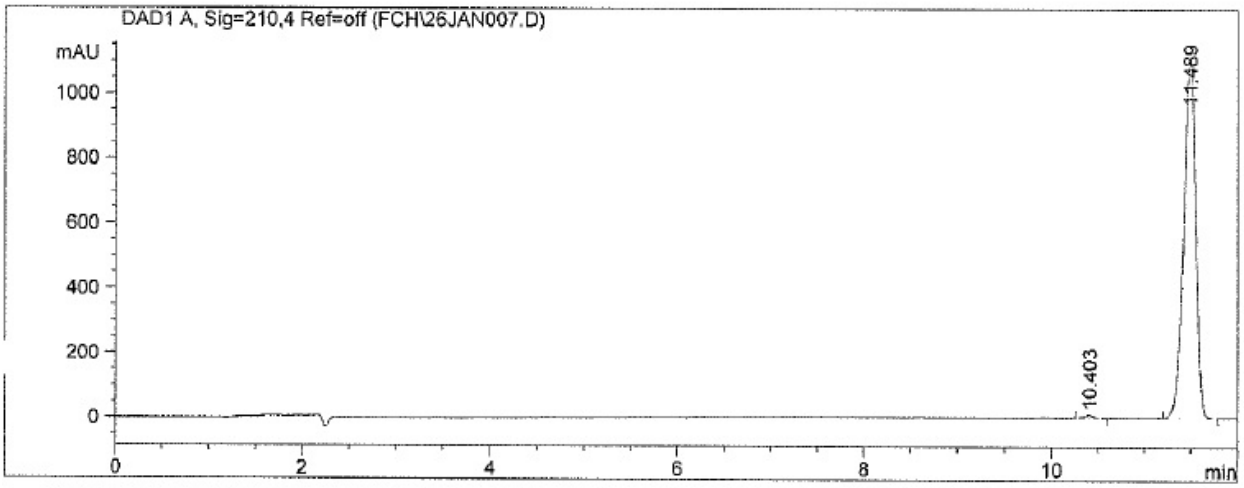
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste

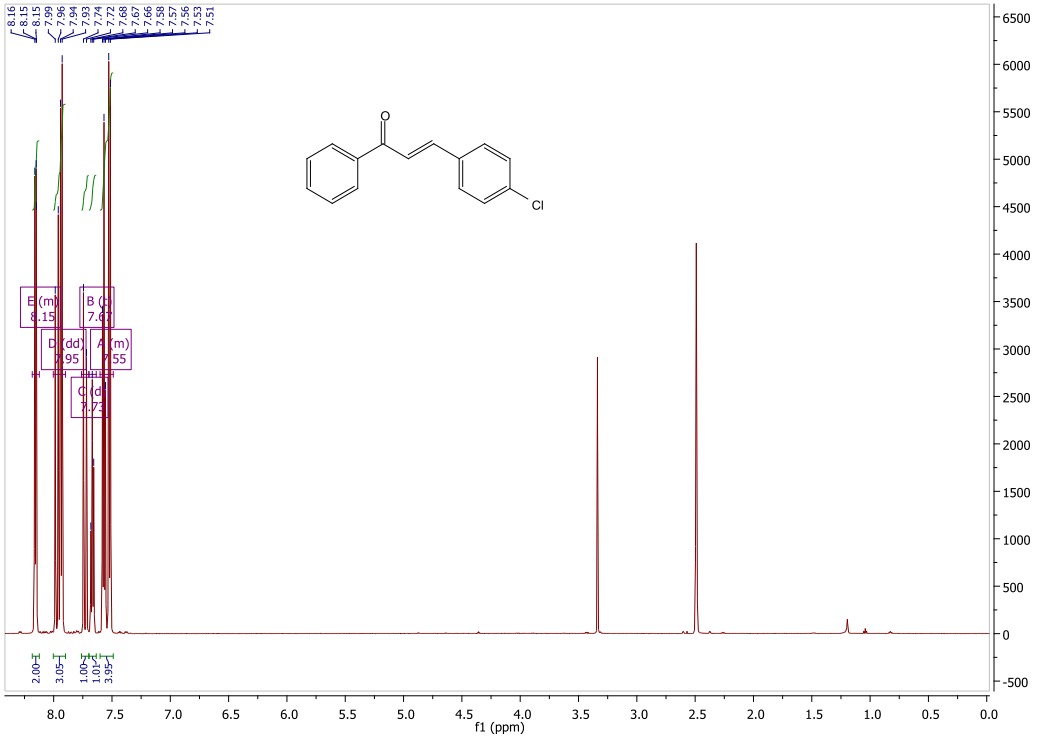


HPLC

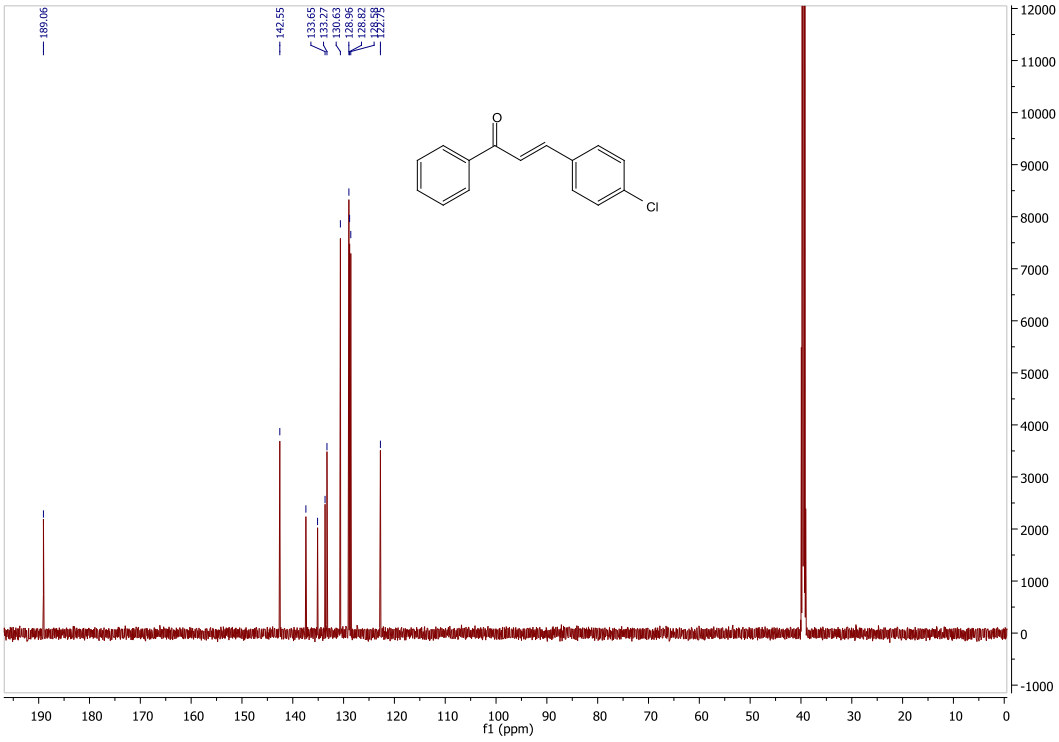


(2*E*)-3-(4-Chlorophenyl)-1-phenylprop-2-en-1-one (1l)

^{1}H NMR



^{13}C NMR



HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
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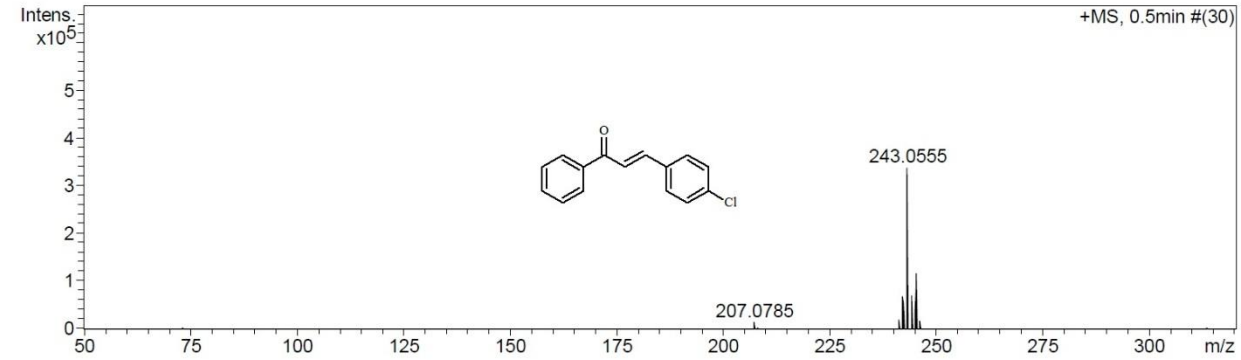
Scan End	1500 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



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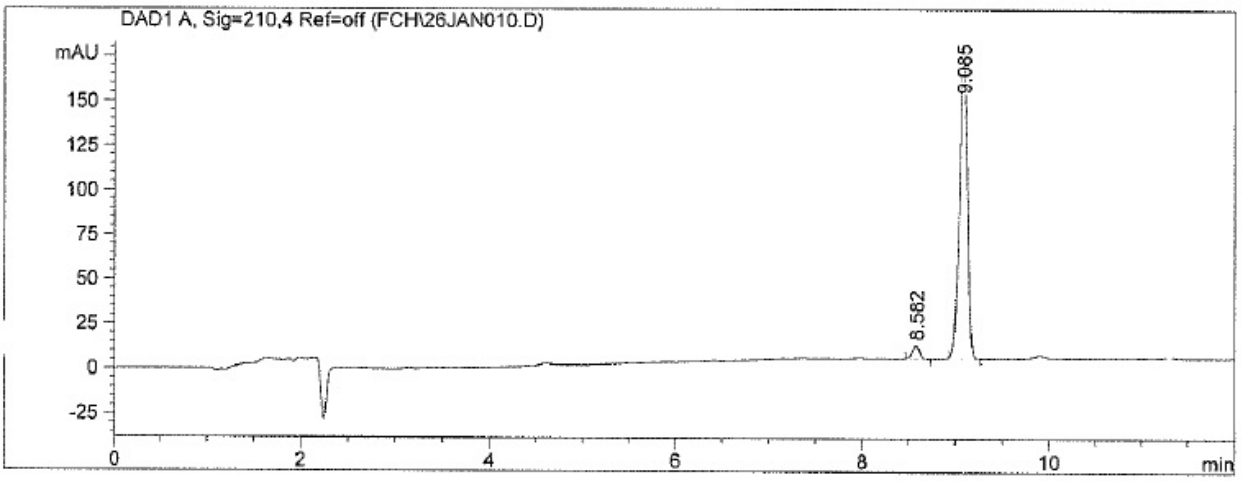

CHAPTER 4: ARTICLE 2

Synthesis and evaluation of 2-benyzlidene-1-tetralone derivatives as monoamine oxidase inhibitors

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ABSTRACT

A series of twenty-three 2-benzylidene-1-tetralone derivatives were synthesised and evaluated for their ability to inhibit human monoamine oxidases A and B (hMAO-A and hMAO-B) in vitro. The 2-benzylidene-1-tetralone derivatives may be viewed as cyclic analogues of the corresponding chalcones, a chemical class which has been shown to inhibit MAO-B potently and with high specificity. The IC_{50} values for the inhibition of the hMAOs show that most 2-benzylidene-1-tetralones possess good inhibitory activity and specificity for hMAO-B. The IC_{50} values for the inhibition of hMAO-B ranged from 0.0064–6.09 µM with one derivative showing no MAO-B inhibition at a maximal tested concentration of 100 µM. The most potent hMAO-B inhibitor, (2*E*)-2-(3,4-dichlorobenzylidene)-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one, proved to be 282-fold more selective for hMAO-B over the hMAO-A isoform. The IC_{50} values for the inhibition of hMAO-A by the series ranged from 0.754–90.4 µM, with four derivatives showing no MAO-A inhibition. It may thus be concluded that 2-benzylidene-1-tetralones are potent and specific hMAO-B inhibitors, and thus suitable leads for the development of therapies for neurodegenerative disorders such as Parkinson's disease.

4.1 Introduction

Monoamine oxidase (MAO) A and B are FAD-dependent enzymes found attached to the outer mitochondrial membrane of neuronal, glial and other mammalian cells (Ferino *et al.*, 2013). While both isoforms catalyse the deamination of monoamine neurotransmitters in the central and peripheral tissues, MAO-A and MAO-B exhibit different tissue distributions. In humans, MAO-A predominates in the gut, placenta and heart, while MAO-B is the principal isoform in platelets and glial cells in the brain (Ramsay, 2012). Both isoforms are expressed in the liver (Fowler *et al.*, 2002). The MAOs are of considerable therapeutic value. MAO-A inhibitors are used for the treatment of depression and MAO-B inhibitors are established therapy for Parkinson's disease (Youdim & Bakhle, 2006; Riederer & Laux, 2011). In depression, MAO-A inhibitors act by blocking the central catabolism of serotonin and possibly norepinephrine, while in Parkinson's disease, MAO-B may act by inhibiting the catabolism of dopamine in the brain. In Parkinson's disease, MAO-B inhibitors are frequently combined with L-dopa, the direct metabolic precursor of dopamine. This combination enhances central dopamine to a greater extent compared to L-dopa monotherapy. By blocking the activity of the MAOs, MAO inhibitors

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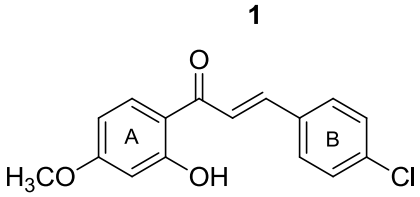
also reduce the formation of hydrogen peroxide, a metabolic by-product of the MAO catalytic cycle. This provides a further motivation for the therapeutic value of MAO inhibitors in Parkinson's disease since hydrogen peroxide may lead to oxidative stress and thus contribute to the neurodegenerative processes. Similarly, MAO-A inhibitors may find future application as treatment for certain cardiomyopathies since hydrogen peroxide formed by MAO-A in the heart has been implicated in age-related cardiac cellular degeneration (Maurel *et al*., 2003).

An important consideration of MAO inhibitors is the reversibility and selectivity of inhibition. Irreversible MAO-A inhibitors are used with caution in the clinic since these compounds may potentiate the sympathomimetic effects of dietary tyramine (Youdim & Bakhle, 2006). Tyramine, present in certain food such as cheese and wine, is metabolised by MAO-A in the gut and peripheral organs, which limits its entry into the systemic circulation. Irreversible MAO-A inhibitors prevent the peripheral breakdown of tyramine leading to excessive systemic concentrations and a potentially fatal increase in blood pressure termed the "cheese reaction" (Finberg, 2014). Reversible MAO-A inhibitors and selective MAO-B inhibitors do not cause tyramine-induced hypertension and are considered safer in this regard. For this reason, MAO inhibitor design focuses on reversible inhibition in preference to irreversible inhibition. It should be noted that irreversible MAO-B inhibitors with a low potential for inhibiting MAO-A have a good safety profile with respect to the cheese reaction.

Chalcones are α,β-unsaturated aromatic ketones with an essentially planar structure due to the presence of carbon atoms with sp^2 hybridization(de Oliveira *et al.*, 2012). Chalcones resemble the open-chain form of the flavonoids and are the biosynthetic precursors of natural flavonoids (Rozmer & Perjési, 2014). Most chalcones are moderately hydrophobic (Mathew *et al.*, 2015), a characteristic important for absorption, blood-brain barrier penetration and pharmacological action in the central nervous system. Chalcones have previously been shown to inhibit the MAOs. Previous studies suggest that potent MAO-B inhibition by chalcones is achieved when the aromatic moieties are substituted with the hydroxy group (A-ring) and halogens (B-ring) (Chimenti *et al.*, 2009). This is exemplified by structure **1** in Figure 4.1. It was also confirmed that bulky substituents (e.g. phenylethoxy, prenyloxy and phenylpropoxy) on the chalcone aromatic rings reduce inhibitor affinity while small groups (e.g. methoxy and hydroxy) yields good MAO-B inhibition activity (Chimenti *et al.*, 2009; Legoabe *et al.*, 2015). The present study aims to contribute to the structure-activity relationships (SARs) for MAO inhibition by chalcone derivatives by investigating the human MAO inhibition properties of a synthetic series of 2-benzylidene-1-tetralone derivatives. 2-Benzylidene-1-tetralone derivatives may be viewed as cyclic analogues of chalcones. With 2-benzylidene-1-tetralones rotation and conformational flexibility are restricted compared to open-chain chalcones, which may impart a greater degree

of selectivity for MAO-B over MAO-A (and other potential biochemical targets). In general, compounds with a lower degree of conformational freedom may be less likely to adopt conformations for interaction with other protein targets.



MAO-B: IC_{50} = 0.0044 µM

Figure 4.1 Examples of chalcones with good MAO inhibitory activities

(Chimenti et al., 2009).

In this study a series of 2-benzylidene-1-tetralone derivatives (Figure 4.2) will thus be synthesised. For this purpose polar functional groups (OH, OMe, NH_2) will be substituted on the A-ring while substitution on the B-ring will be carried out with halogens (F, Cl, Br) and other functional groups such as CN, OH, CH_3, $N(CH_3)_2$ and OMe.

4.2 Results and discussion

4.2.1 The synthesis of the 2-benzylidene-1-tetralone derivatives

The 2-benzylidene-1-tetralone derivatives were synthesised in low to good yields (26–76%) following two protocols (Figure 4.2), employing either acid or base catalysis. All reagents were commercially available except for 7-hydroxy-1-tetralone, which was synthesised by hydrolysis of 7-methoxy-1-tetralone in the presence of $AlCl_3$ as described in literature (Legoabe *et al.*, 2014). To synthesise the 2-benzylidene-1-tetralone derivatives, the appropriately substituted 1-tetralone derivatives and an aldehyde were reacted in the presence of a base (KOH) or an acid (HCl). The derivatives were purified by crystallisation from an appropriate solvent. The structures of the products were verified by ^{1}H NMR, ^{13}C NMR and mass spectrometry, while the purities were estimated by HPLC. The physical data are cited in the supplementary information. The ^{1}H NMR spectra displayed all the expected signals, integration values and chemical shifts. In particular, sharp singlets, representing the vinyl protons of the 2-benzylidene moiety were observed at 7.53–7.86 ppm. The absorptions of the vinylic protons occur at low field due to the proximity of the carbonyl group which exerts an anisotropic effect. Dimmock and coworkers observed these signals in the region of 7.15–7.95 ppm while Böhler and Tamm observed the vinyl proton signal at 7.7 ppm for the *cis* conformation whereas that of the *trans* conformation

was at δ 6.7 ppm. The most characteristic signals on the ^{13}C NMR spectra are those of the carbonyl carbon and the sp^3 carbons of ring-C, in the range 183.8–187.9 ppm and 26.7–28.8 ppm, respectively.

4.2.2 MAO inhibitory potencies of the 2-benzylidene-1-tetralone

The inhibitory activities of the 2-benzylidene-1-tetralone derivatives were determined by using the commercially available recombinant human MAO enzymes. As enzyme substrate, the non-specific MAO-A/B substrate, kynuramine, was used. Kynuramine is oxidised by the MAOs to yield a fluorescent product, 4-hydroxyquinoline, which may be measured by fluorescence spectrophotometry (Novaroli *et al.*, 2005). By measuring the rate of kynuramine oxidation in the presence of various inhibitor concentrations (0.003–100 µM) sigmoidal plots of enzyme catalytic rate versus the logarithm of the inhibitor concentration were constructed from which IC_{50} values were estimated. The results of the inhibition studies are given in Table 4-1.

The 2-benzylidene-1-tetralone derivatives are moderately potent inhibitors of MAO-A, with **2p** being the most potent inhibitor of the series (IC_{50} = 0.754 µM). Only two derivatives (**2m** and **2p**) possess IC_{50} values for the inhibition for MAO-A <1 µM. The 2-benzylidene-1-tetralone derivatives are, however, specific inhibitors of the MAO-B isoform with all derivatives except **2c** displaying higher potency inhibition for MAO-B compared to MAO-A. In this respect, ten derivatives exhibited IC_{50} values for the inhibition of MAO-B <0.1 µM. The most potent MAO-B inhibitor, derivative **2u**, exhibited an IC_{50} value of 0.0064 µM. This inhibitor is therefore significantly more potent than the reference MAO-B inhibitor, lazabemide (IC_{50} = 0.091 µM).

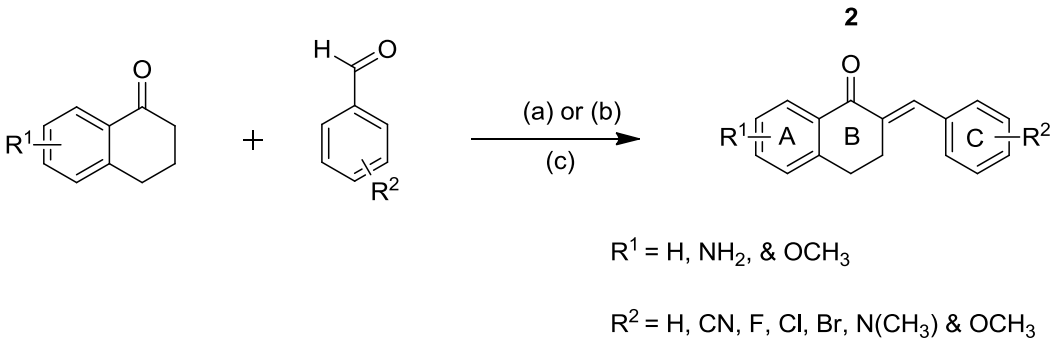


Figure 4.2: Synthetic route to the 2-benzylidene-1-tetralone derivatives 2a–w.

Reagents and conditions: (a) KOH/MeOH, rt, 10–18 h; (b) HCl/MeOH, reflux, 1–6 h; (c) Water.

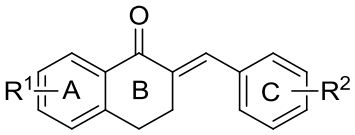
For the inhibition of MAO-B, interesting SARs may be derived. (1) Substituents on the A- and B-rings of 2-benzylidene-1-tetralone is a requirement for high potency inhibition since the

derivative lacking these (**2a**) is a relatively weak MAO-B inhibitor (IC_{50} = 6.09 µM). (2) Similarly derivatives that are unsubstituted on the B-ring while bearing a substituent on the A-ring (**2d**, **2g–j**) are relatively weaker MAO-B inhibitors (IC_{50} > 0.115 µM). A substituent on the B-ring therefore significantly enhances MAO-B inhibition, even when the A-ring is unsubstituted (e.g. **2b**). In general however, addition of an appropriate substituent on the A-ring, such as the 7-hydroxy group, yields higher potency inhibition compared to derivatives substituted on only the B-ring (compare **2n** vs. **2o**; **2k** vs. **2e**). (3) Amino substitution on the A-ring is, however, not well tolerated since **2c** is not an MAO-B inhibitor (compare with **2b**). (4) Hydroxy substitution of the A-ring on the other hand yields high potency MAO-B inhibitors as exemplified by **2u** (IC_{50} = 0.0064 µM), the most potent inhibitor of the series. With 7-hydroxy substitution on the A-ring, a variety of substituents and substitution patterns on ring-B yields good potency MAO-B inhibition. This is exemplified by the fluoro (**2k**), chloro (**2m**, **2n**, **2u**), bromo (**2p**, **2q**), methyl (**2s**), dimethylamine (**2t**) and methoxy (**2w**) substituted derivatives, all possessing IC_{50} < 0.082 µM. (5) When comparing hydroxy and methoxy substitution on the A-ring, 6-hydroxy substitution appears to be most optimal for MAO-B inhibition (compare **2g**–**2j**).

4.3 Conclusion

This study shows that 2-benzylidene-1-tetralones, which may be viewed as cyclic analogues of chalcones, are MAO-B specific inhibitors. With the appropriate substitution pattern high potency inhibitors such as **2u** (IC_{50} = 0.0064 µM) may be obtained. In this study 7-hydroxy substitution on the A-ring combined with substitution on the B-ring with a variety of substituents (F, Cl, Br, CH_3, $N(CH_3)_2$, OCH_3) yielded particularly potent MAO-B inhibitors. In fact all 2-benzylidene-1-tetralone derivatives with this substitution pattern exhibited IC_{50} < 0.109 µM. Considering the structure of human MAO-B, a potential explanation for this behaviour may be proposed. The MAO-B active site is mostly hydrophobic with a small polar area in proximity to the FAD cofactor, the region where the amine group of a substrate binds. It may be hypothesised that the 7-hydroxy substituent of the A-ring binds in this region, establishing polar interactions with residues and waters in the region of the FAD co-factor. Support for this view is X-ray crystallography evidence that the OH of *trans*,*trans*-farnesol binds in proximity to the FAD, where amine groups of substrates are expected to bind. The substituent on the B-ring, in contrast, is expected to interact via Van der Waals interactions with the hydrophobic regions of the MAO-B active site, distal to the FAD. Thus **2u**, which possesses two chloro substituents, is the most active MAO-B inhibitor, suggesting that the contribution of hydrophobic interactions to the stabilisation of an inhibitor in MAO-B, is additive. In conclusion, 2-benzylidene-1-tetralones represent potent inhibitors of MAO-B and could be suitable leads for the development of drugs for the treatment of Parkinson's disease.

Table 4-1: The IC_{50} values for the inhibition of recombinant hMAO-A and hMAO-B by 2-benzylidene-1-tetralone derivatives. The values for the reference inhibitors, lazabemide and toloxatone, are also given.



Compound	cLogP[a]	R^1	R^2	hMAO-A (IC_{50} µM)	hMAO-B (IC_{50} µM)	SI[b]
2a	4.85 ± 0.44	H	H	No Inhibition	6.09 ± 0.486	-
2b	4.42 ± 0.57	H	*m*-CN	13.4 ± 2.60	0.065 ± 0.0018	208
2c	2.99 ± 0.96	6-NH_2	*m*-CN	10.9 ± 1.84	No Inhibition	-
2d	4.77 ± 0.84	6-NH_2	H	No Inhibition	1.89 ± 0.203	-
2e	4.20 ± 0.72	H	*p*-F	90.4 ± 5.77	0.755 ± 0.162	120
2f	4.97 ± 0.56	H	*m*-F	83.2 ± 8.84	1.08 ± 0.268	76.8
2g	4.11 ± 0.83	6-OH	H	19. 3 ± 1.91	0.115 ± 0.026	168
2h	4.20 ± 0.65	5-OH	H	20.9 ± 2.39	0.425 ± 0.100	49.3
2i	4.20 ± 0.65	7-OH	H	2.78 ± 0.404	0.325 ± 0.0095	8.55
2j	3.57 ± 0.83	7-OMe	H	1.95 ± 0.064	0.707 ± 0.088	2.77
2k	4.20 ± 0.72	7-OH	*p*-F	1.92 ± 0.174	0.043 ± 0.0035	44.6
2l	4.12 ± 0.91	7-OH	*m*-F	4.40 ± 0.623	0.109 ± 0.0064	40.3
2m	4.72 ± 0.85	7-OH	*p*-Cl	0.960 ± 0.054	0.024 ± 0.0062	40.7
2n	4.80 ± 0.67	7-OH	*m*-Cl	1.84 ± 0.245	0.037 ± 0.0040	49.8
2o	5.46 ± 0.47	H	*m*-Cl	No Inhibition	0.593 ± 0.105	-
2p	5.09 ± 0.72	7-OH	*p*-Br	0.754 ± 0.053	0.0125 ± 0.0020	60.1
2q	4.46 ± 0.93	7-OH	*m*-Br	1.87 ± 0.118	0.042 ± 0.008	44.6
2r	3.66 ± 0.66	7-OH	*p*-OH	3.95 ± 0.073	1.90 ± 0.095	2.09
2s	4.70 ± 0.71	7-OH	*p*-Me	1.22 ± 0.052	0.041 ± 0.0027	29.9
2t	4.66 ± 0.65	7-OH	*p*-$N(Me)_2$	36.7 ±.326	0.047 ± 0.019	787
2u	5.20 ± 0.68	7-OH	*m,p*-diCl	1.79 ± 0.130	0.0064 ± 0.00015	282
2v	4.80 ± 0.46	H	*m*-OMe	No Inhibition	0.650 ± 0.197	-
2w	4.14 ± 0.66	7-OH	*p*-OMe	1.01 ± 0.058	0.082 ± 0.013	12.2
Lazabemide	-	-	-	202 ± 26	0.091 ± 0.015	2219
Toloxatone	-	-	-	3.92 ± 0.015	-	-

[a]Calculated with ACD/ChemSketch; [b]Selectivity index for the inhibition of hMAO-B, SI = IC_{50} (hMAO-A)/IC_{50} (hMAO-B). All values are reported as mean ± standard deviation (SD) of triplicate experiments. Reference inhibitor (lazabemide and toloxatone) values are obtained from literature (Petzer *et al.*, 2013).

4.4 Experimental

4.4.1 Materials and methods

The reagents and solvents used for the synthetic procedures were obtained from Sigma-Aldrich and were used without further purification. Column chromatography was carried out with silica gel 60 (Fluka, 0.063–0.200 mm particle size) while thin-layer chromatography was performed on 0.20 mm thick aluminium coated silica gel sheets (Macherey-Nagel). Developed TLC sheets were visualised under UV light (254 and 366 nm) or by staining with iodine vapour. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III 600 spectrometer at 600 MHz and 151 MHz, respectively, and $CDCl_3$ or DMSO-*d6* served as NMR solvents. Chemical shifts are reported in parts per million (*∂*) and were referenced to the residual solvent signal ($CDCl_3$: 7.26 and 77.16 ppm for ^{1}H and ^{13}C, respectively; DMSO-*d6*: 2.50 and 39.52 ppm for ^{1}H and ^{13}C, respectively). Spin multiplicities are given as s (singlet), d (doublet), t (triplet), dd (doublet of doublets) dt (doublet of triplets), tt (triplet of triplets), q (quartet), qn (quintet), m (multiplet) or brs (broad singlet). Melting points (mp) were determined on a Buchi B-545 melting point apparatus and are uncorrected. Chemical purity was determined by high performance liquid chromatography (HPLC). HPLC analyses were performed using an Agilent 1200 series HPLC system equipped with a quaternary pump and an Agilent 1200 series diode array detector. A Venusil XBP C18 column (4.60×150mm, 5µm) was used for separation and the mobile phase consisted initially of 30% acetonitrile and 70% MilliQ water at a flow rate of 1 mL/min. At the start of each HPLC run a solvent gradient program was initiated by linearly increasing the composition of the acetonitrile in the mobile phase to 85% acetonitrile over a period of 5 min. Each HPLC run lasted 15 min and a time period of 5 min was allowed for equilibration between runs. A volume of 20 µL of solutions of the test compounds in acetonitrile (1 mM) was injected into the HPLC system and the eluent was monitored at wavelengths of 210, 254 and 300 nm.

Fluorescence spectrophotometry was carried out with a Varian Cary Eclipse fluorescence spectrophotometer (Agilent Technologies). Microsomes from insect cells containing recombinant human MAO-A and MAO-B (5 mg/mL) were obtained from Sigma-Aldrich and were pre-aliquoted and stored at −80 ^{o}C. Kynuramine dihydrobromide, the MAO substrate, was also obtained from Sigma-Aldrich.

4.4.2 Chemical synthesis

4.4.2.1 Synthesis of 7-hydroxy-1-tetralone

7-Methoxy-1-tetralone (25.5 mmol) and anhydrous aluminium trichloride (63.8 mmol) were dissolved in toluene (15 mL). The reaction mixture was heated under reflux for 1.5 h and was subsequently cautiously quenched with 30 mL water. The resultant mixture was extracted to ethyl acetate (2 × 100 mL) and the combined organic phases were washed with water (2 × 30 mL). The organic phase was dried over anhydrous magnesium sulphate and the solvent was evaporated with a rotary evaporator to yield an orange-brown solid. The crude was recrystallized from ethyl acetate. The yield of 88.6% was obtained.

4.4.2.2 Synthesis 2-benzylidene-1-tetralones derivatives (2a–w)

The 2-benzylidene-1-tetralone derivatives **2a**–**f** and **2p** were synthesised via the acid catalysed Claisen-Schmidt condensation reaction of an appropriately substituted 1-tetralone (1.850 mmol) and an aldehyde (2.035 mmol). These reactions were conducted in 15 mL of methanol containing 22.5 mL HCl (conc.). 2-Benzylidene-1-tetralone derivatives **2g**–**o** and **2q**–**w** were synthesised by base catalysis of the reaction between a 1-tetralone (2.052 mmol) and an aldehyde (2.257 mmol). These reactions were conducted in methanol (5 mL) containing potassium hydroxide (4.10 mmol). The acid catalysed reactions were stirred under reflux for 1–6 h while the base catalysed reactions were stirred for 10–18 h at room temperature. With the exceptions of **2n** and **2q**, which were extracted to ethyl acetate (2 × 50 mL), the products were precipitated by addition of a minimum of 20 mL water. The crude products were collected by filtration and recrystallised from appropriate solvents as indicated below.

(2*E*)-2-Benzylidene-3,4-dihydronaphthalen-1(2*H*)-one (2a)

The title compound is a product of 3,4-dihydronaphthalen-1(2*H*)-one and benzaldehyde in a yield of 67.4%: yellow crystals, mp 106.0–107.3 ^{o}C (ethanol). ^{1}H NMR ($CDCl_3$) δ ppm 8.12 (d, *J* = 7.7 Hz, 1H), 7.86 (s, 1H), 7.50 – 7.30 (m, 7H), 7.26 – 7.20 (m, 1H), 3.15 – 3.08 (m, 2H), 2.93 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 187.90, 143.21, 136.64, 135.81, 135.43, 133.44, 133.26, 129.86, 128.53, 128.42, 128.20, 128.16, 127.00, 28.85, 27.16. APCI-HRMS *m/z*: calcd for $C_{17}H_{14}O$ $[M+H]^+$, 235.1117, found: 235.1121. Purity (HPLC): 97.9%.

3-[(*E*)-(1-Oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl]benzonitrile (2b)

The title compound is a product of 3,4-dihydronaphthalen-1(2*H*)-one and 3-formylbenzonitrile in a yield of 73.1%: brown crystals, mp 138.9–140.0 ^{o}C (ethanol). ^{1}H NMR ($CDCl_3$) δ ppm 8.11 (d, *J* = 7.7 Hz, 1H), 7.76 (s, 1H), 7.67 (s, 1H), 7.65 – 7.58 (m, 2H), 7.55 – 7.46 (m, 2H), 7.36 (t, *J* =

7.5 Hz, 1H), 7.28 – 7.22 (m, 1H), 3.05 (dd, *J* = 9.1, 3.8 Hz, 2H), 2.96 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 187.25, 143.05, 137.58, 137.07, 133.97, 133.64, 133.60, 133.04, 132.77, 131.64, 129.37, 128.30, 128.28, 127.20, 118.44, 112.78, 28.65, 27.11. APCI-HRMS *m/z* calcd for $C_{18}H_{13}NO$ $[M+H]^+$, 260.1070, found 260.1064. Purity (HPLC): 96.8%.

3-[(*E*)-(7-Amino-1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl]benzonitrile (2c)

The title compound is a product of 6-amino-3,4-dihydronaphthalen-1(2*H*)-one and 3-formylbenzonitrile in a yield of 31.2%: orange powder, mp 226.3–229.9 °C (ethanol). ^{1}H NMR (DMSO-d_6) δ ppm 7.92 (s, 1H), 7.79 (dd, *J* = 15.2, 7.8 Hz, 2H), 7.71 (d, *J* = 8.6 Hz, 1H), 7.63 (t, *J* = 7.8 Hz, 1H), 7.53 (s, 1H), 6.51 (dd, *J* = 8.6, 2.0 Hz, 1H), 6.34 (s, 1H), 6.23 (s, 2H), 2.93 (t, *J* = 5.9 Hz, 2H), 2.74 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ ppm 183.79, 154.19, 145.73, 138.55, 137.12, 134.24, 132.89, 131.59, 130.94, 130.26, 129.70, 121.32, 118.65, 112.71, 111.70, 110.56, 28.36, 26.70. APCI-HRMS *m/z* calcd for $C_{18}H_{14}N_2O$ $[M+H]^+$, 275.1179, found 275.1164. Purity (HPLC): 87.5%.

(2*E*)-6-Amino-2-benzylidene-3,4-dihydronaphthalen-1(2*H*)-one (2d)

The title compound is a product of 6-amino-3,4-dihydronaphthalen-1(2*H*)-one and benzaldehyde in a yield of 51.3%: brown shinny crystals, mp 230.0–233.6 °C (ethanol). ^{1}H NMR (DMSO-d_6) δ ppm 2.79 (t, *J* =6.40 Hz, 2 H) 2.99 (t, *J* =5.83 Hz, 2 H) 4.92 (br. s., 2 H) 6.68 (s, 1 H) 6.80 (dd, *J* =8.28, 1.88 Hz, 1 H) 7.32–7.39 (m, 1 H) 7.40–7.50 (m, 4 H) 7.60 (s, 1 H) 7.81 (d, *J* =8.66 Hz, 1 H); ^{13}C NMR (DMSO-d_6) δ ppm 184.81, 148.48, 145.49, 136.10, 135.60, 134.24, 129.92, 129.80, 128.59, 128.50, 124.98, 115.69, 114.58, 28.32, 26.74. APCI-HRMS *m/z* calcd for $C_{17}H_{15}NO$ $[M+H]^+$, 250.1226, found 250.1226. Purity (HPLC): 98.8%.

(2I)-2-(4-Fluorobenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (2e)

The title compound is a product of 3,4-dihydronaphthalen-1(2*H*)-one and 4-fluorobenzaldehyde in a yield of 60.0%: yellow crystals, mp 114.6–115.2 °C (ethanol). ^{1}H NMR ($CDCl_3$) δ ppm 8.11 (d, *J* = 7.8 Hz, 1H), 7.81 (s, 1H), 7.48 (td, *J* = 7.5, 1.2 Hz, 1H), 7.44 – 7.32 (m, 3H), 7.24 (d, *J* = 6.1 Hz, 1H), 7.09 (t, *J* = 8.6 Hz, 2H), 3.12 – 3.05 (m, 2H), 2.96 – 2.90 (m, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 187.72, 163.43, 161.77, 143.09, 135.49, 135.22, 133.37, 133.32, 131.77, 131.72, 128.21, 128.16, 127.05, 115.61, 115.47, 28.75, 27.09. APCI-MS *m/z* calcd for $C_{17}H_{13}FO$ $[M+H]^+$, 253.1023, found 253.1029. Purity (HPLC): 97.5%.

(2*E*)-2-(3-Fluorobenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (2f)

The title compound is a product of 3,4-dihydronaphthalen-1(2*H*)-one and 4-fluorobenzaldehyde in a yield of 58.9%: pale yellow crystals, mp 88.3–89.0 °C (ethanol). ^{1}H NMR ($CDCl_3$) δ ppm

8.11 (d, *J* = 7.8 Hz, 1H), 7.79 (s, 1H), 7.48 (td, *J* = 7.5, 1.1 Hz, 1H), 7.36 (ddd, *J* = 10.4, 9.4, 6.2 Hz, 2H), 7.27 – 7.21 (m, 1H), 7.19 (d, *J* = 7.7 Hz, 1H), 7.11 (d, *J* = 9.8 Hz, 1H), 7.04 (td, *J* = 8.4, 2.0 Hz, 1H), 3.13 – 3.06 (m, 2H), 2.95 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 187.62, 163.43, 161.80, 143.17, 137.98, 136.46, 135.13, 133.43, 133.27, 130.00, 128.22, 127.09, 125.67, 116.26, 115.46, 28.74, 27.13. APCI-HRMS *m/z* calcd for $C_{17}H_{13}FO$ $[M+H]^+$, 253.1023, found 253.1052. Purity (HPLC): 96.8%.

(2*E*)-2-Benzylidene-6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2g)

The title compound is a product of 6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and benzaldehyde in a yield of 25.9%: purple crystal, mp 199.8–200.8 ^{o}C (cyclohexane). ^{1}H NMR (DMSO-d_6) δ ppm 10.44 (s, 1H), 7.85 (d, *J* = 8.6 Hz, 1H), 7.63 (s, 1H), 7.51 – 7.40 (m, 4H), 7.37 (t, *J* = 7.3 Hz, 1H), 6.77 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.66 (d, *J* = 2.1 Hz, 1H), 3.01 (dd, *J* = 9.2, 3.6 Hz, 2H), 2.82 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ ppm 185.26, 162.32, 146.06, 135.89, 135.48, 134.48, 130.30, 129.77, 128.55, 128.51, 125.08, 114.78, 113.94, 28.24, 26.74. APCI-HRMS *m/z* calcd for $C_{17}H_{14}O_2$ $[M+H]^+$, 251.1067, found 251.1047. Purity (HPLC): 96.8%.

(2*E*)-2-Benzylidene-5-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2h)

The title compound is a product of 5-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and benzaldehyde in a yield of 71.8%: brown shinny crystal, mp 173.2–179.2 ^{o}C (dichloromethane). ^{1}H NMR (DMSO-d_6) δ ppm 9.85 (s, 1H), 7.66 (s, 1H), 7.51 (d, *J* = 7.5 Hz, 2H), 7.45 (ddd, *J* = 7.7, 4.2, 3.3 Hz, 3H), 7.38 (t, *J* = 7.3 Hz, 1H), 7.20 (t, *J* = 7.9 Hz, 1H), 7.07 (dd, *J* = 7.9, 1.0 Hz, 1H), 3.03 (dd, *J* = 9.2, 3.7 Hz, 2H), 2.81 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ ppm 187.09, 154.37, 135.62, 135.31, 135.20, 134.03, 130.12, 129.89, 128.69, 128.59, 127.06, 119.28, 117.98, 26.10, 21.13. APCI-HRMS *m/z* calcd for $C_{17}H_{14}O_2$ $[M+H]^+$, 251.1067, found 251.1056. Purity (HPLC): 99.1%.

(2*E*)-2-Benzylidene-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2i)

The title compound is a product of 7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and benzaldehyde in a yield of 47.5%: orange crystal, mp 160.3–160.7 ^{o}C (dichloromethane). ^{1}H NMR (DMSO-d_6) δ ppm 9.67 (s, 1H), 7.67 (s, 1H), 7.50 (d, *J* = 7.5 Hz, 2H), 7.45 (t, *J* = 7.6 Hz, 2H), 7.41 – 7.32 (m, 2H), 7.17 (d, *J* = 8.3 Hz, 1H), 6.99 (dd, *J* = 8.2, 2.7 Hz, 1H), 3.02 (t, *J* = 5.7 Hz, 2H), 2.80 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ ppm 186.77, 156.25, 135.63, 135.47, 135.31, 134.12, 133.69, 129.88, 129.75, 128.73, 128.59, 121.43, 112.59, 27.12, 27.00. APCI-HRMS *m/z* calcd for $C_{17}H_{14}O_2$ $[M+H]^+$, 251.1067, found 251.1048. Purity (HPLC): 99.4%.

(2*E*)-2-Benzylidene-7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one (2j)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one and benzaldehyde in a yield of 51.2%: pale yellow crystals, mp 121.5–122.7 ^{o}C (dichloromethane). ^{1}H NMR ($CDCl_3$) δ ppm 7.85 (s, 1H), 7.61 (d, *J* = 2.8 Hz, 1H), 7.41 (dt, *J* = 15.2, 7.4 Hz, 4H), 7.33 (dd, *J* = 9.9, 4.2 Hz, 1H), 7.15 (d, *J* = 8.3 Hz, 1H), 7.06 (dd, *J* = 8.3, 2.8 Hz, 1H), 3.85 (s, 3H), 3.09 (td, *J* = 6.7, 1.6 Hz, 2H), 2.90 – 2.84 (m, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 187.80, 158.62, 136.68, 135.92, 135.85, 135.43, 134.23, 129.84, 129.42, 128.49, 128.40, 121.50, 110.26, 55.52, 28.02, 27.34. APCI-HRMS *m/z* calcd for $C_{18}H_{16}O_2$ $[M+H]^+$, 265.1223, found 265.1212. Purity (HPLC): 99.2%.

(2*E*)-2-(4-Fluorobenzylidene)-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2k)

The title compound is a product of 7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 4-fluorobenzaldehyde in a yield of 70.2%: yellow crystals, mp 199.9–200.9 ^{o}C (ethanol). ^{1}H NMR (DMSO-d_6) δ ppm 9.66 (s, 1H), 7.65 (s, 1H), 7.57 (dd, *J* = 8.5, 5.7 Hz, 2H), 7.33 (d, *J* = 2.7 Hz, 1H), 7.28 (t, *J* = 8.8 Hz, 2H), 7.17 (d, *J* = 8.3 Hz, 1H), 6.98 (dd, *J* = 8.2, 2.7 Hz, 1H), 3.00 (t, *J* = 5.8 Hz, 2H), 2.80 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ ppm 186.66, 162.85, 161.22, 156.25, 135.47, 134.40, 134.08, 133.66, 132.22, 132.16, 129.75, 121.45, 115.65, 115.51, 112.59, 27.04, 26.89. APCI-HRMS *m/z* calcd for $C_{17}H_{13}FO_2$ $[M+H]^+$, 269.0972, found 269.0962. Purity (HPLC): 99%.

(2*E*)-2-(3-Fluorobenzylidene)-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2l)

The title compound is a product of 7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 3-fluorobenzaldehyde in a yield of 64.7%: orange shinny crystals, mp 149.1–150.2 ^{o}C (ethanol/cyclohexane). ^{1}H NMR (DMSO-d_6) δ ppm 9.68 (s, 1H), 7.64 (s, 1H), 7.52 – 7.44 (m, 1H), 7.34 (dd, *J* = 10.1, 5.3 Hz, 3H), 7.21 (ddd, *J* = 21.9, 14.2, 5.2 Hz, 2H), 6.99 (dd, *J* = 8.2, 2.7 Hz, 1H), 3.01 (dd, *J* = 9.0, 3.6 Hz, 2H), 2.81 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ ppm 186.63, 162.90, 161.28, 156.27, 137.75, 136.78, 134.21, 133.56, 130.56, 129.80, 126.02, 121.58, 116.39, 115.40, 112.58, 27.02, 26.96. APCI-HRMS (*m/z*) calcd for $C_{17}H_{13}FO_2$ $[M+H]^+$, 269.0972, found 269.0976. Purity (HPLC): 98.7%.

(2*E*)-2-(4-Chlorobenzylidene)-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2m)

The title compound is a product of 7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 4-clorobenzaldehyde in a yield of 63.2%: peach shinny crystals, mp 197.2–198.3 ^{o}C (ethanol/cyclohexane). ^{1}H NMR (DMSO-d_6) δ ppm 9.67 (s, 1H), 7.63 (s, 1H), 7.51 (q, *J* = 8.6 Hz, 4H), 7.33 (d, *J* = 2.7 Hz, 1H), 7.17 (d, *J* = 8.3 Hz, 1H), 6.99 (dd, *J* = 8.2, 2.7 Hz, 1H), 2.99 (t,

J = 5.7 Hz, 2H), 2.80 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ ppm 186.60, 156.26, 136.27, 134.19, 134.13, 134.10, 133.59, 133.29, 131.66, 129.77, 128.61, 121.52, 112.58, 27.03, 26.95. APCI-HRMS *m/z* calcd for $C_{17}H_{13}ClO_2$ $[M+H]^+$, 285.0677, found 285.0652. Purity (HPLC): 99%.

(2I)-2-(3-Chlorobenzylidene)-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2n)

The title compound is a product of 7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 3-clorobenzaldehyde in a yield of 29.0%: brown crystals, mp 150.7–151.8 ^{o}C (dichloromethane). ^{1}H NMR (DMSO-d_6) δ ppm 9.68 (s, 1H), 7.62 (s, 1H), 7.56 (s, 1H), 7.50 – 7.41 (m, 3H), 7.33 (d, *J* = 2.7 Hz, 1H), 7.18 (d, *J* = 8.3 Hz, 1H), 6.99 (dd, *J* = 8.2, 2.7 Hz, 1H), 3.00 (t, *J* = 5.7 Hz, 2H), 2.81 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ ppm 186.59, 156.27, 137.52, 136.96, 134.20, 133.79, 133.53, 133.29, 130.39, 129.81, 129.31, 128.44, 128.39, 121.60, 112.57, 27.03, 26.96. APCI-HRMS *m/z* calcd for $C_{17}H_{13}ClO_2$ $[M+H]^+$, 285.0677, found 285.0665. Purity (HPLC): 98.2%.

(2*E*)-2-(3-Chlorobenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (2o)

The title compound is a product of 3,4-dihydronaphthalen-1(2*H*)-one and 3-chlorobenzaldehyde in a yield of 76.4%: white crystals, mp: 112.1–113.2 ^{o}C (ethanol). ^{1}H NMR ($CDCl_3$) δ ppm 8.11 (d, *J* = 7.8 Hz, 16H), 7.76 (s, 16H), 7.48 (td, *J* = 7.5, 0.9 Hz, 17H), 7.41 – 7.21 (m, 104H), 3.47 (s, 4H), 3.08 (dd, *J* = 9.1, 3.8 Hz, 35H), 2.94 (t, *J* = 6.4 Hz, 35H); ^{13}C NMR ($CDCl_3$) δ ppm 187.57, 143.17, 137.62, 136.61, 134.91, 134.35, 133.45, 133.25, 129.71, 129.48, 128.47, 128.26, 128.23, 127.96, 127.10, 28.76, 27.15. APCI-HRMS *m/z* calcd for $C_{17}H_{13}ClO$ $[M+H]^+$, 269.0728, found 269.0751. Purity (HPLC): 100%.

(2*E*)-2-(4-Bromobenzylidene)-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2p)

The title compound is a product of 7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 4-bromobenzaldehyde in a yield of 51.1%: yellow shinny crystals, mp 226.4–227.0 ^{o}C (ethanol). ^{1}H NMR (DMSO-d_6) δ ppm 9.67 (s, 1H), 7.67 – 7.57 (m, 3H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.33 (d, *J* = 2.6 Hz, 1H), 7.18 (d, *J* = 8.3 Hz, 1H), 6.99 (dd, *J* = 8.2, 2.7 Hz, 1H), 2.99 (t, *J* = 5.8 Hz, 2H), 2.80 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ ppm 186.61, 156.26, 136.33, 134.53, 134.16, 134.14, 133.58, 131.90, 131.53, 129.78, 122.04, 121.53, 112.58, 27.03, 26.96. APCI-HRMS *m/z* calcd for $C_{17}H_{13}BrO_2$ $[M+H]^+$, 329.0172 found 329.0145. Purity (HPLC): 100%.

(2*E*)-2-(3-Bromobenzylidene)-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2q)

The title compound is a product of 7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 3-bromobenzaldehyde in a yield of 36.9%: yellow granules, mp 149.6–150.0 ^{o}C (ethanol). ^{1}H NMR (DMSO-d_6) δ ppm 9.67 (s, 1H), 7.69 (s, 1H), 7.64 – 7.55 (m, 2H), 7.50 (d, *J* = 7.7 Hz, 1H), 7.40

(t, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 2.6 Hz, 1H), 7.18 (d, *J* = 8.3 Hz, 1H), 6.99 (dd, *J* = 8.2, 2.7 Hz, 1H), 2.99 (t, *J* = 5.8 Hz, 2H), 2.81 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ ppm 186.55, 156.25, 137.79, 136.95, 134.18, 133.72, 133.52, 132.14, 131.31, 130.62, 129.79, 128.72, 121.84, 121.59, 112.56, 27.03, 26.94. APCI-HRMS *m/z* calcd for $C_{17}H_{13}BrO_2$ $[M+H]^+$, 329.0172, found: 329.0167. Purity (HPLC): 97.7%.

(2*E*)-7-Hydroxy-2-(4-hydroxybenzylidene)-3,4-dihydronaphthalen-1(2*H*)-on (2r)

The title compound is a product of 7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 4-hydroxybenzaldehyde in a yield of 39.2%: brown crystals, mp 249.5–249.7 ^{o}C (ethyl acetate). ^{1}H NMR (DMSO-d_6) δ ppm 9.92 (s, 1H), 9.60 (s, 1H), 7.61 (s, 1H), 7.39 (d, *J* = 8.5 Hz, 2H), 7.31 (d, *J* = 2.6 Hz, 1H), 7.16 (d, *J* = 8.3 Hz, 1H), 6.96 (dd, *J* = 8.2, 2.7 Hz, 1H), 6.84 (d, *J* = 8.6 Hz, 2H), 3.02 (t, *J* = 5.9 Hz, 2H), 2.79 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ ppm 186.61, 158.35, 156.17, 136.17, 133.96, 133.82, 132.49, 132.08, 129.57, 126.16, 121.04, 115.51, 112.58, 27.01, 27.01. APCI-HRMS *m/z* calcd for $C_{17}H_{14}O_3$ $[M+H]^+$, 267.1016, found 267.1027. Purity (HPLC): 100%.

(2*E*)-7-Hydroxy-2-(4-methylbenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (2s)

The title compound is a product of 7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 4-dimethylbenzaldehyde in a yield of 60.3%: yellow crystals, mp 181.0–181.7 ^{o}C (ethanol). ^{1}H NMR (DMSO-d_6) δ ppm 9.64 (s, 1H), 7.64 (s, 1H), 7.41 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 2.6 Hz, 1H), 7.26 (d, *J* = 7.9 Hz, 2H), 7.17 (d, *J* = 8.3 Hz, 1H), 6.98 (dd, *J* = 8.2, 2.6 Hz, 1H), 3.02 (t, *J* = 5.8 Hz, 2H), 2.79 (t, *J* = 6.4 Hz, 2H), 2.33 (s, 3H); ^{13}C NMR (DMSO-d_6) δ ppm 186.71, 156.22, 138.52, 135.60, 134.81, 134.01, 133.75, 132.46, 129.96, 129.68, 129.18, 121.30, 112.58, 27.08, 27.01, 20.97. APCI-HRMS *m/z* calcd for $C_{18}H_{16}O_2$ $[M+H]^+$, 265.1223, found 265.1224. Purity (HPLC): 100%.

(2*E*)-2-[4-(Dimethylamino)benzylidene]-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2t)

The title compound is a product of 7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 4-(dimethylamino)benzaldehyde in a yield of 66.3%: orange crystals, mp 235.4–245.6 ^{o}C (ethanol). ^{1}H NMR (DMSO-d_6) δ ppm 9.58 (s, 1H), 7.62 (s, 1H), 7.42 (d, *J* = 8.7 Hz, 2H), 7.30 (d, *J* = 2.6 Hz, 1H), 7.15 (d, *J* = 8.2 Hz, 1H), 6.94 (dd, *J* = 8.2, 2.7 Hz, 1H), 6.76 (d, *J* = 8.4 Hz, 2H), 3.04 (t, *J* = 6.0 Hz, 2H), 2.97 (s, 6H), 2.78 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ ppm 186.35, 156.15, 150.42, 136.89, 134.21, 133.59, 131.96, 130.66, 129.43, 120.75, 112.59, 111.86, 27.12, 27.01. APCI-HRMS *m/z* calcd $C_{19}H_{19}NO_2$ $[M+H]^+$, 294.1489, found 294.1487. Purity (HPLC): 98.5%.

(2*E*)-2-(3,4-Dichlorobenzylidene)-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2u)

The title compound is a product of 7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 3,4-dichlobenzaldehyde in a yield of 58.1%: orange crystals, mp 183.6–184.6 ^{o}C (dichloromethane). ^{1}H NMR (DMSO-d_6) δ ppm 9.68 (s, 1H), 7.77 (d, *J* = 1.5 Hz, 1H), 7.69 (d, *J* = 8.3 Hz, 1H), 7.59 (s, 1H), 7.49 (dd, *J* = 8.4, 1.5 Hz, 1H), 7.33 (d, *J* = 2.7 Hz, 1H), 7.18 (d, *J* = 8.3 Hz, 1H), 6.99 (dd, *J* = 8.2, 2.7 Hz, 1H), 2.99 (t, *J* = 5.7 Hz, 2H), 2.81 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ ppm 186.44, 156.27, 137.40, 136.10, 134.19, 133.45, 132.76, 131.52, 131.32, 131.08, 130.65, 129.87, 129.81, 121.64, 112.55, 26.95, 26.92. APCI-HRMS *m/z* calcd for $C_{17}H_{12}Cl_2O_2$ $[M+H]^+$, 319.0287, found 319.0282. Purity (HPLC): 96.1%.

(2*E*)-2-(4-Methoxybenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (2v)

The title compound is a product of 3,4-dihydronaphthalen-1(2*H*)-one and 3-methoxybenzaldehyde in a yield of 62.1%: yellow crystals, mp 75.4–77.9 ^{o}C (ethanol). ^{1}H NMR ($CDCl_3$) δ ppm 8.14 – 8.09 (m, 1H), 7.82 (s, 1H), 7.47 (td, *J* = 7.5, 1.3 Hz, 1H), 7.38 – 7.29 (m, 2H), 7.23 (d, *J* = 7.7 Hz, 1H), 7.02 (d, *J* = 7.6 Hz, 1H), 6.95 (s, 1H), 6.89 (dd, *J* = 8.2, 2.2 Hz, 1H), 3.82 (s, 3H), 3.12 (td, *J* = 6.6, 1.6 Hz, 2H), 2.93 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 187.86, 159.48, 143.24, 137.16, 136.49, 135.70, 133.43, 133.27, 129.43, 128.20, 128.17, 127.00, 122.26, 115.31, 114.04, 55.27, 28.84, 27.23. APCI-HRMS *m/z* calcd for $C_{18}H_{16}O_2$ $[M+H]^+$, 265.1223, found 265.1229. Purity (HPLC): 97.1%.

(2*E*)-7-Hydroxy-2-(4-methoxybenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (2w)

The title compound is a product of 7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and 4-methoxybenzaldehyde in a yield of 63.8%: yellow crystals, mp 184.2–185.2 ^{o}C (dichloromethane). ^{1}H NMR (DMSO-d_6) δ ppm 9.63 (s, 1H), 7.64 (s, 1H), 7.49 (d, *J* = 8.7 Hz, 2H), 7.32 (d, *J* = 2.7 Hz, 1H), 7.16 (d, *J* = 8.3 Hz, 1H), 7.01 (d, *J* = 8.7 Hz, 2H), 6.97 (dd, *J* = 8.2, 2.7 Hz, 1H), 3.79 (s, 3H), 3.03 (t, *J* = 5.8 Hz, 2H), 2.79 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ ppm 186.63, 159.72, 156.20, 135.60, 133.88, 133.86, 133.47, 131.82, 129.61, 127.71, 121.17, 114.10, 112.58, 55.27, 27.03, 26.99. APCI-HRMS *m/z* calcd for $C_{18}H_{16}O_3$ $[M+H]^+$, 281.1172, found 281.1163. Purity (HPLC): 99.7%.

4.5 Determination of IC_{50} values for the inhibition of the MAOs

The IC_{50} values for the inhibition of MAO-A and MAO-B were determined using the recombinant human enzymes as described in literature (Strydom *et al*., 2011). All enzymatic reactions were carried out in 96-well microtiter plates in potassium phosphate buffer (100 mM, pH 7.4) which was made isotonic with KCl. The final volumes of the reactions were 200 µL and contained (1) MAO-A (0.0075 mg protein/mL) or MAO-B (0.015 mg protein/mL), (2) the mixed MAO-A/B substrate kynuramine (50 µM) and (3) the test inhibitors (0.003–100 µM). Stock solutions of the test inhibitors were prepared in DMSO and added to the reactions to yield a final concentration of 4% (v/v) DMSO. The reactions were incubated in a convection oven at 37 ^{o}C for 20 min and terminated with the addition of 80 µl NaOH (2N). The concentrations of the MAO generated 4-hydroxyquinoline in the reactions were measured by fluorescence spectrophotometry (λ_{ex} = 310 nm, λ_{em} = 400 nm) (Novaroli *et al.,* 2005). To quantify 4-hydroxyquinoline, a linear calibration curve was constructed from solutions of authentic 4-hydroxyquinoline (0.0469–1.5 µM) dissolved in the reaction buffer. The rates of kynuramine oxidation were calculated and the rate data were fitted to the one site competition model incorporated into the Prism software package (GraphPad). From the resulting sigmoidal curves (catalytic rate versus the logarithm of the inhibitor concentration), the IC_{50} values were estimated. All experiments were carried out in triplicate and the IC_{50} values are expressed as mean ± standard deviation (SD).

4.6 Acknowledgements

The NMR and MS spectra were recorded by André Joubert and Johan Jordaan of the SASOL Centre for Chemistry, North-West University. This work is based on the research supported in part by the Medical Research Council and National Research Foundation of South Africa (Grant specific unique reference numbers (UID) 85642, 96180, 96135). The Grant holders acknowledge that opinions, findings and conclusions or recommendations expressed in any publication generated by the NRF supported research are that of the authors, and that the NRF accepts no liability whatsoever in this regard.

REFERENCES

Böhler, P. & Tamm, C. 1967. The homo-isoflavones, a new class of natural product. Isolation and structure of eucomin and eucomol. *Tetrahedron letters*, 8(36):3479-3483.

Chimenti, F., Fioravanti, R., Bolasco, A., Chimenti, P., Secci, D., Rossi, F., Matilde, Y., Orallo, F., Ortuso, F. & Alcaro, S. 2009. Chalcones: a valid scaffold for monoamine oxidases inhibitor. *Journal of medicinal chemistry*, 52(9):2818-2824.

de Oliveira, G.R., de Oliveira, H.C.B., Silva, W.A., da Silva, V.H.C., Sabino, J.R. & Martins, F.T. 2012. Structure and theoretical approaches to a chalcone derivative. *Structural chemistry*, 23(6):1667-1676.

Dimmock, J.R., Kandepu, N.M., Nazarali, A.J., Kowalchuk, T.P., Motaganahalli, N., Quail, J.W., Mykytiuk, P.A., Audette, G.F., Prasad, L., Perjesi, P., Allen, T.M., Santos, C.L., Szydlowski, J., De Clercq, E. & Balzarini, J. 1999. Conformational and quantitative structure-activity relationship study of cytotoxic 2-arylidenebenzocycloalkanones. *Journal of medicinal chemistry*, 42(8):1358-1366.

Ferino, G., Cadoni, E., Matos, M.J., Quezada, E., Uriarte, E., Santana, L., Vilar, S., Tatonetti, N.P., Yanez, M., Vina, D., Picciau, C., Serra, S. & Delogu, G. 2013. MAO inhibitory activity of 2-arylbenzofurans versus 3-arylcoumarins: synthesis, in vitro study, and docking calculations. *ChemMedChem*, 8(6):956-966.

Finberg, J.P.M. 2014. Update on the pharmacology of selective inhibitors of MAO-A and MAO-B: focus on modulation of CNS monoamine neurotransmitter release. *Pharmacology & therapeutics*, 143(2):133-152.

Fowler, J.S., Logan, J., Wang, G.J., Volkow, N.D., Zhu, W., Franceschi, D., Pappas, N., Ferrieri, R., Shea, C., Garza, V., Xu, Y., MacGregor, R.R., Schlyer, D., Gatley, S.J., Ding, Y. & Alexoff, D. 2002. PET imaging of monoamine oxidase B in peripheral organs in humans. *Journal of nuclear medicine*, 43(10):1331-1338.

Legoabe, L.J., Petzer, A. & Petzer, J.P. 2014. α-Tetralone derivatives as inhibitors of monoamine oxidase. *Bioorganic & medicinal chemistry letters*, 24(12):2758-2763.

Legoabe, L.J., Petzer, A. & Petzer, J.P. 2015. The synthesis and evaluation of C7-substituted α-tetralone derivatives as inhibitors of monoamine oxidase. *Chemical biology & drug design*, 86(4):895-904.

Mathew, B., Mathew, G.E., Uçar, G., Baysal, I., Suresh, J., Vilapurathu, J.K., Prakasan, A., Suresh, J.K. & Thomas, A. 2015. Development of fluorinated methoxylated chalcones as selective monoamine oxidase-B inhibitors: synthesis, biochemistry and molecular docking studies. *Bioorganic chemistry*, 62:22-29.

Maurel, A., Hernandez, C., Kunduzova, O., Bompart, G., Cambon, C., Parini, A. & Francés, B. 2003. Age-dependent increase in hydrogen peroxide production by cardiac monoamine oxidase A in rats. *American journal of physiology - heart & circulatory physiology* 284(4):460-467.

Novaroli, L., Reist, M., Favre, E., Carotti, A., Catto, M. & Carrupt, P.-A. 2005. Human recombinant monoamine oxidase B as reliable and efficient enzyme source for inhibitor screening. *Bioorganic & medicinal chemistry*, 13(22):6212-6217.

Petzer, A., Pienaar, A. & Petzer, J.P. 2013. The interactions of caffeine with monoamine oxidase. *Life sciences*, 93(7):283-287.

Ramsay, R.R. 2012. Monoamine oxidases: the biochemistry of the proteins as targets in medicinal chemistry and drug discovery. *Current topics in medicinal chemistry*, 12(20):2189-2209.

Riederer, P. & Laux, G. 2011. MAO-inhibitors in Parkinson's disease. *Experimental neurobiology*, 20(1):1-17.

Rozmer, Z. & Perjési, P. 2014. Naturally occurring chalcones and their biological activities. *Phytochemistry reviews*, 15(1):87-120.

Strydom, B., Bergh, J.J. & Petzer, J.P. 2011. 8-Aryl- and alkyloxycaffeine analogues as inhibitors of monoamine oxidase. *European journal of medicinal chemistry*, 46(8):3474-3485.

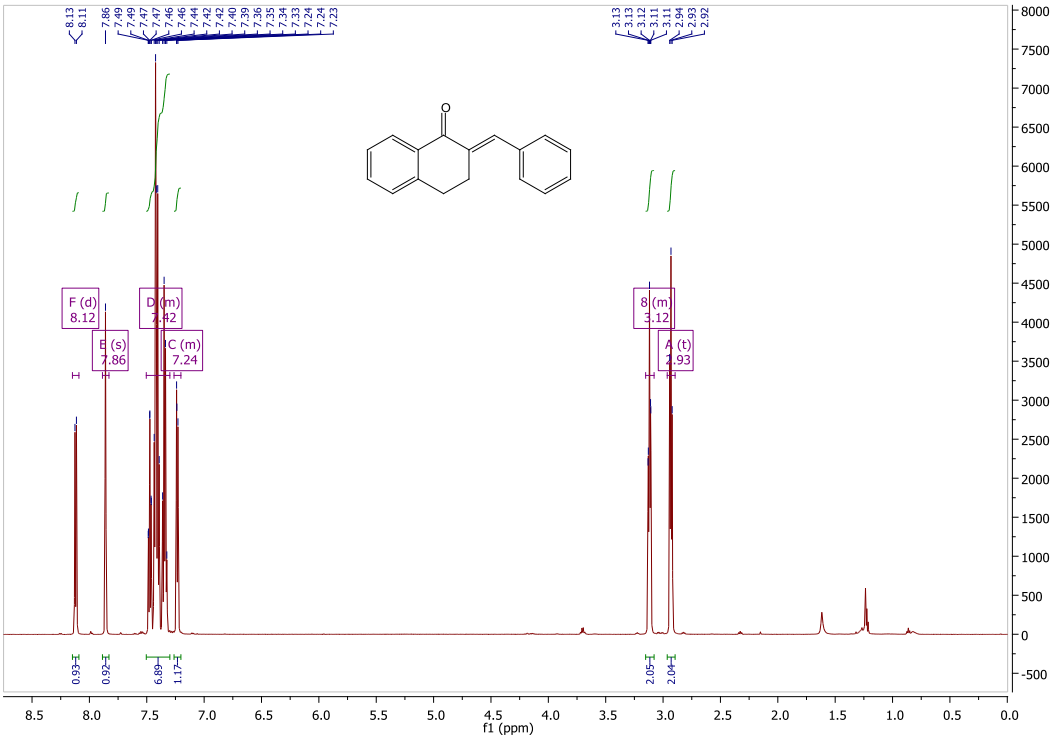
Youdim, M.B.H. & Bakhle, Y.S. 2006. Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness. *British journal of pharmacology*, 147(Suppl. 1):S287-S296.

APPENDIX B: SPECTRA

^{1}H NMR, ^{13}C NMR, HRMS and HPLC

(2*E*)-2-Benzylidene-3,4-dihydronaphthalen-1(2*H*)-one (2a)

^{1}H NMR



HRMS

Acquisition Parameter

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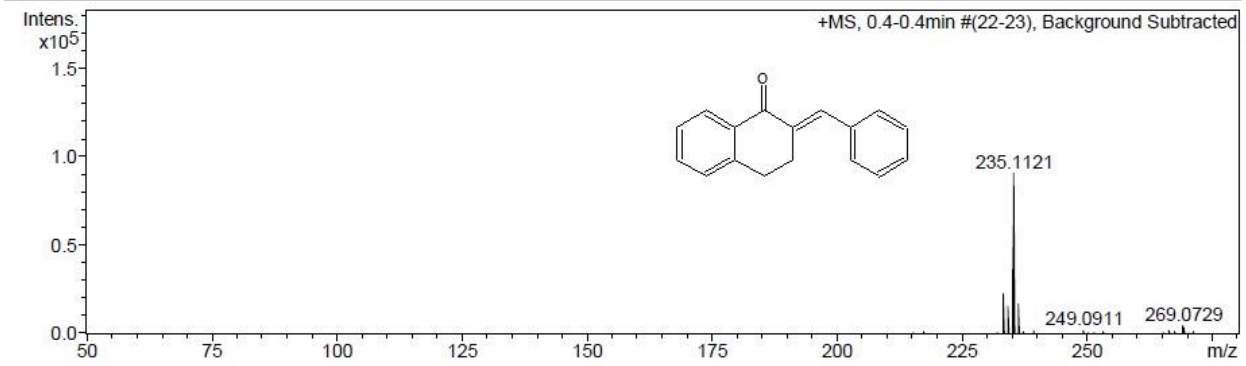
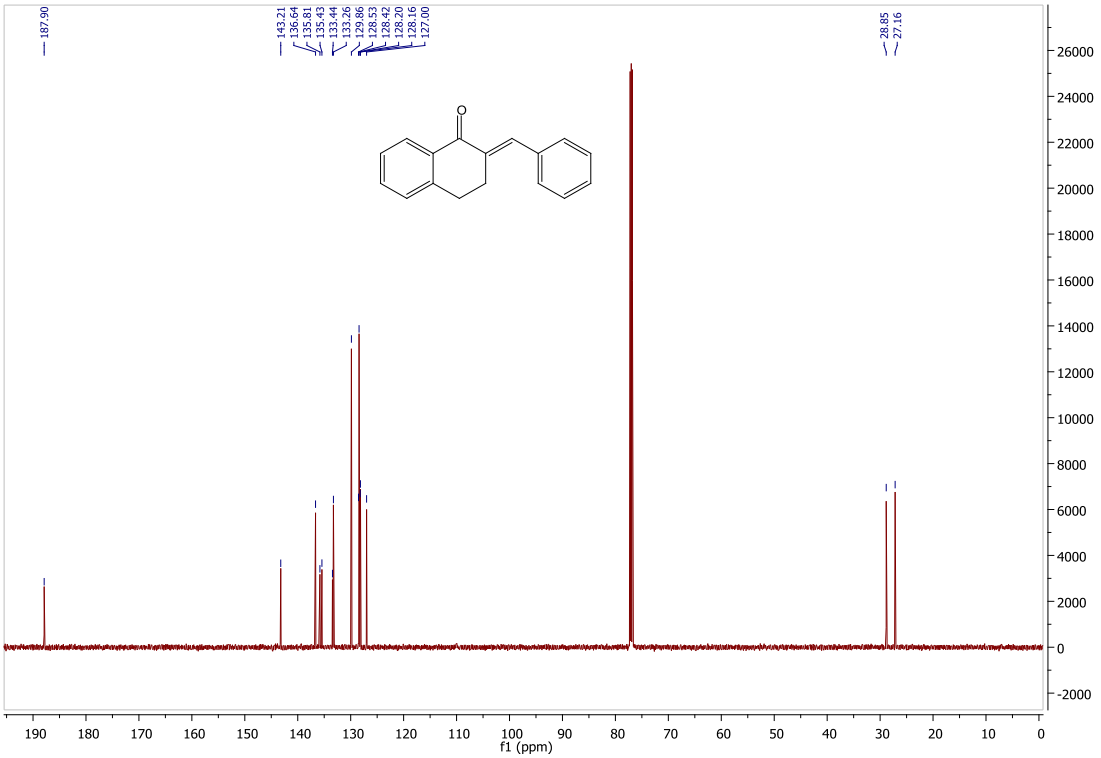
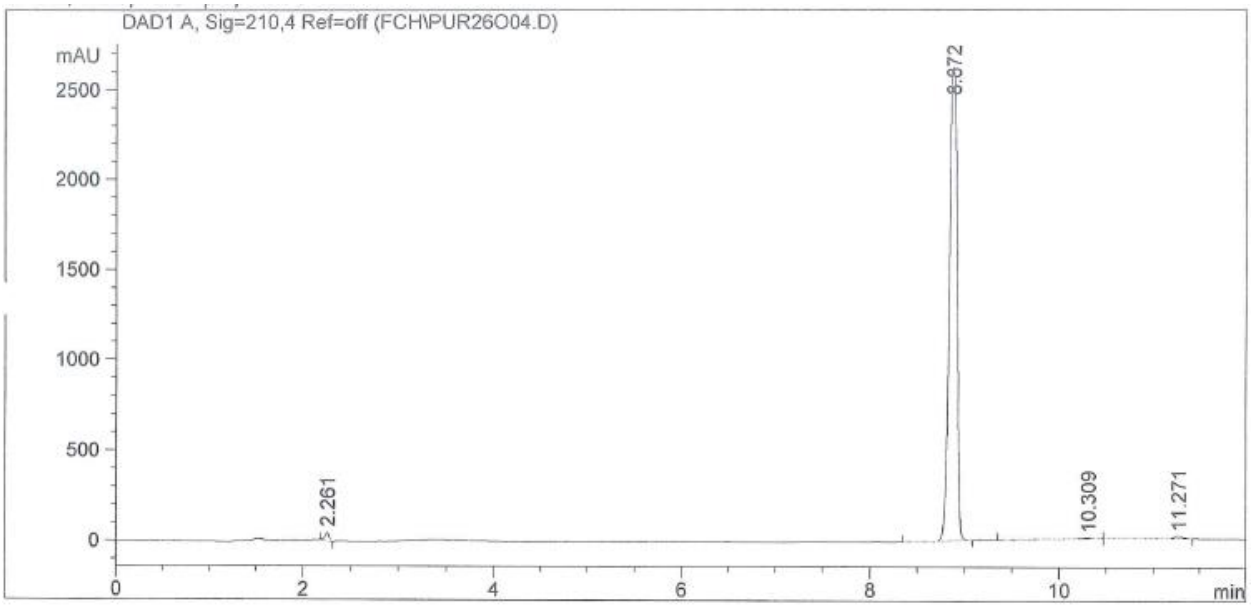
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste



^{13}C NMR

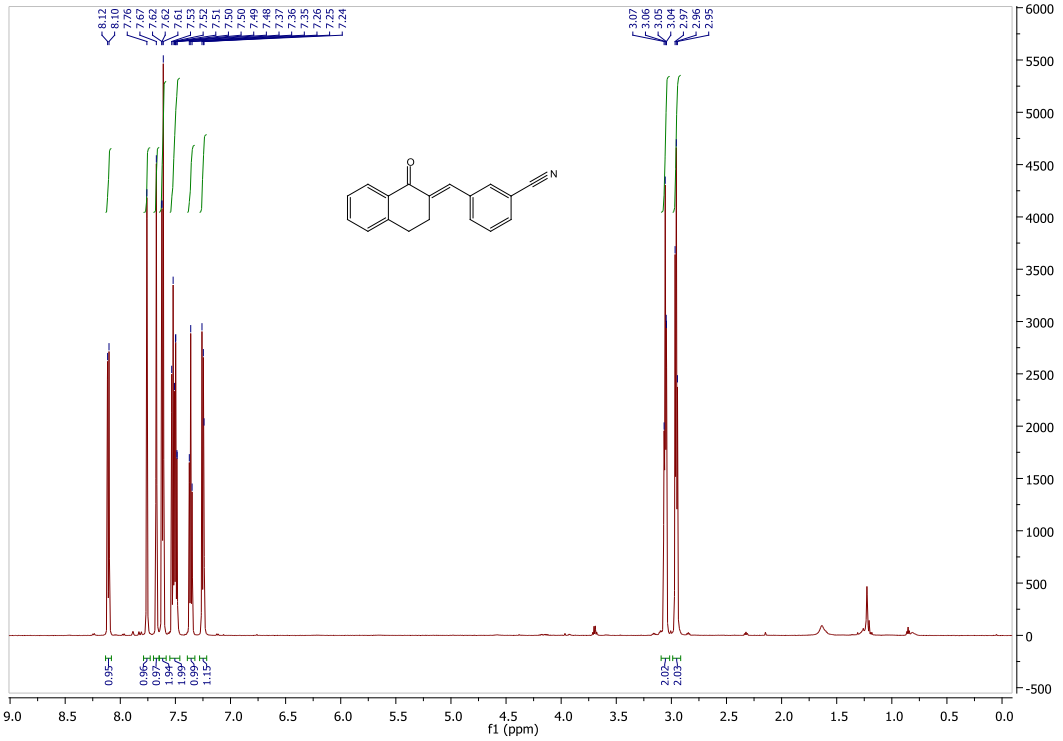

187.90
143.21
136.64
135.81
135.43
133.44
133.26
129.86
128.53
128.42
128.20
126.16
127.00
28.85
27.16
f1 (ppm)

HPLC

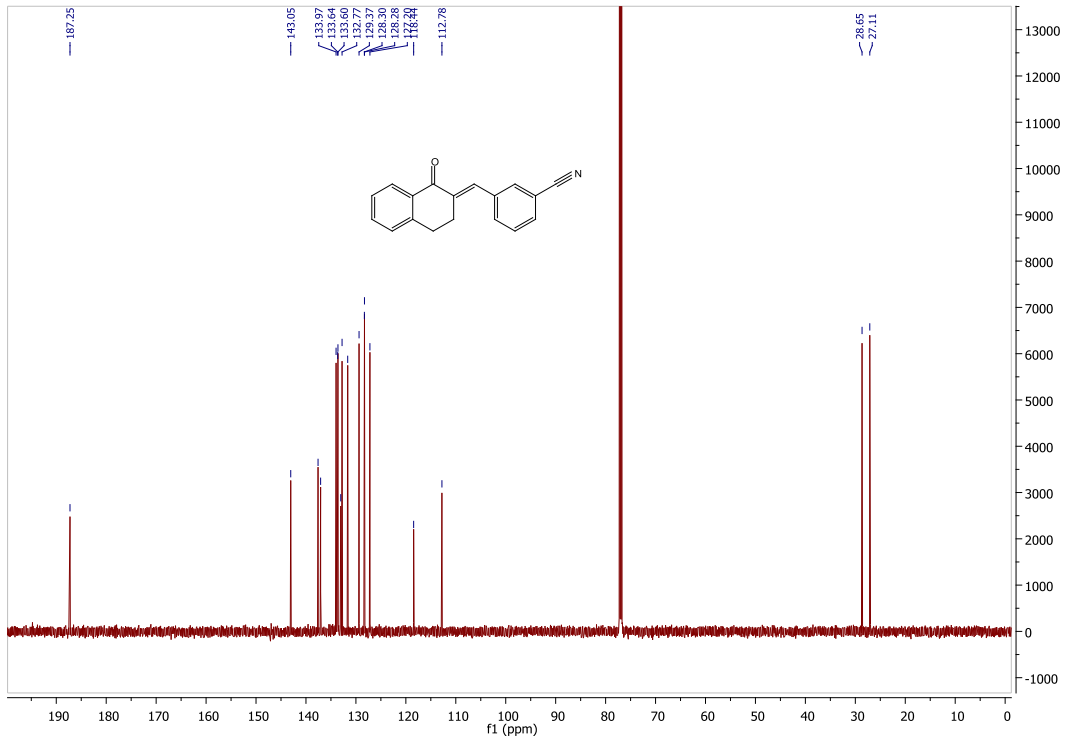

DAD1 A, Sig=210,4 Ref=off (FCH\PUR26O04.D)
mAU
8.872
2.261
10.309
11.271
min

3-[(*E*)-(1-Oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl]benzonitrile (2b)

^{1}H NMR



^{13}C NMR



HRMS

Acquisition Parameter

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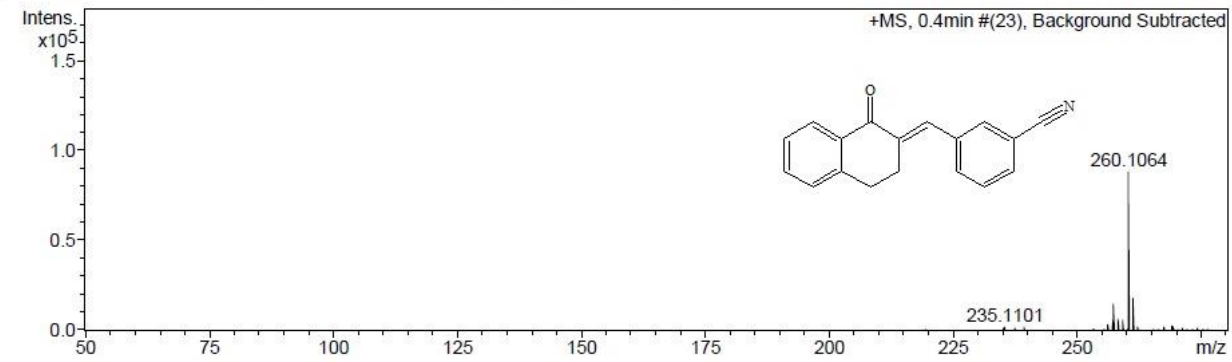
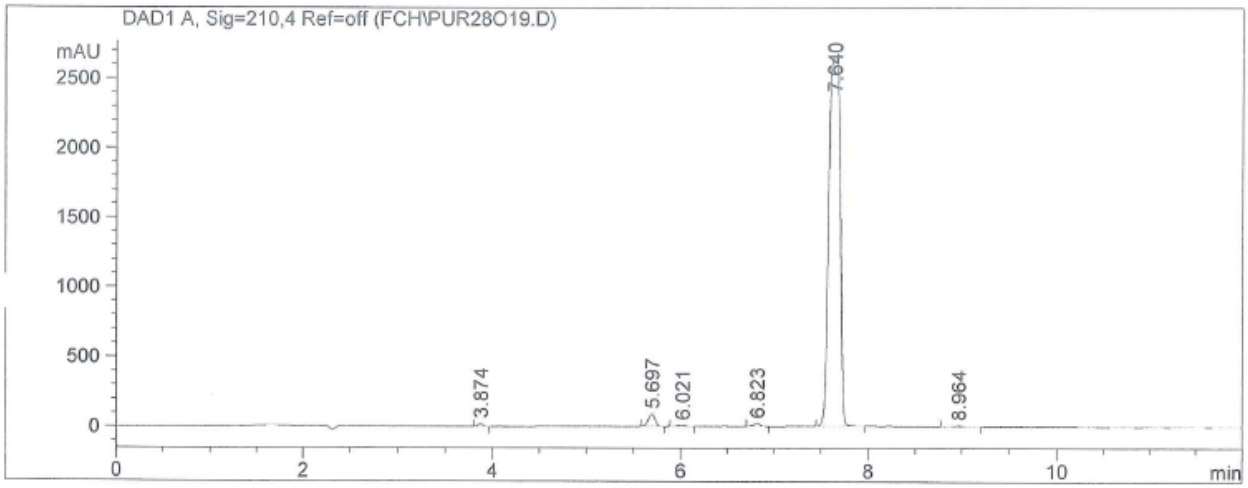
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste

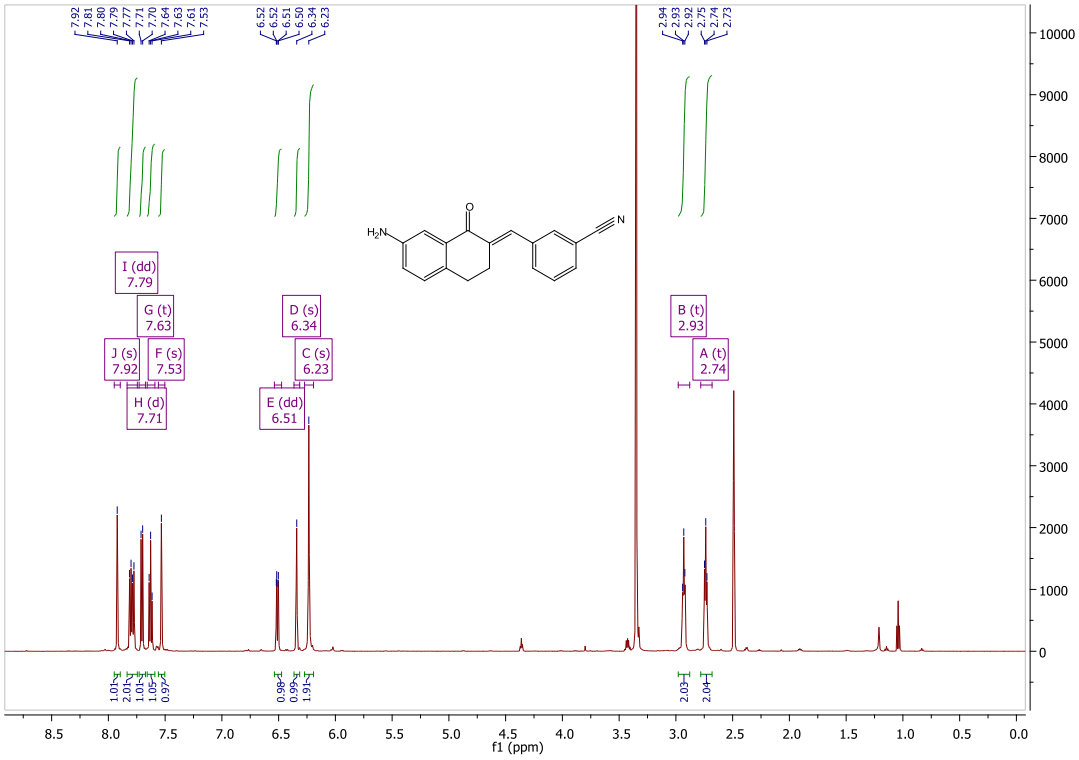


HPLC

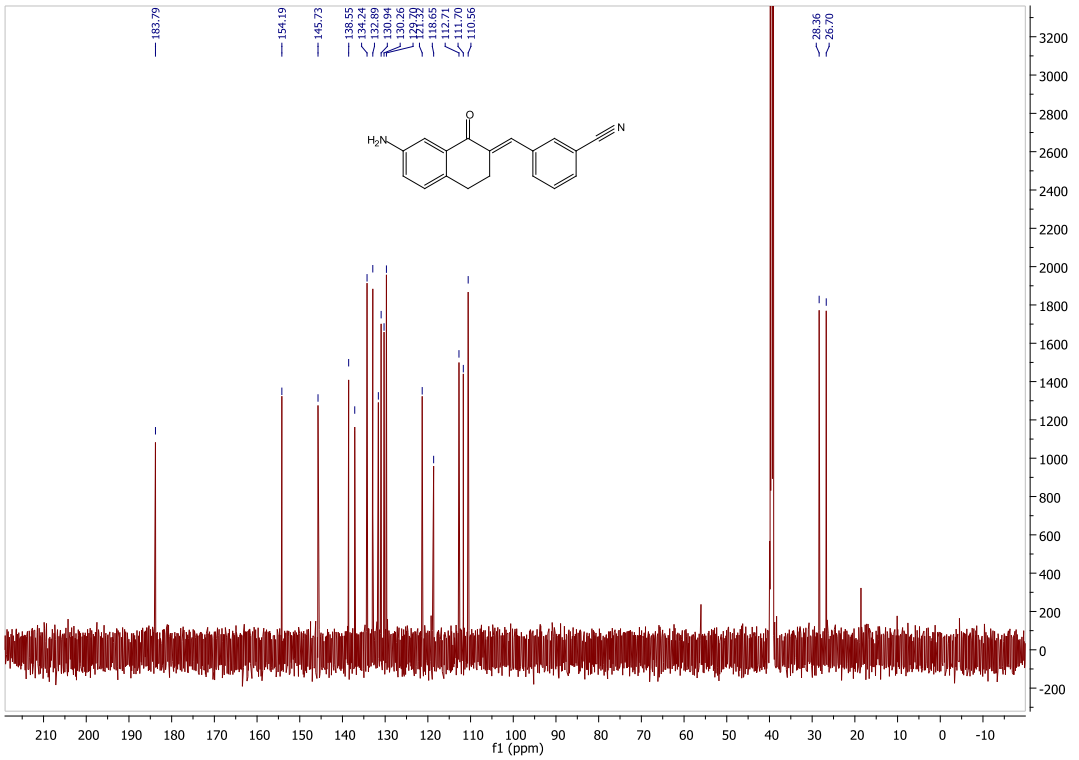


3-[(*E*)-(7-Amino-1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl]benzonitrile (2c)

^{1}H NMR



^{13}C NMR



HRMS

Acquisition Parameter

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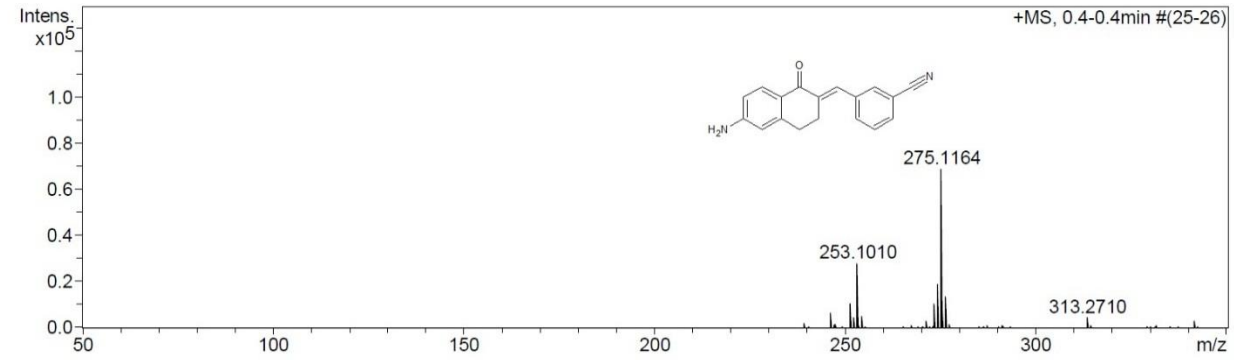
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste



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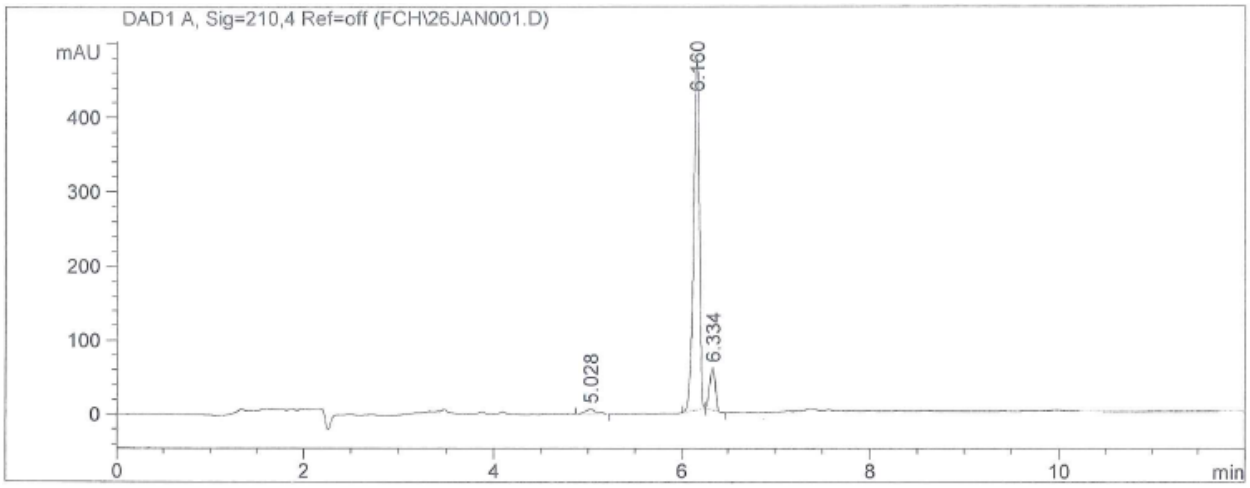
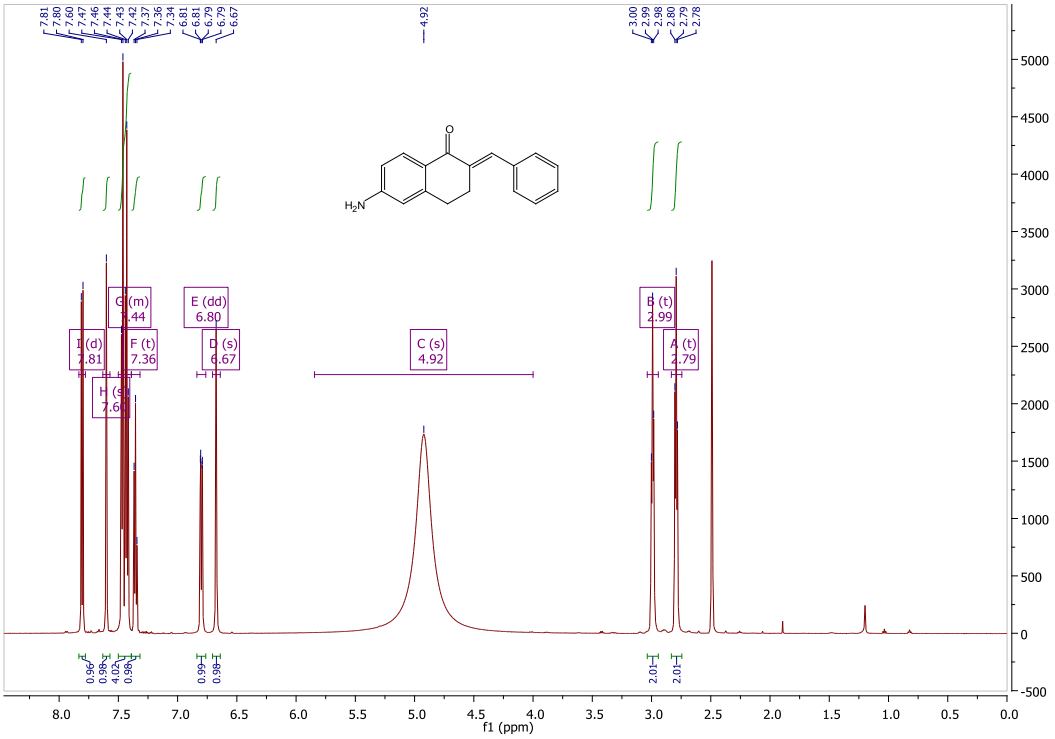
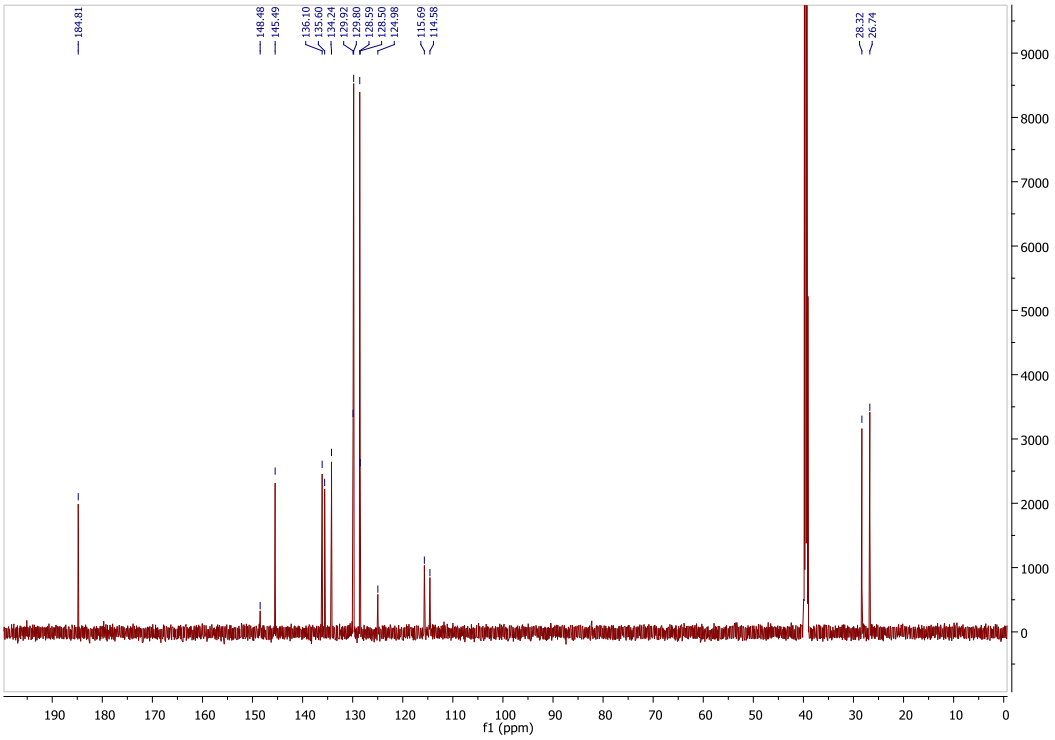


(2*E*)-6-Amino-2-benzylidene-3,4-dihydronaphthalen-1(2*H*)-one (2d)

^{1}H NMR



^{13}C NMR



HRMS

Acquisition Parameter

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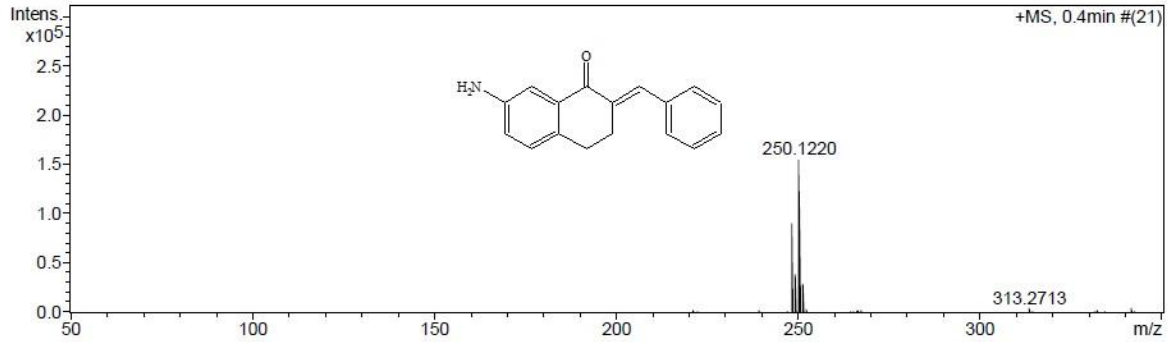

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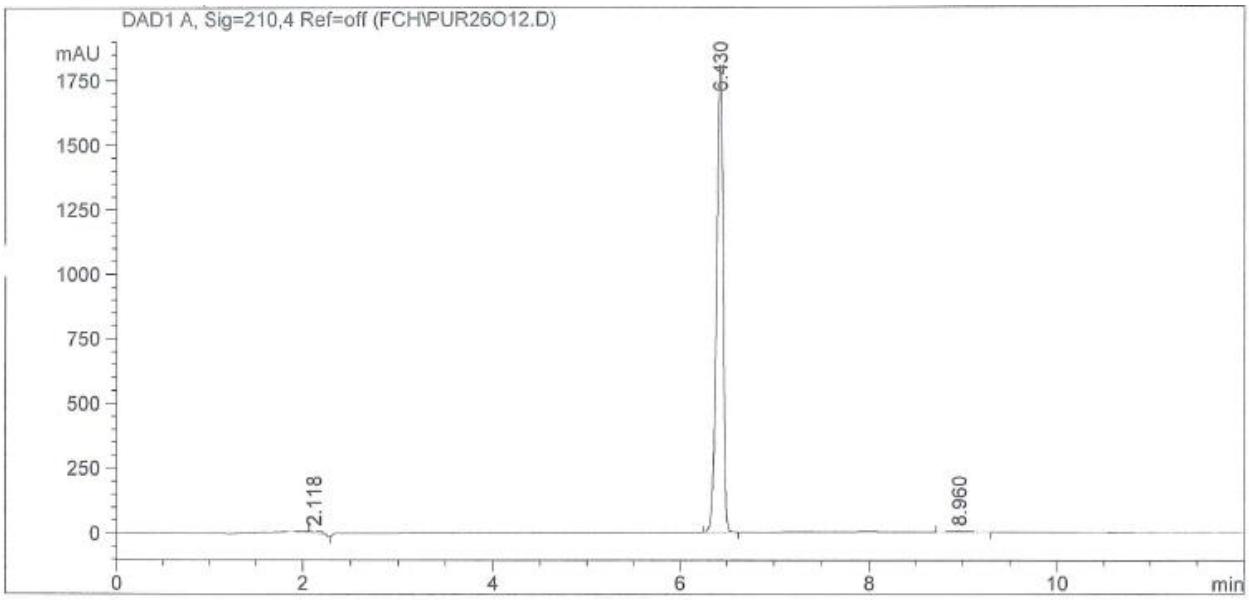
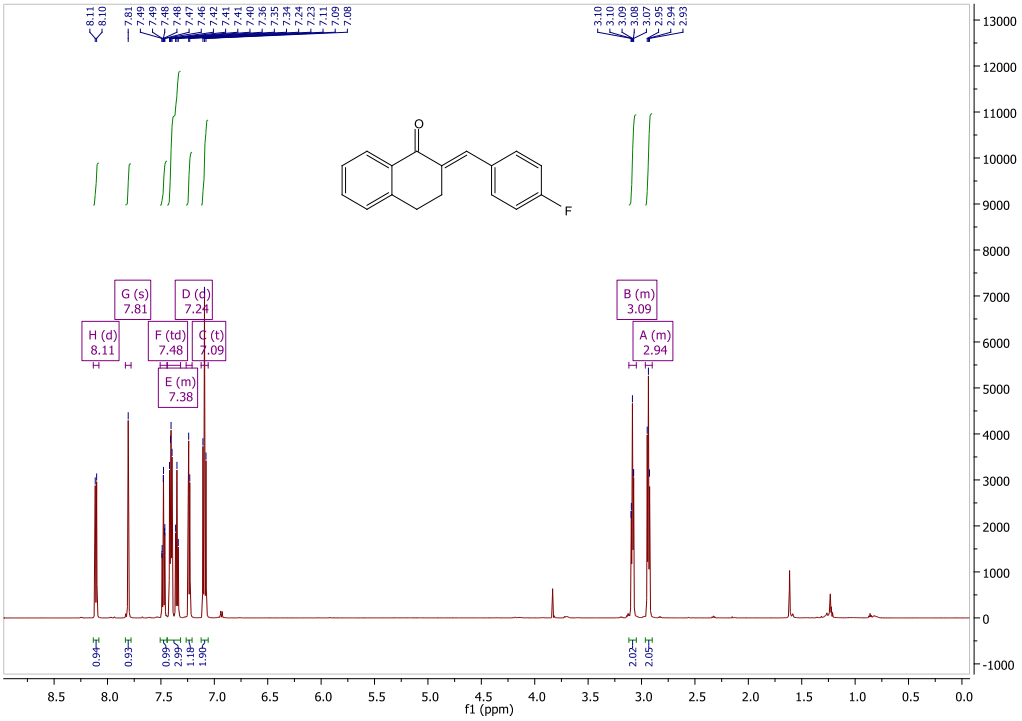
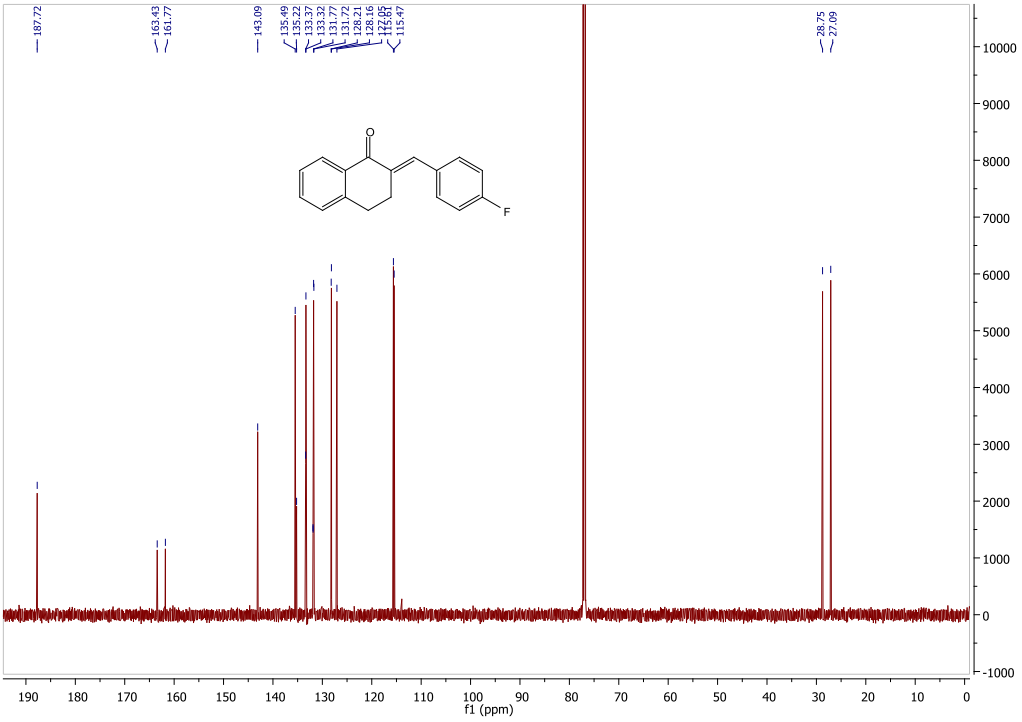


(2*E*)-2-(4-Fluorobenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (2e)

^{1}H NMR



^{13}C NMR



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Acquisition Parameter

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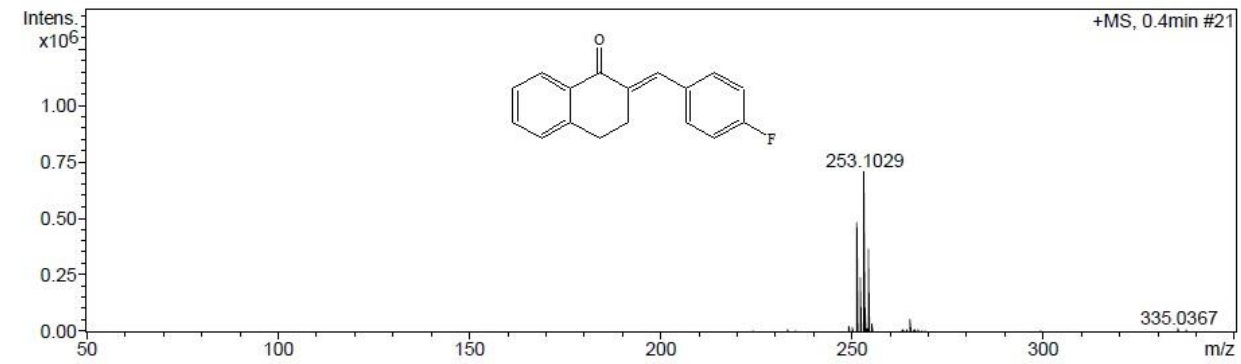

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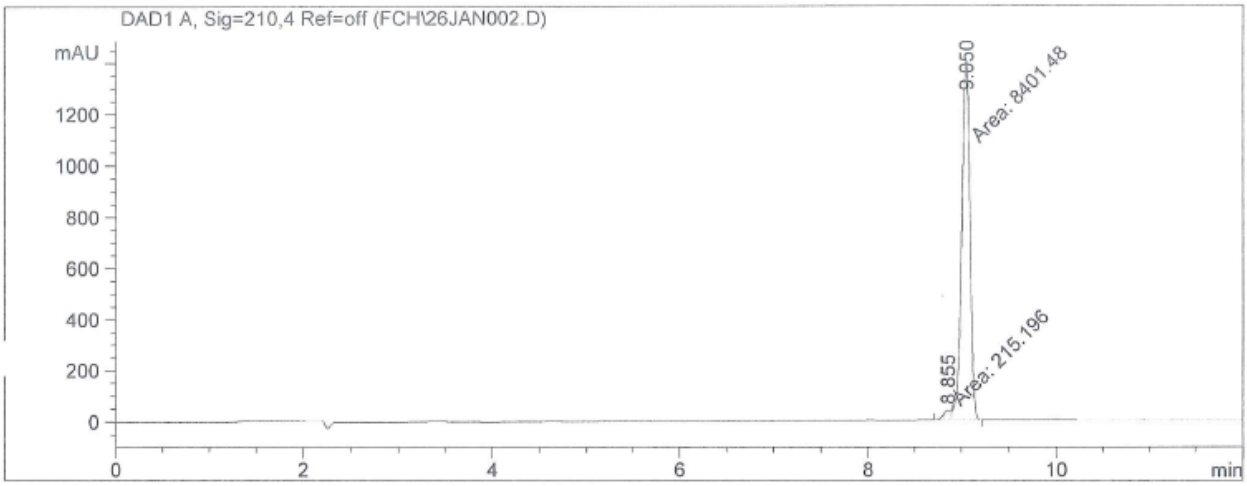
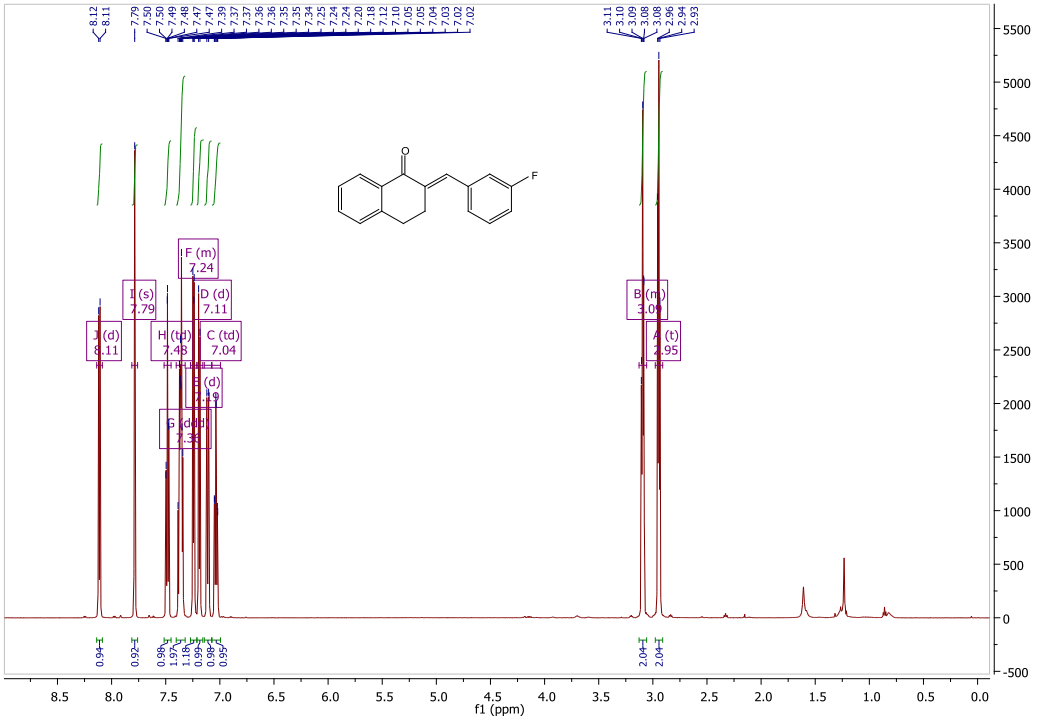
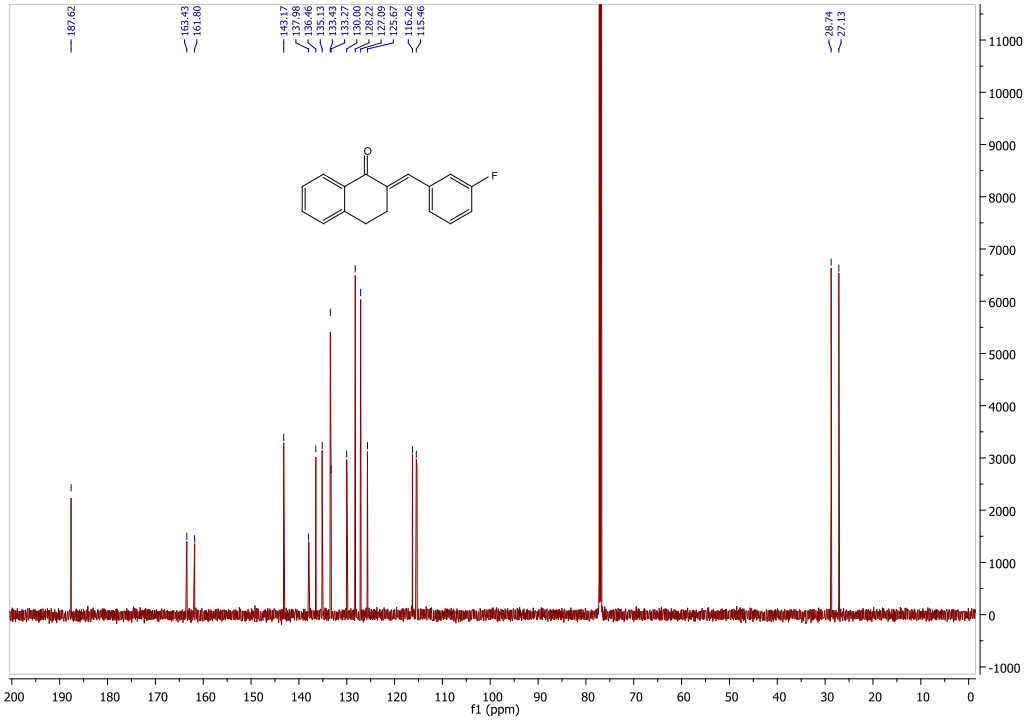


(2*E*)-2-(3-Fluorobenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (2f)

^{1}H NMR



^{13}C NMR



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Acquisition Parameter

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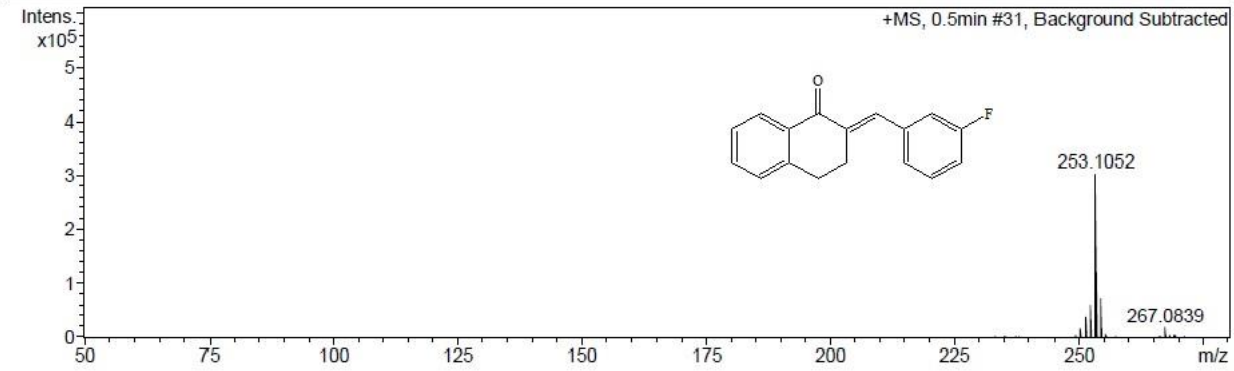

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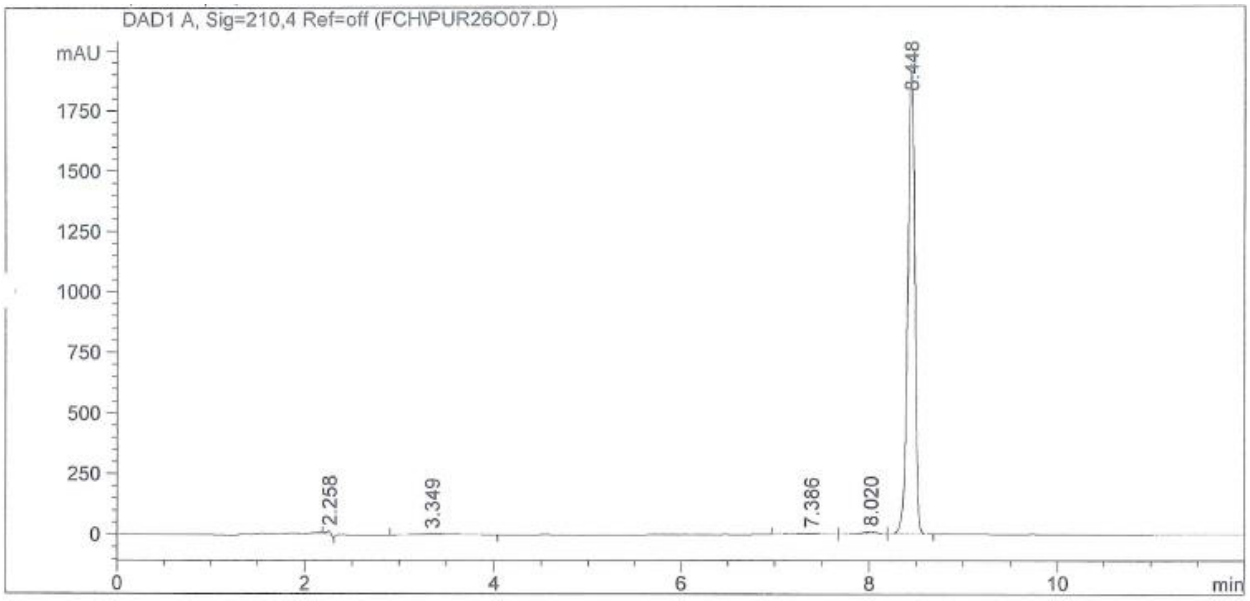
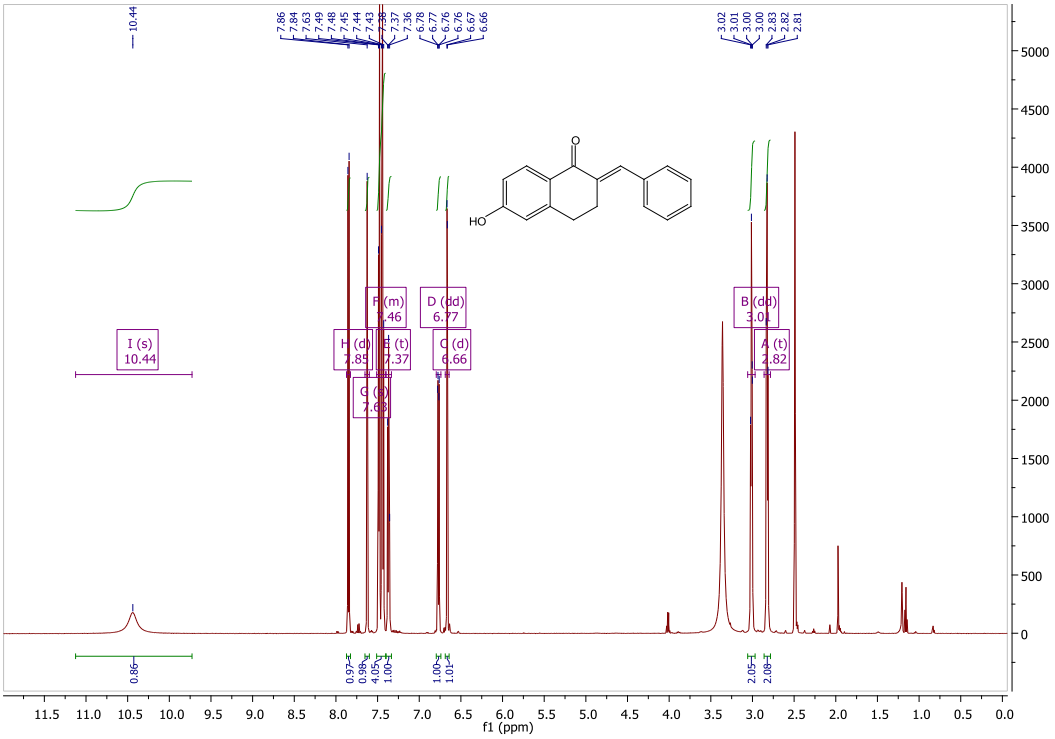


(2*E*)-2-Benzylidene-6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2g)

^{1}H NMR



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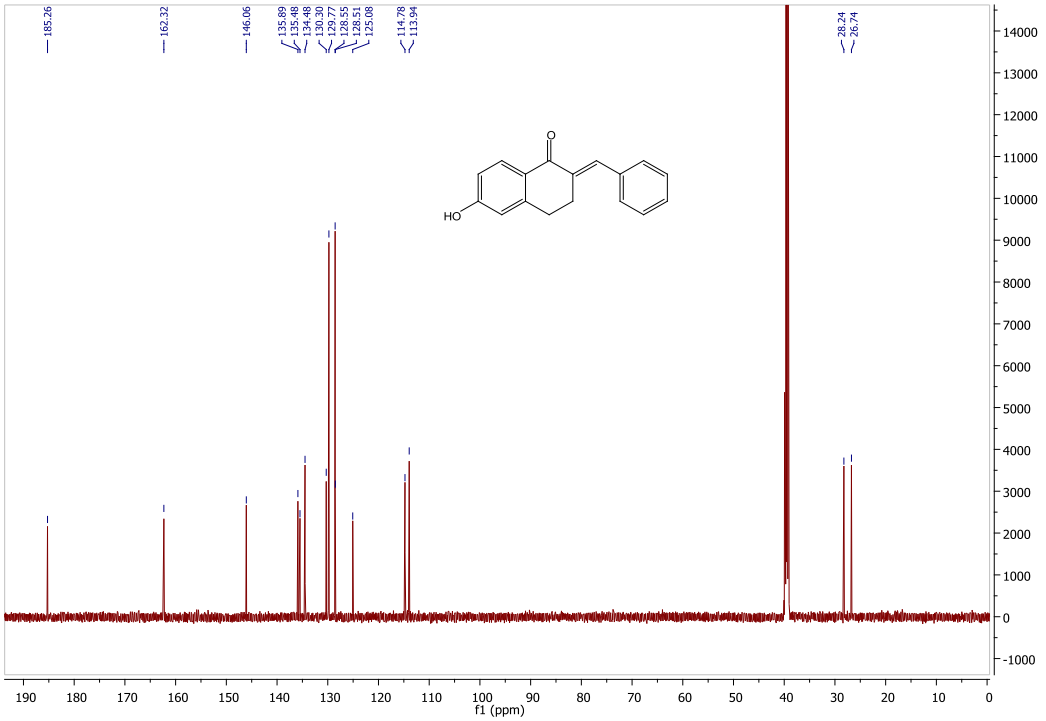

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Acquisition Parameter

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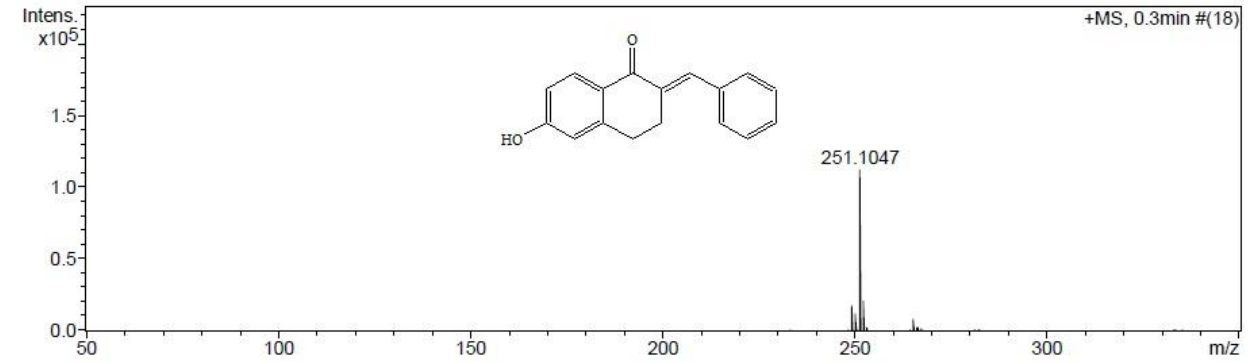

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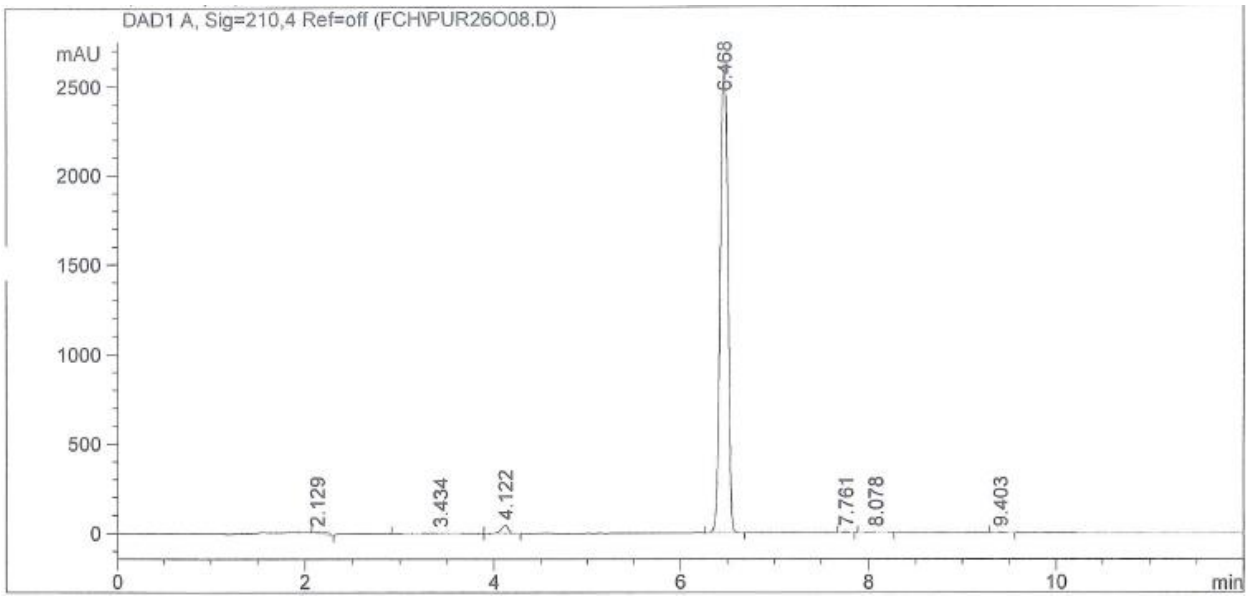
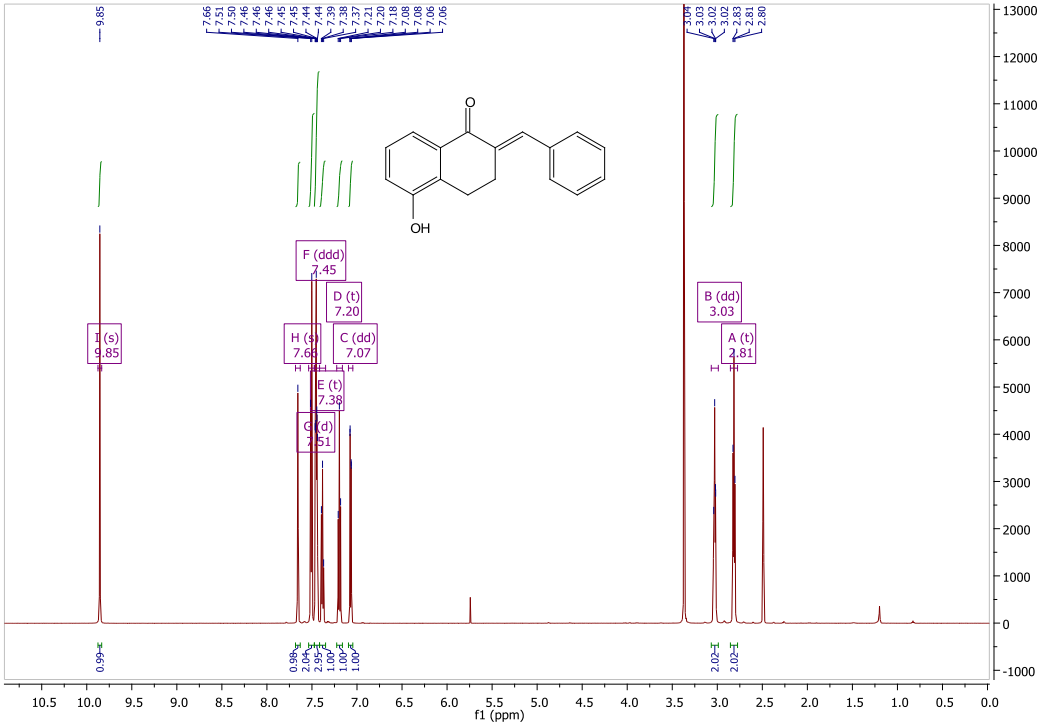


(2*E*)-2-Benzylidene-5-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2h)

^{1}H NMR



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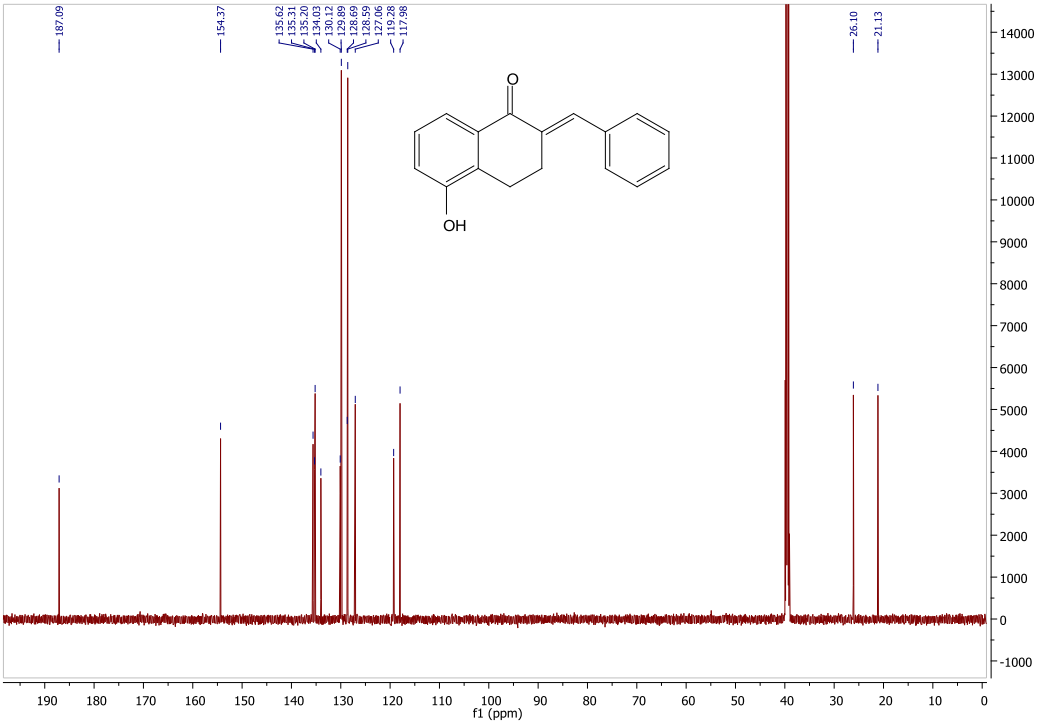

HRMS

Acquisition Parameter

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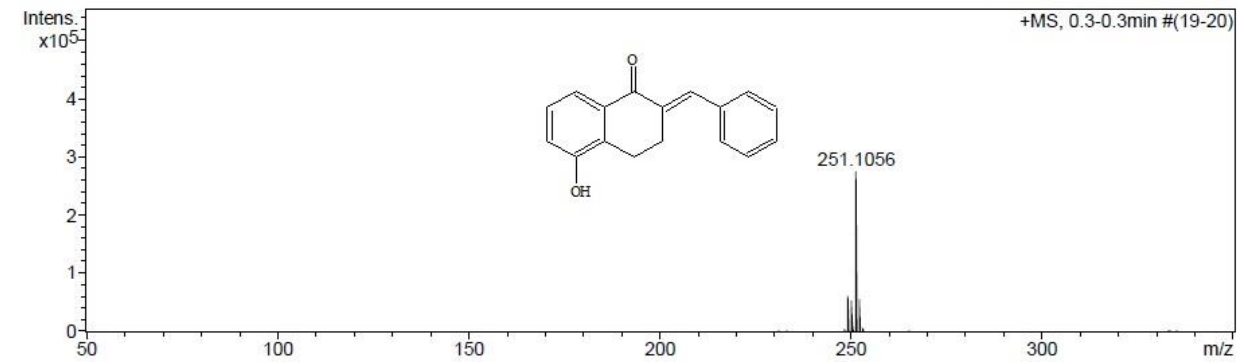
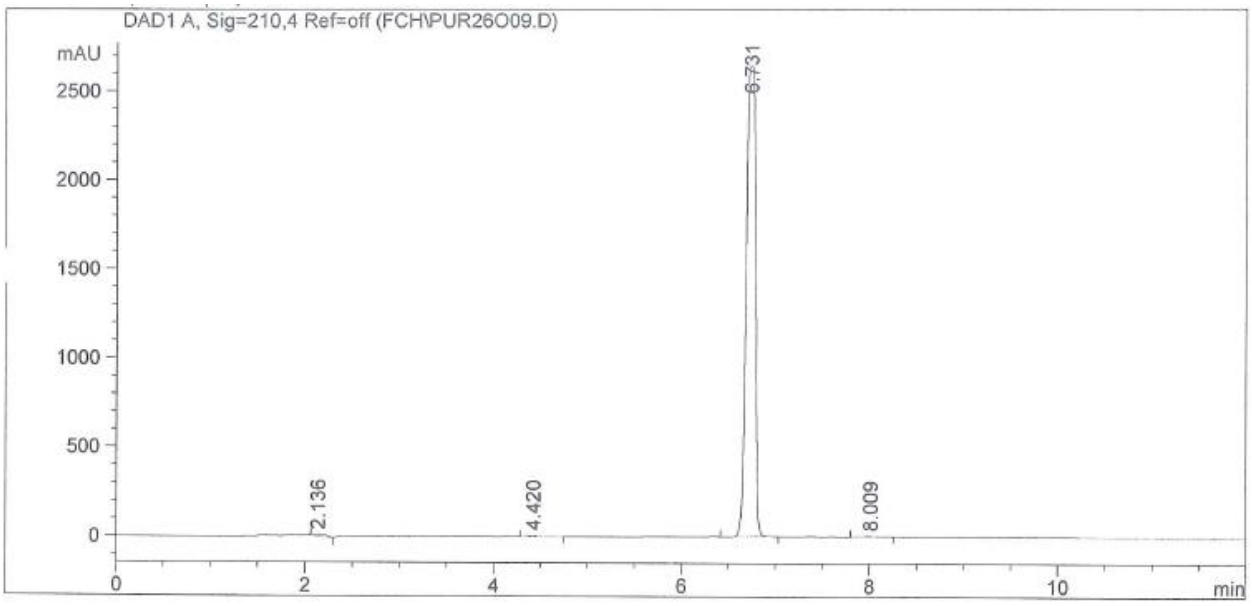


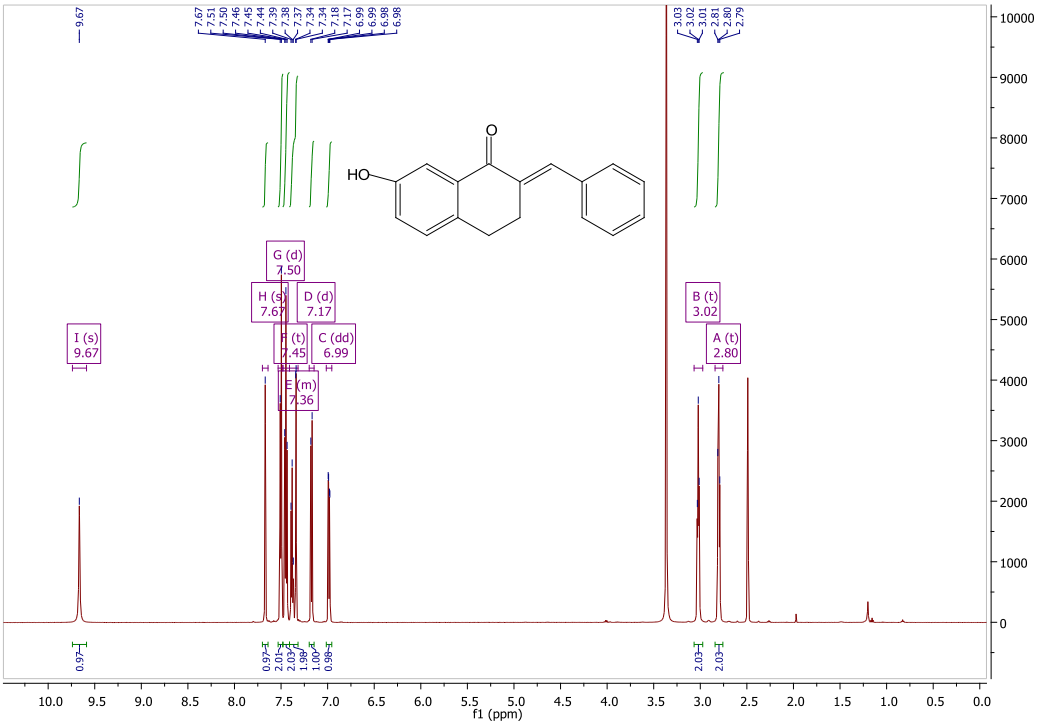
HPLC



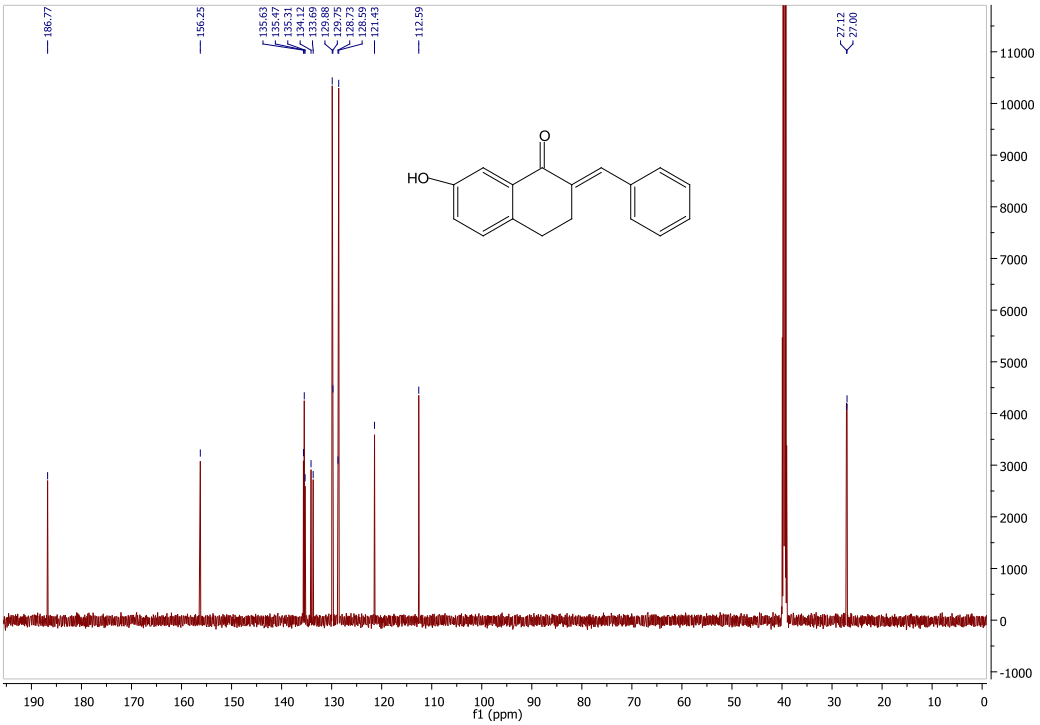
I

(2*E*)-2-Benzylidene-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2i)

^{1}H NMR



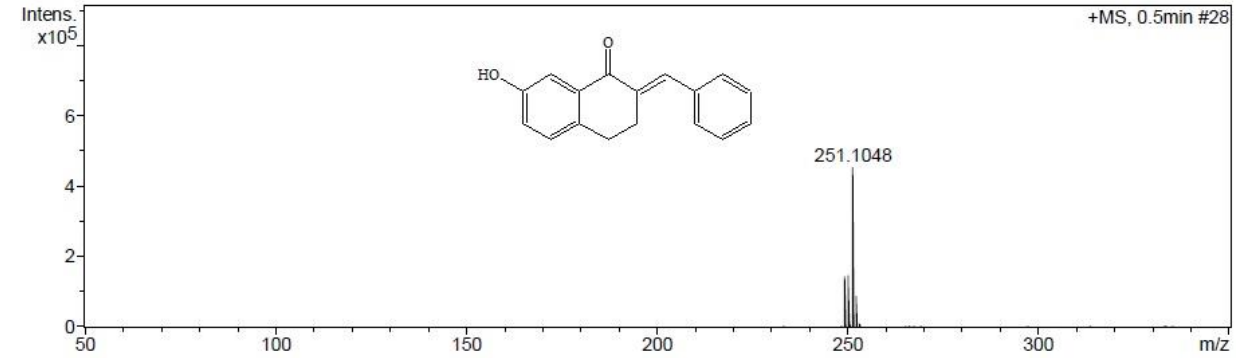
^{13}C NMR



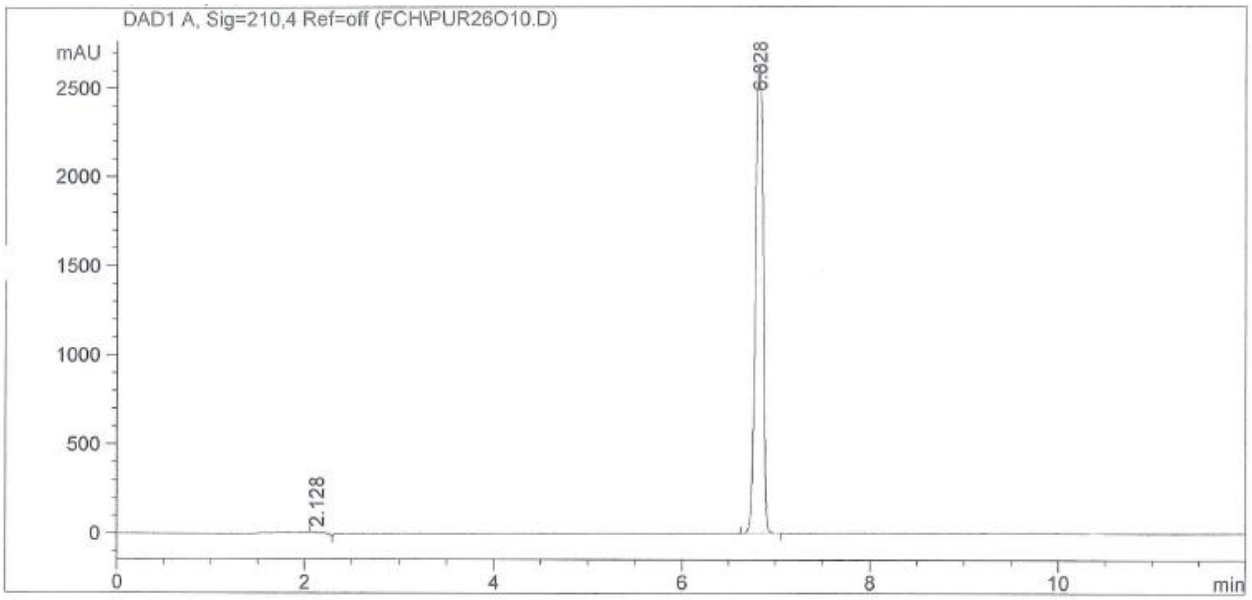
HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste

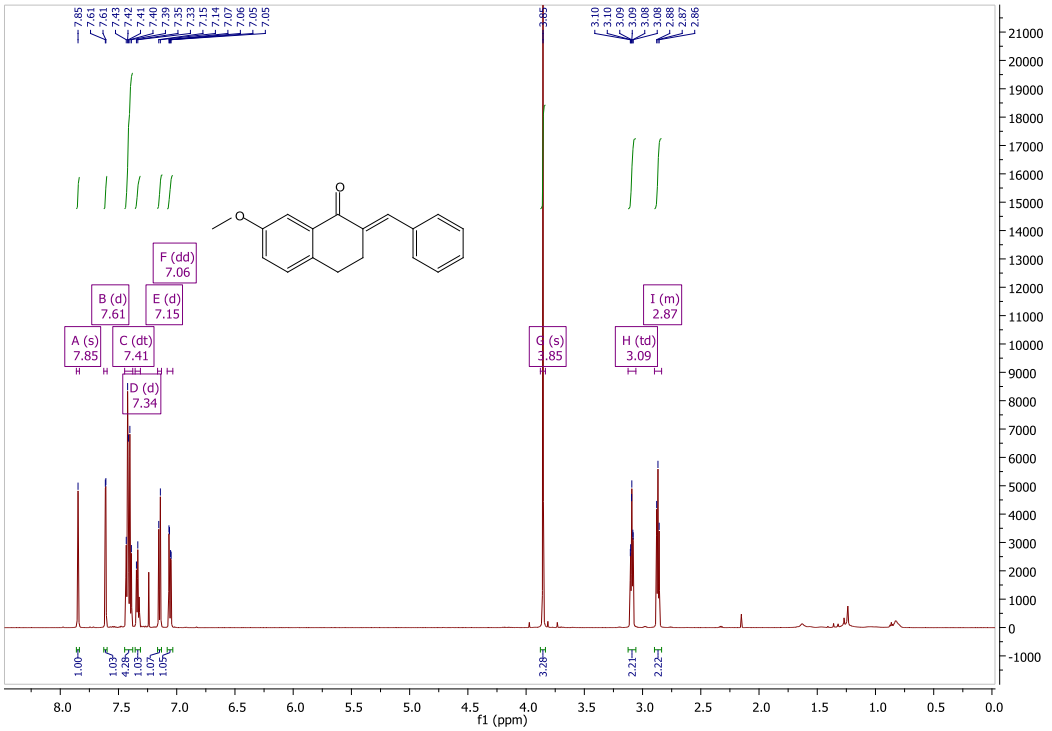


HPLC

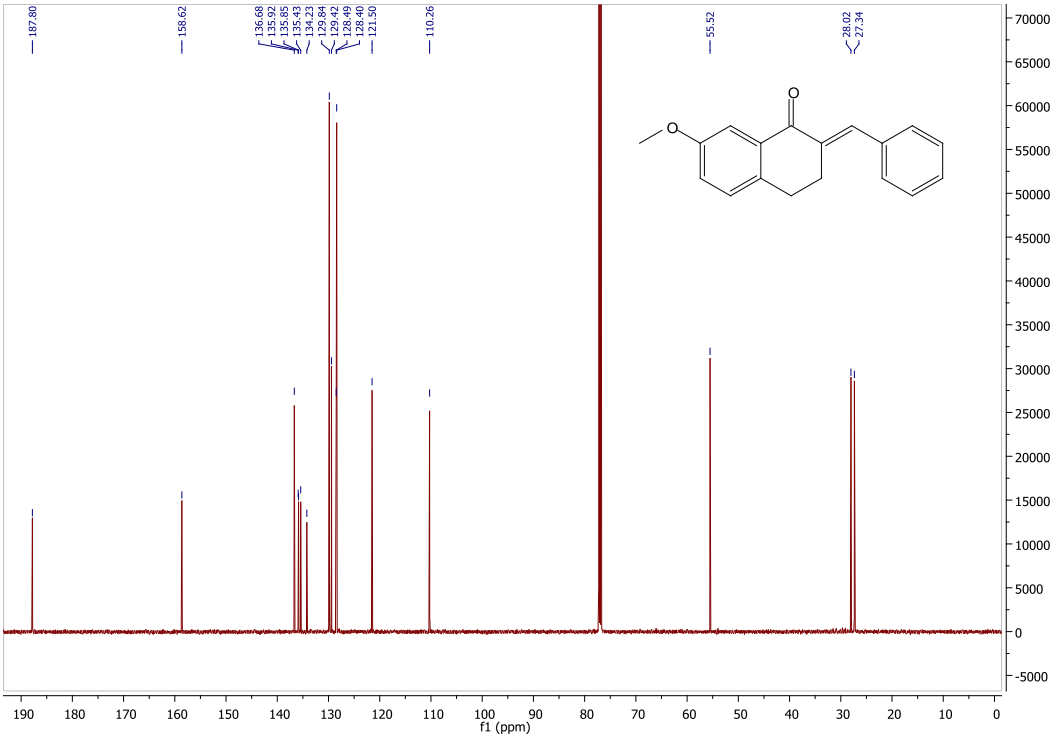


(2*E*)-2-Benzylidene-7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one (2j)

^{1}H NMR



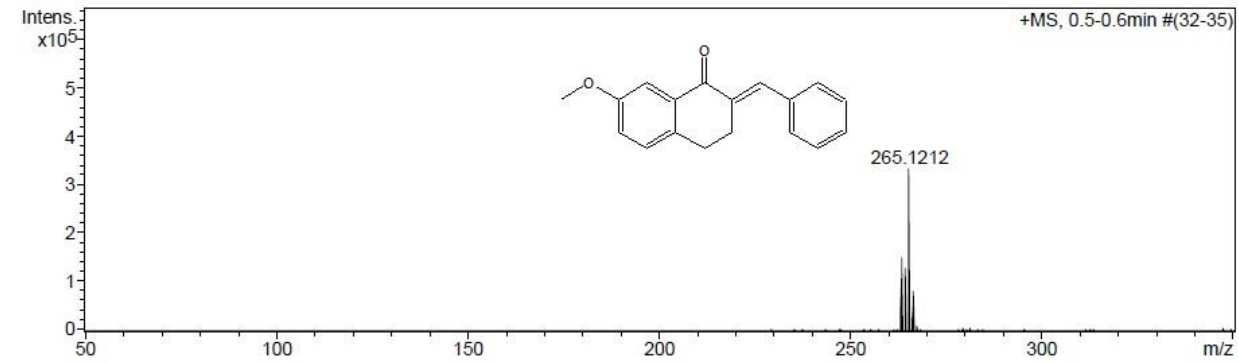
^{13}C NMR



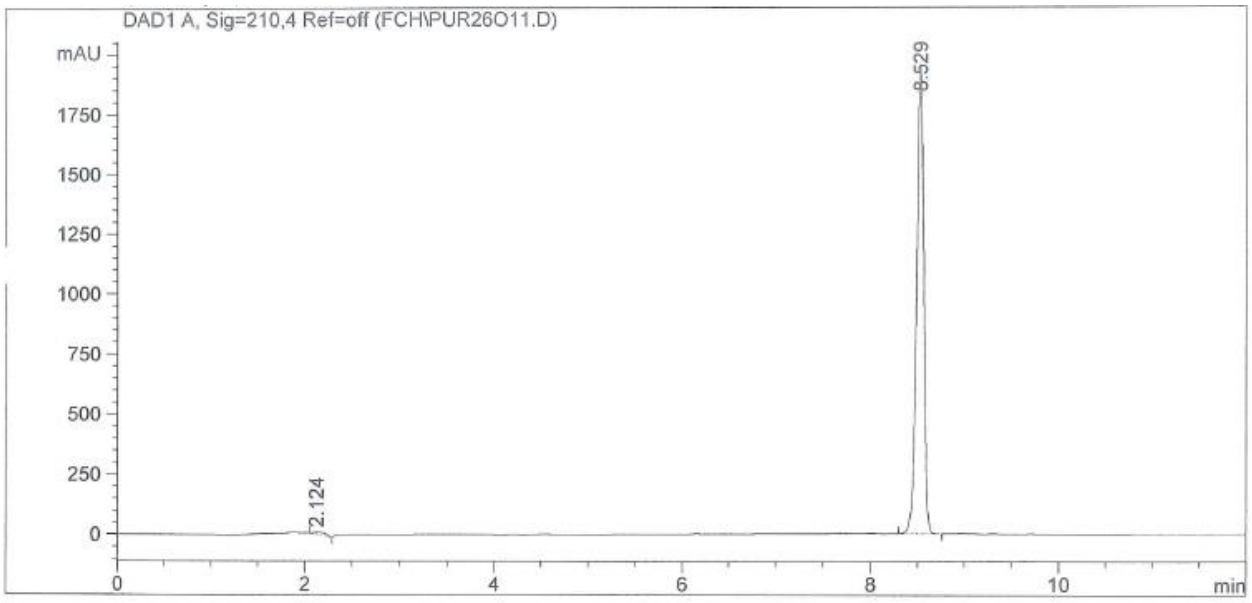
HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste

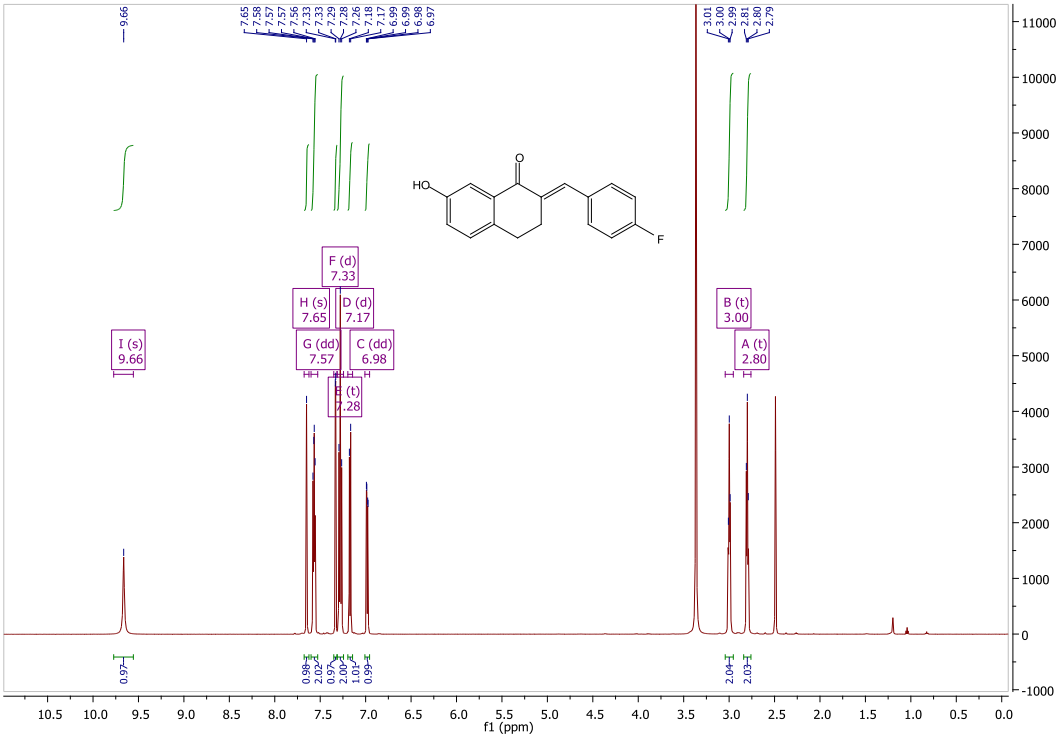


HPLC

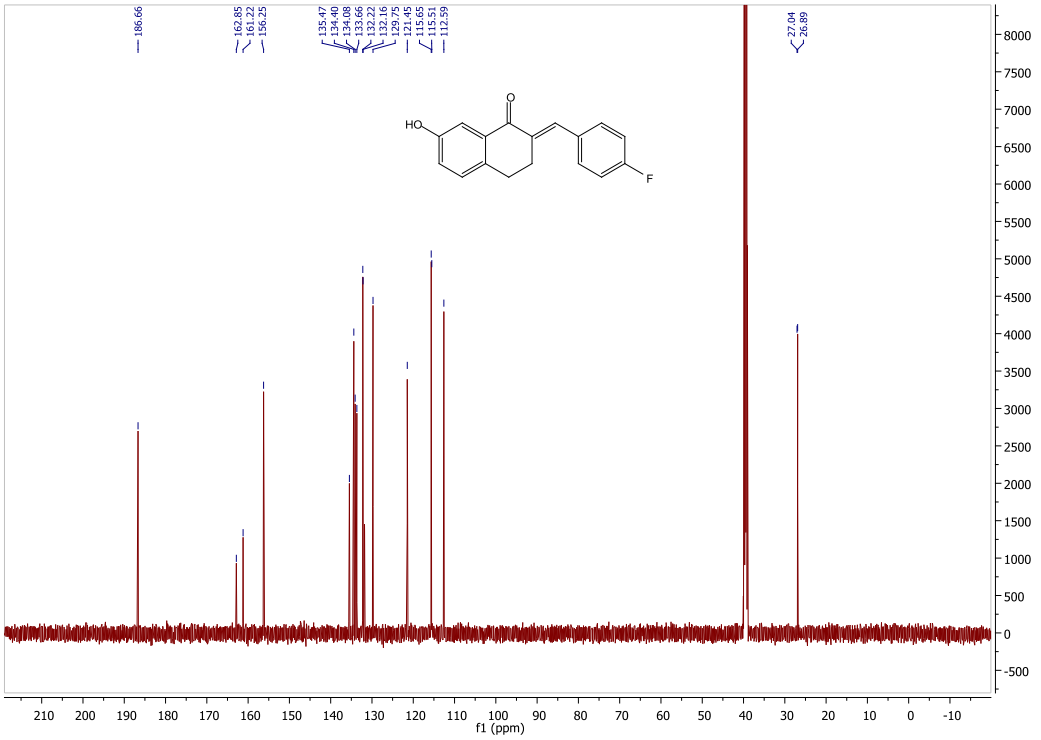


(2*E*)-2-(4-Fluorobenzylidene)-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2k)

^{1}H NMR



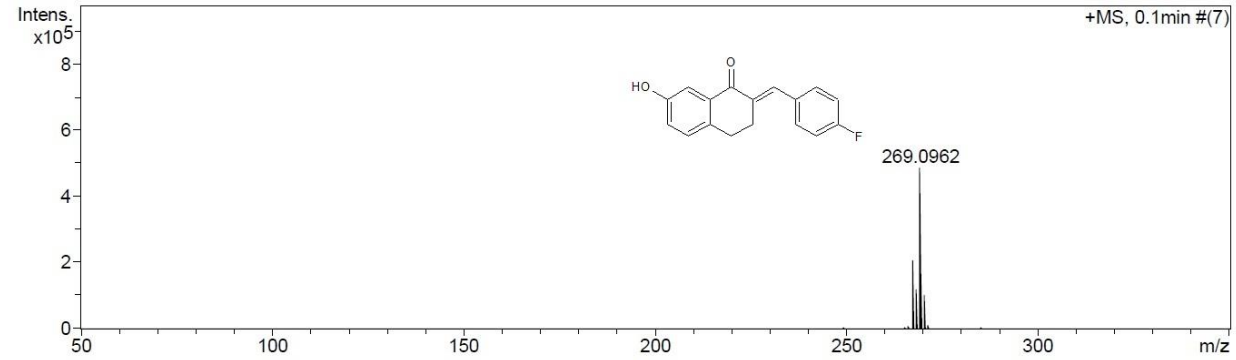
^{13}C NMR



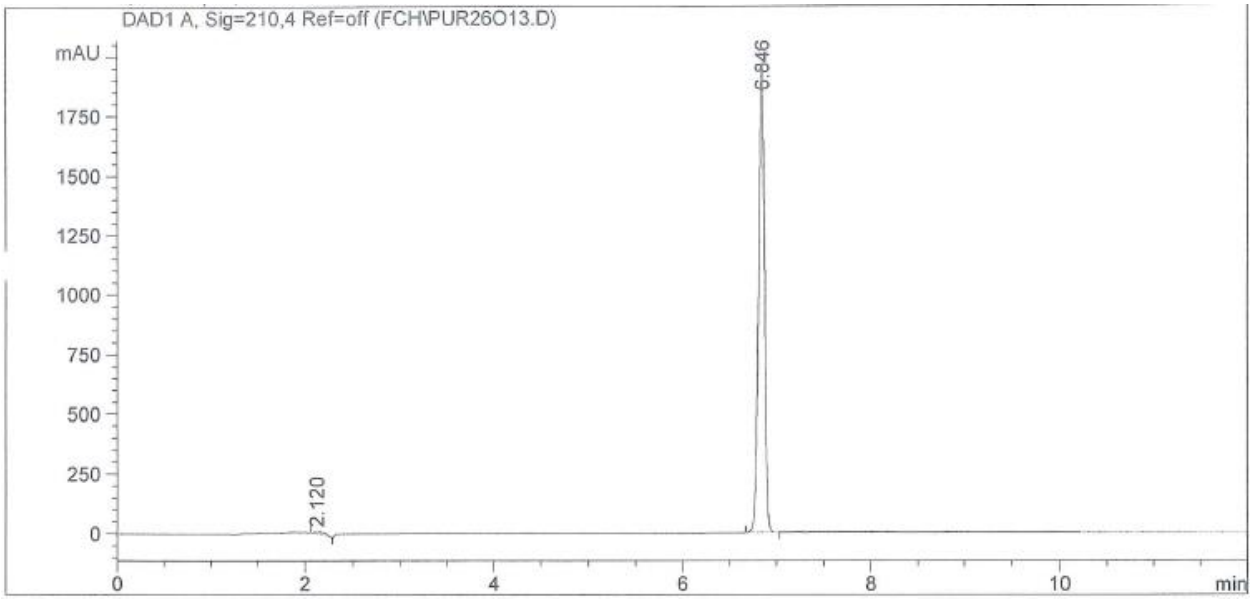
HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste

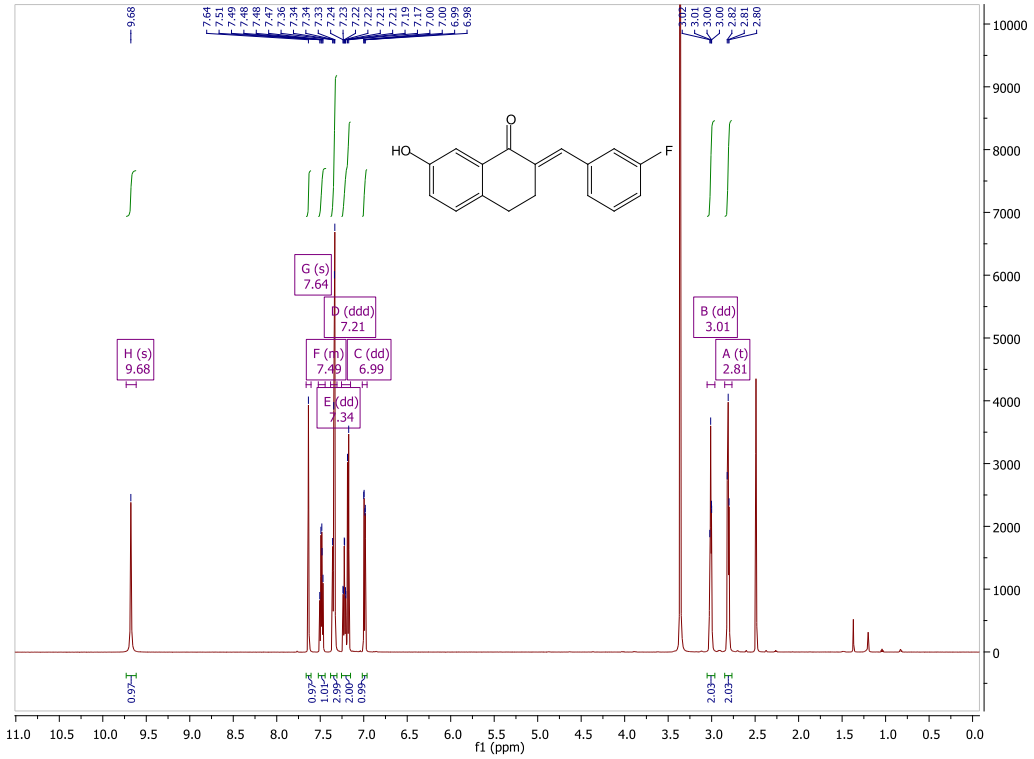


HPLC

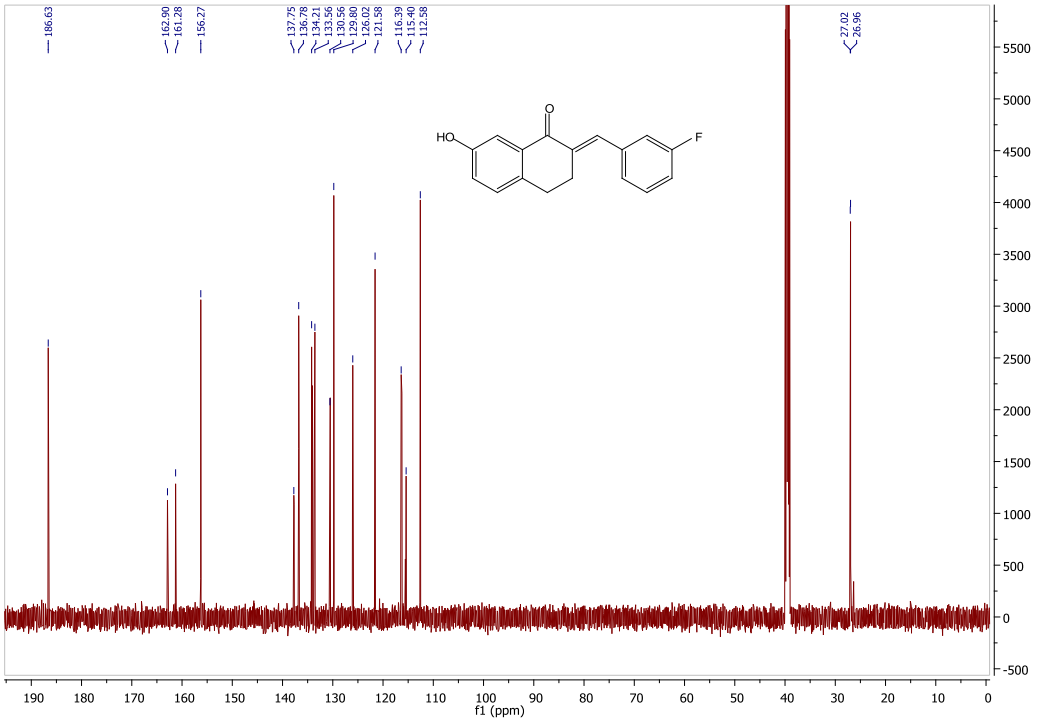


(2*E*)-2-(3-Fluorobenzylidene)-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2l)

^{1}H NMR



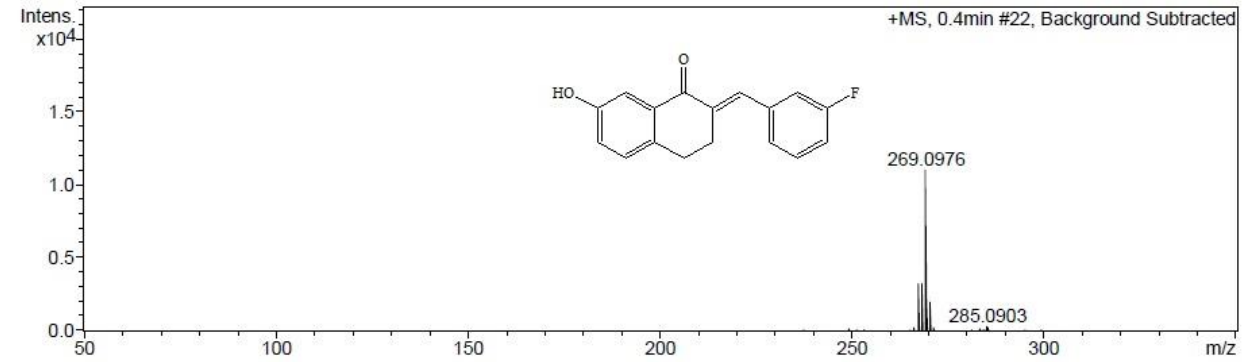
^{13}C NMR



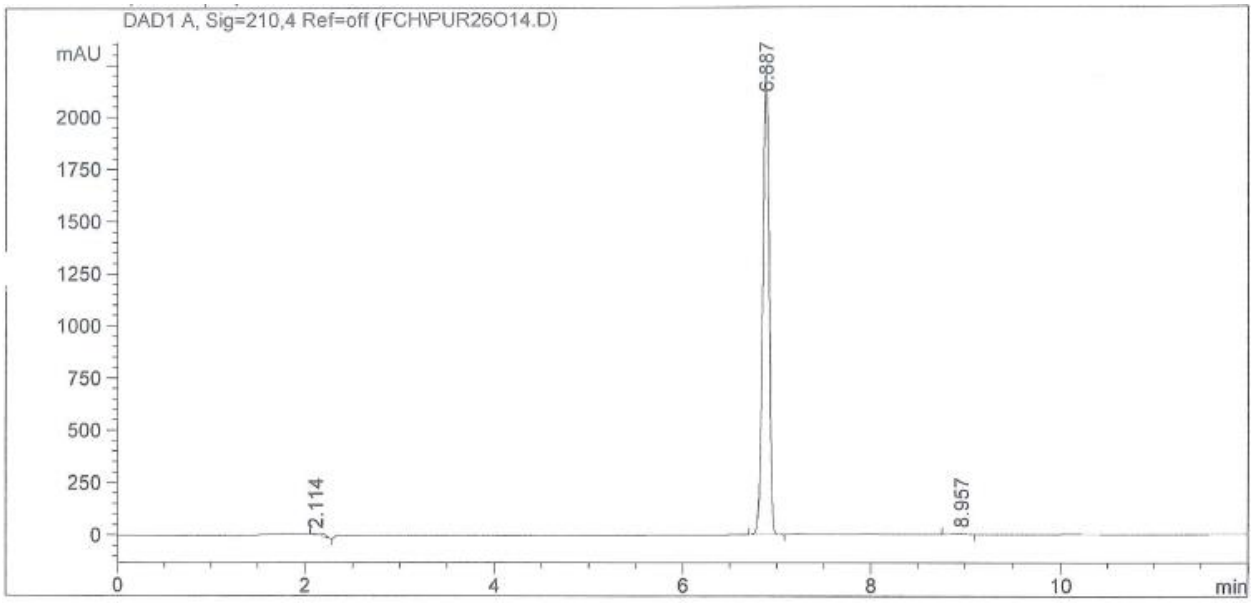
HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste

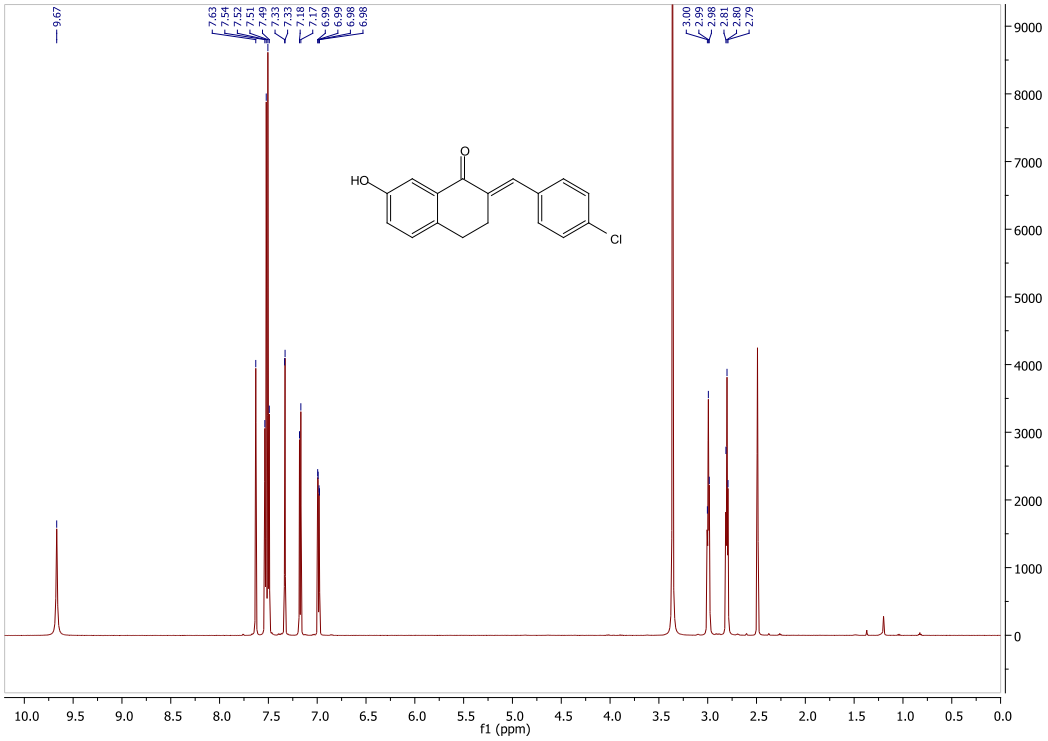


HPLC

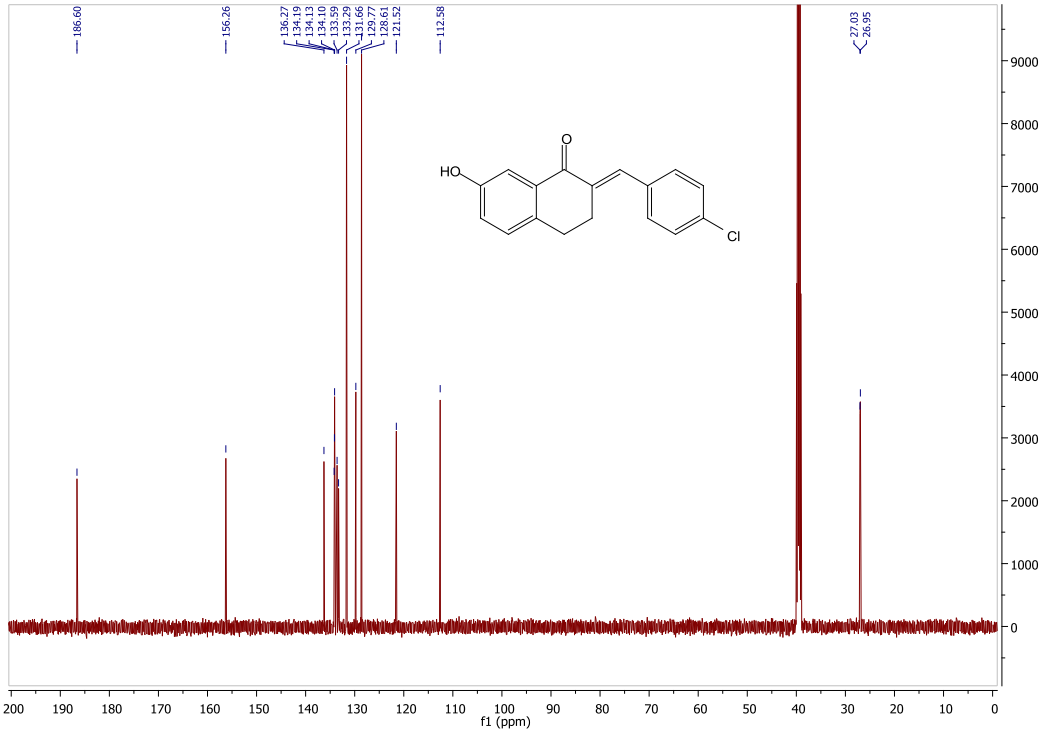


(2*E*)-2-(4-Chlorobenzylidene)-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2m)

^{1}H NMR



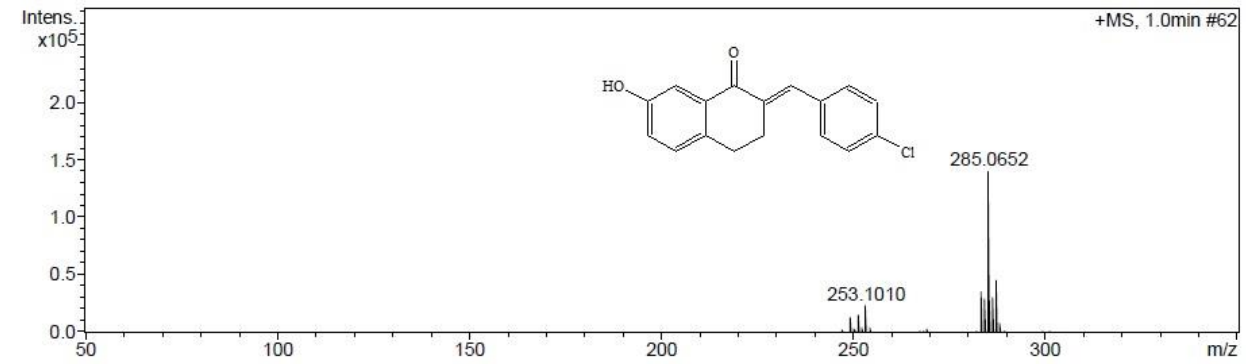
^{13}C NMR



HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste



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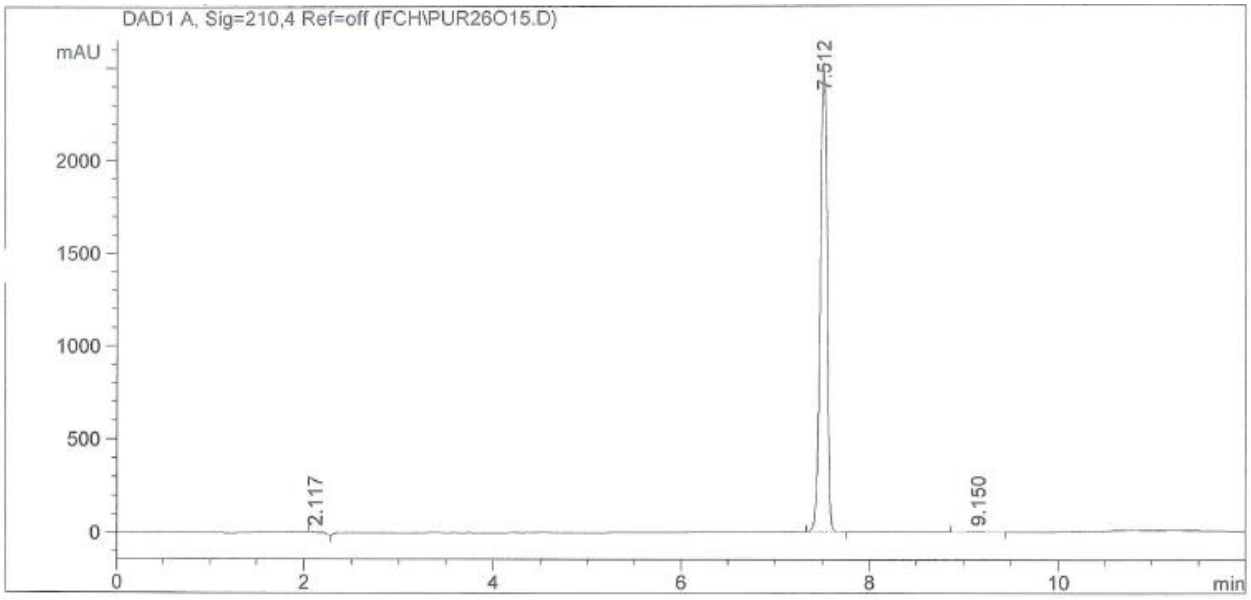
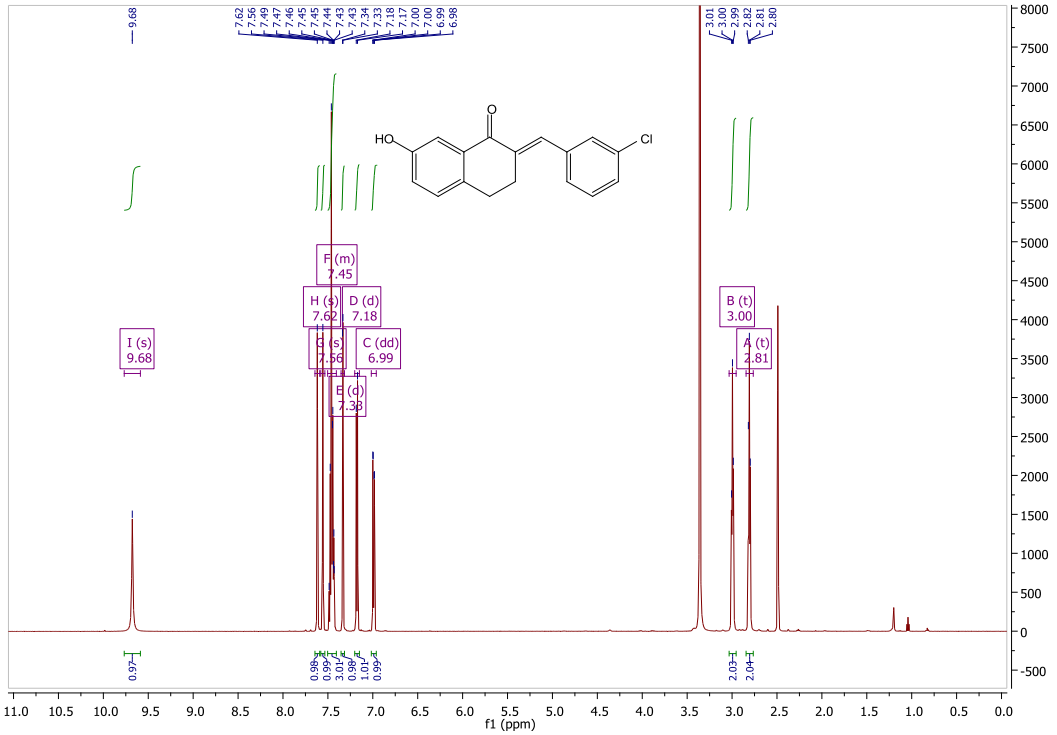
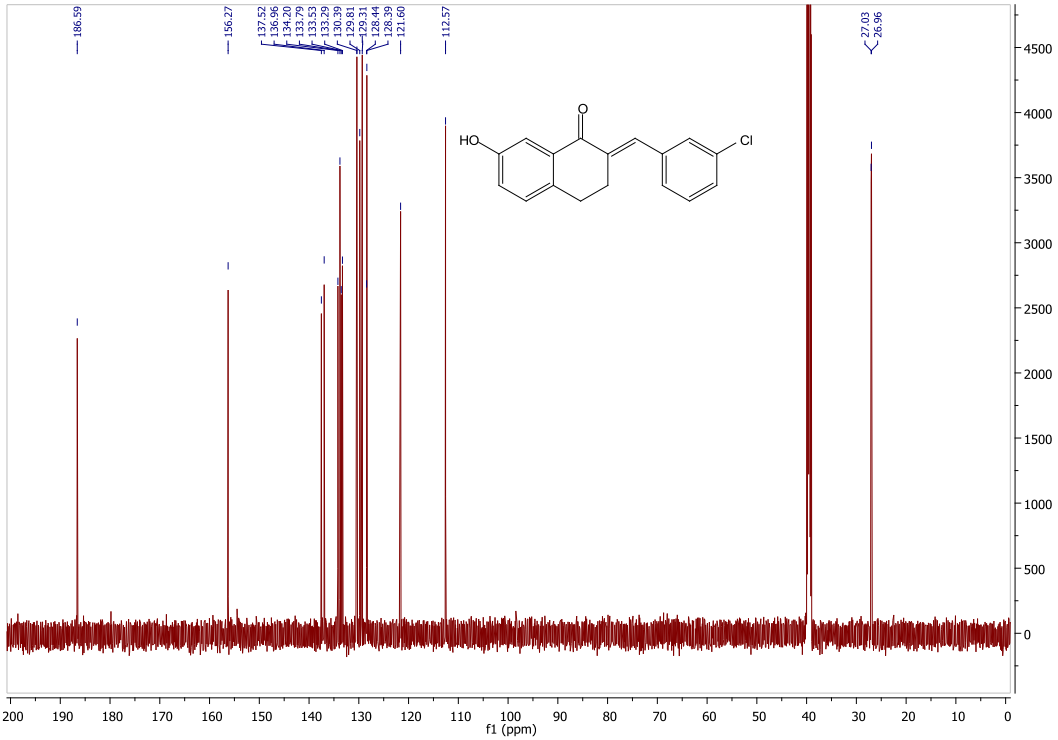


(2*E*)-2-(3-Chlorobenzylidene)-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2n)

^{1}H NMR



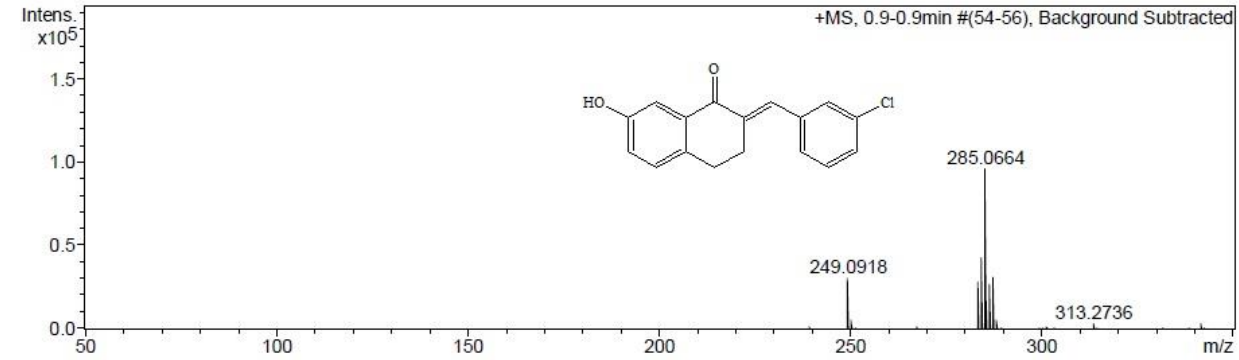
^{13}C NMR



HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste



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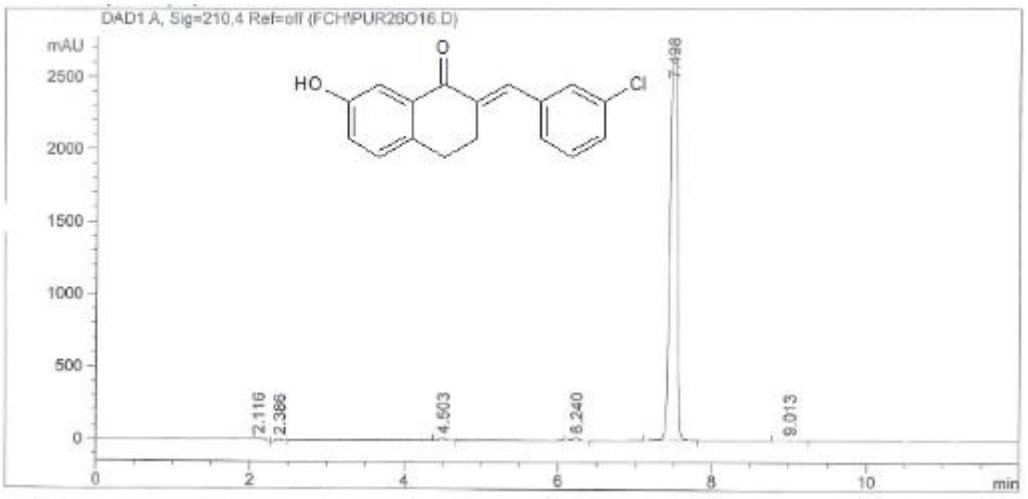
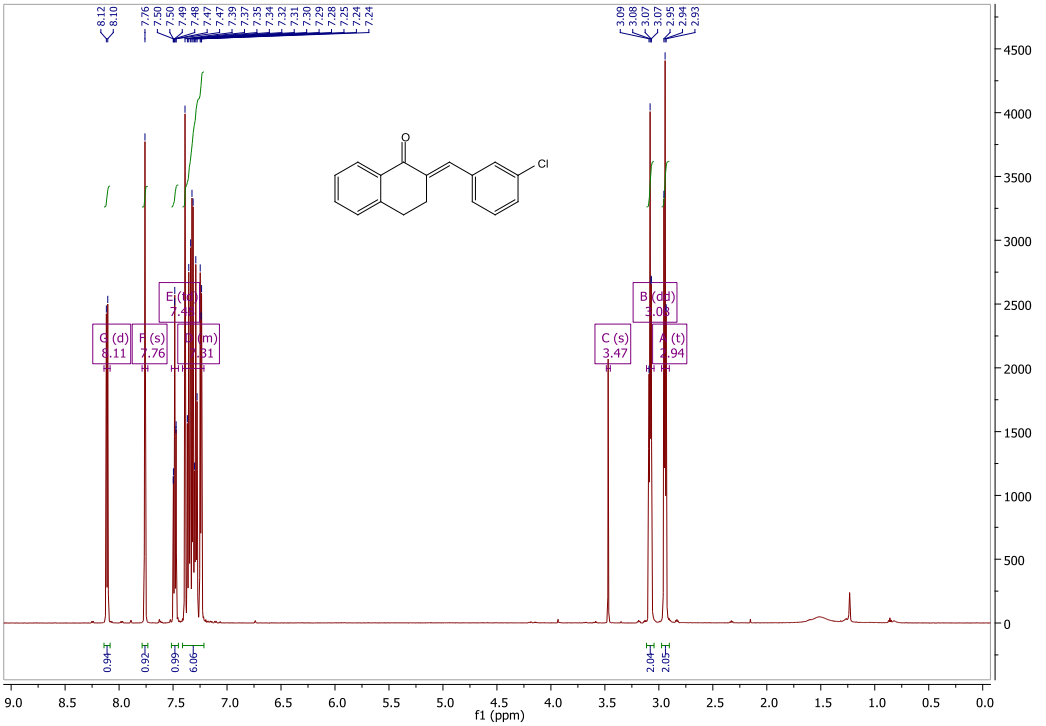
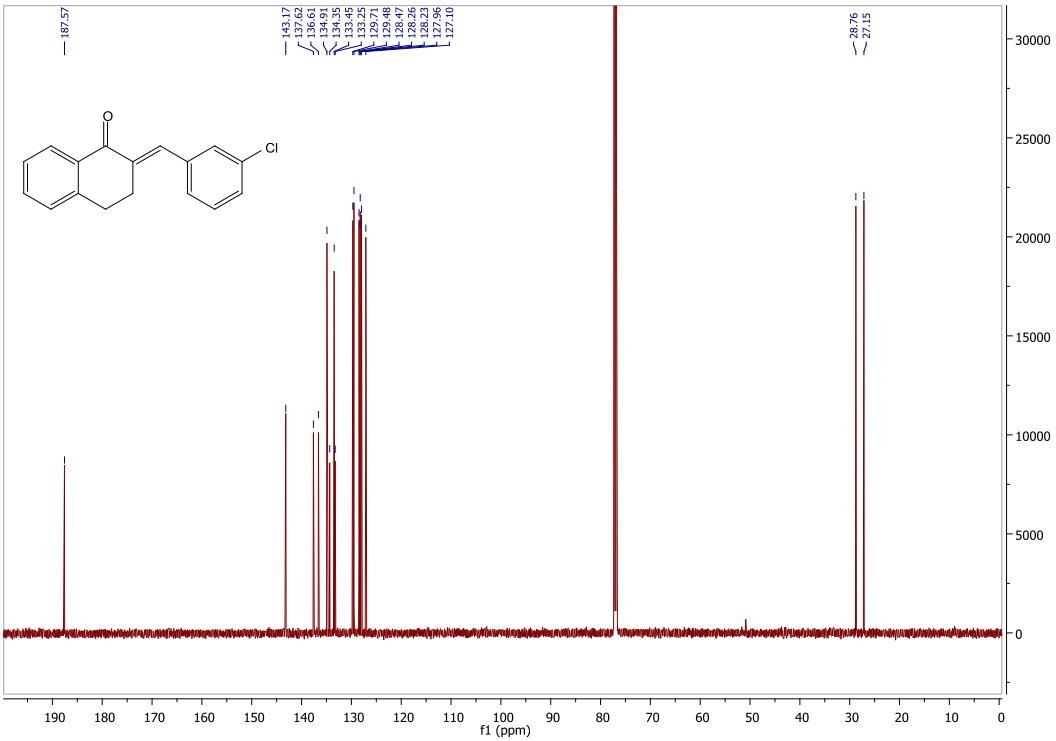


(2*E*)-2-(3-Chlorobenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (2o)

^{1}H NMR



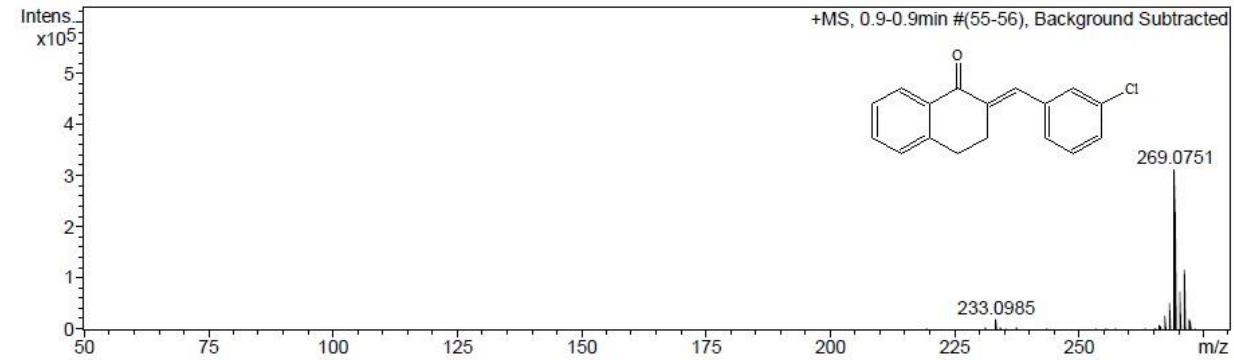
^{13}C NMR



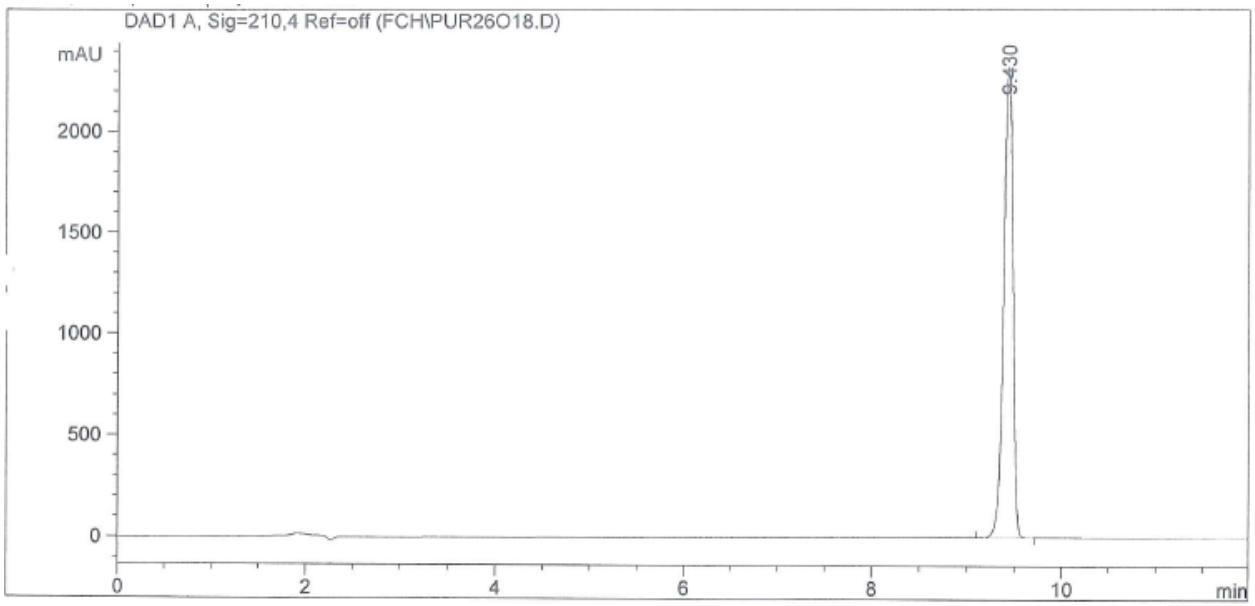
HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste

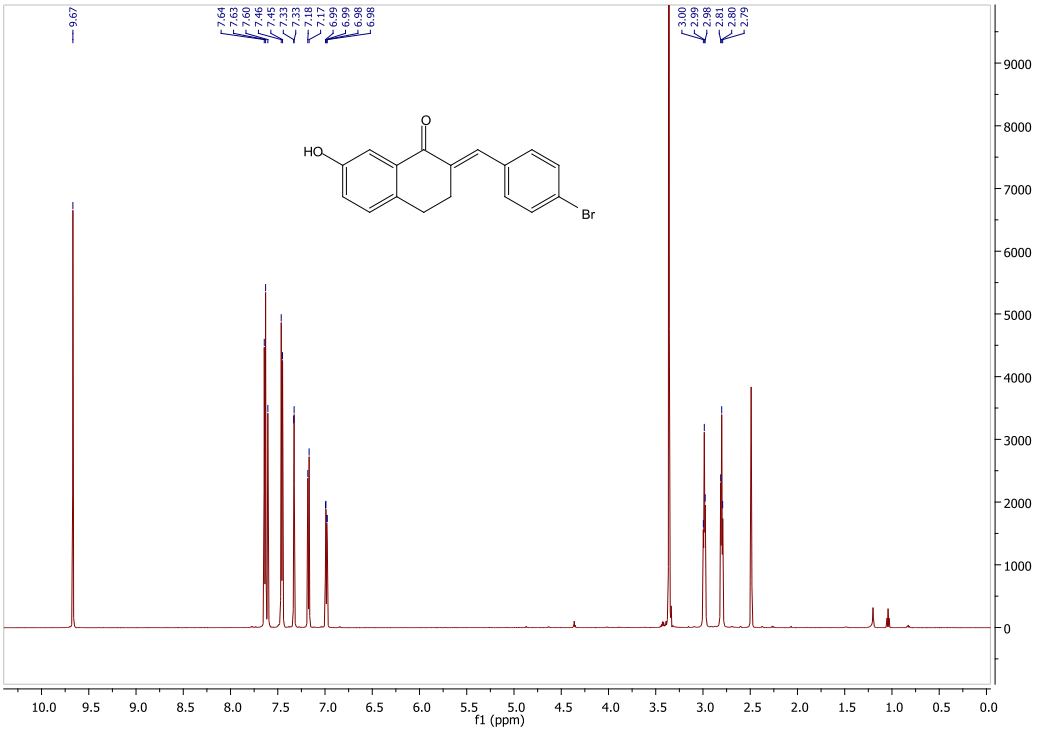


HPLC

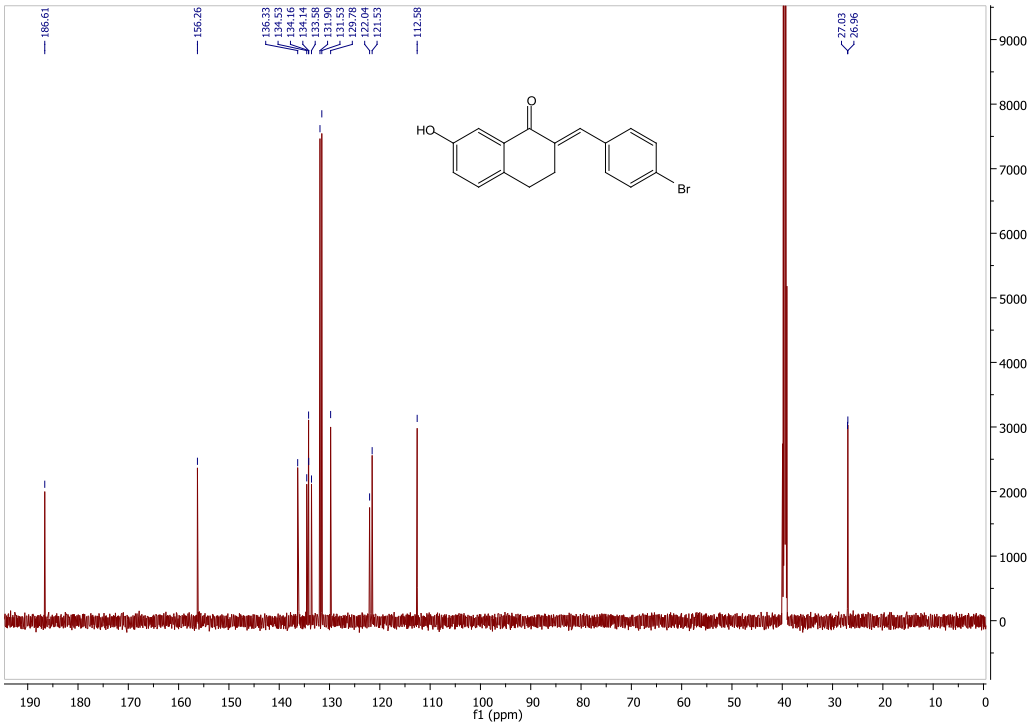


(2*E*)-2-(4-Bromobenzylidene)-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2p)

^{1}H NMR



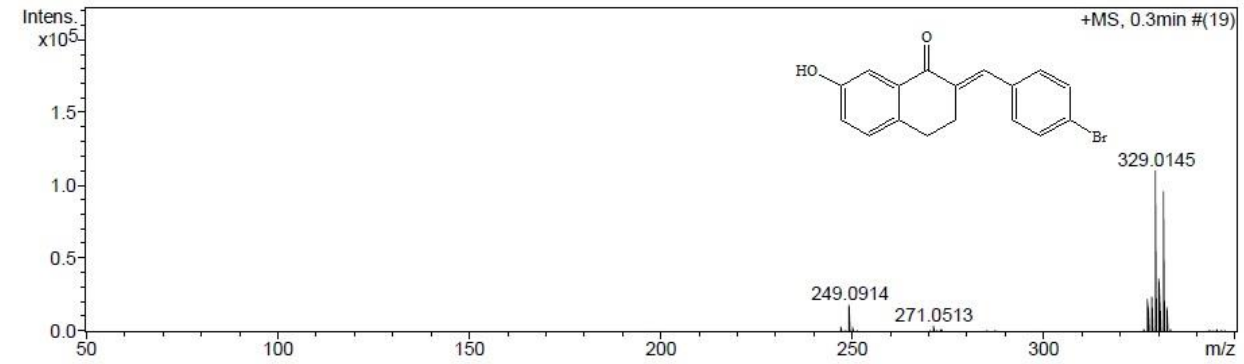
^{13}C NMR



HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste



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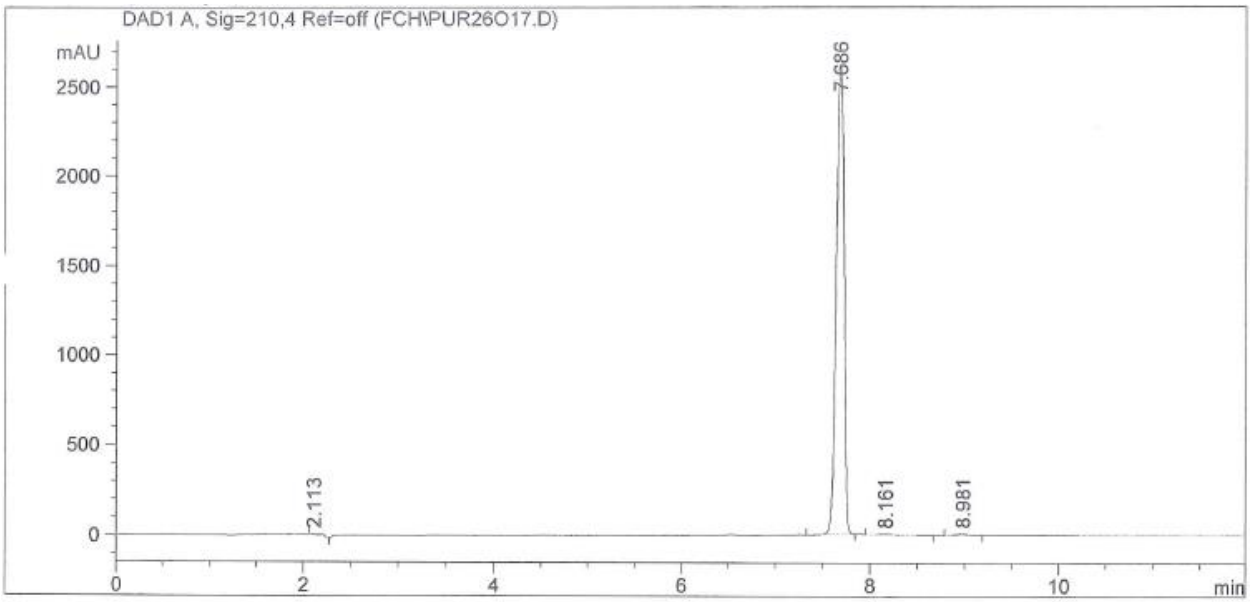
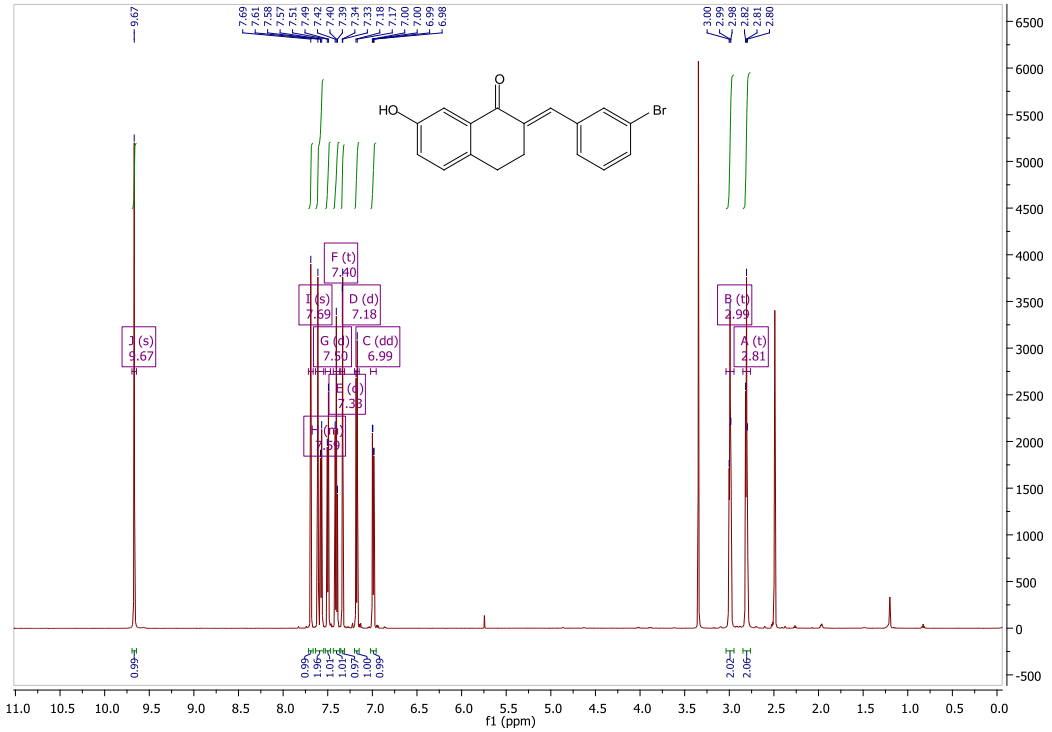
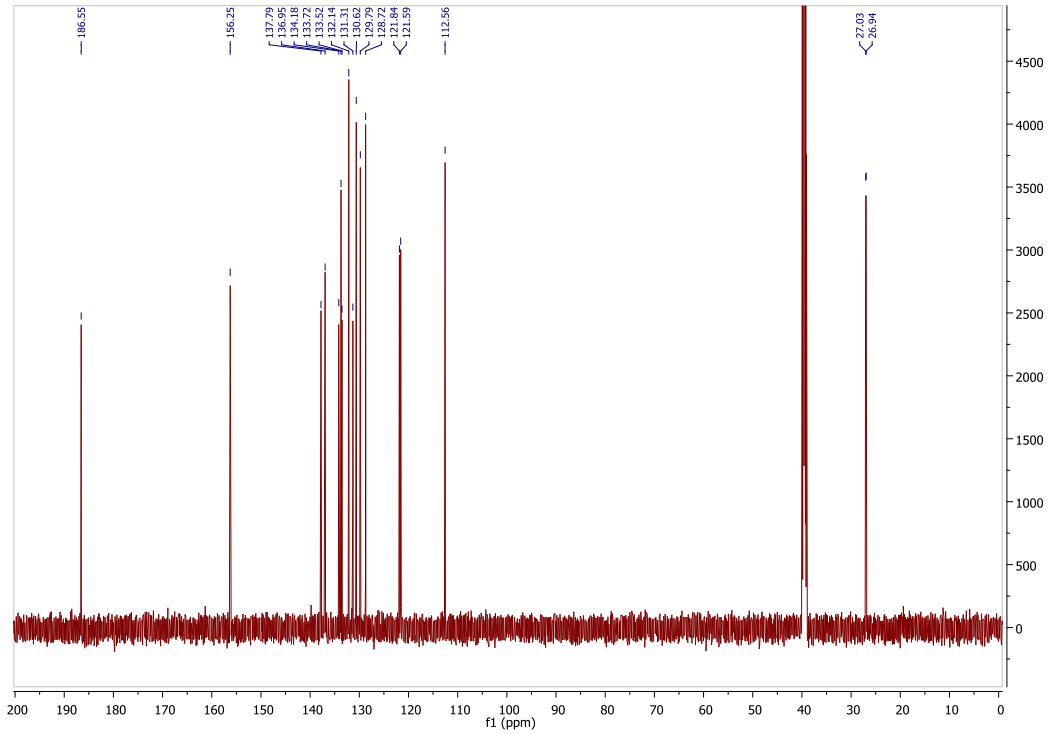


(2*E*)-2-(3-Bromobenzylidene)-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2q)

^{1}H NMR



^{13}C NMR



HRMS

Acquisition Parameter

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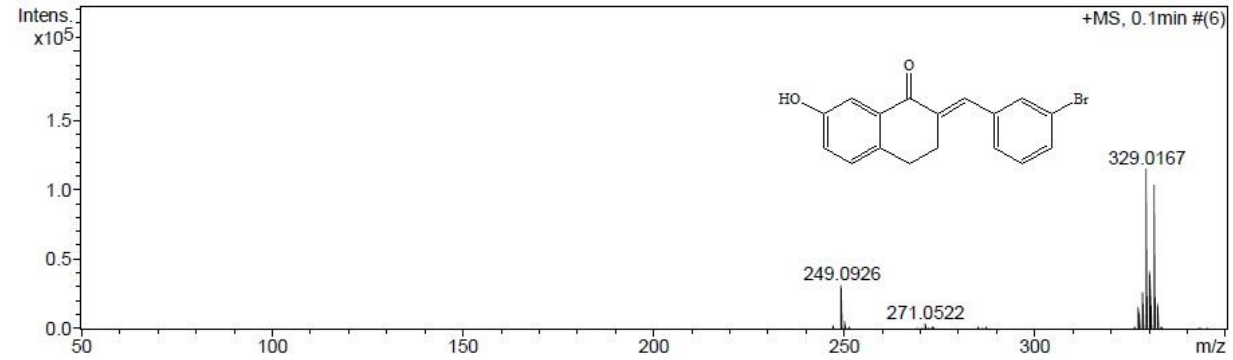
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste



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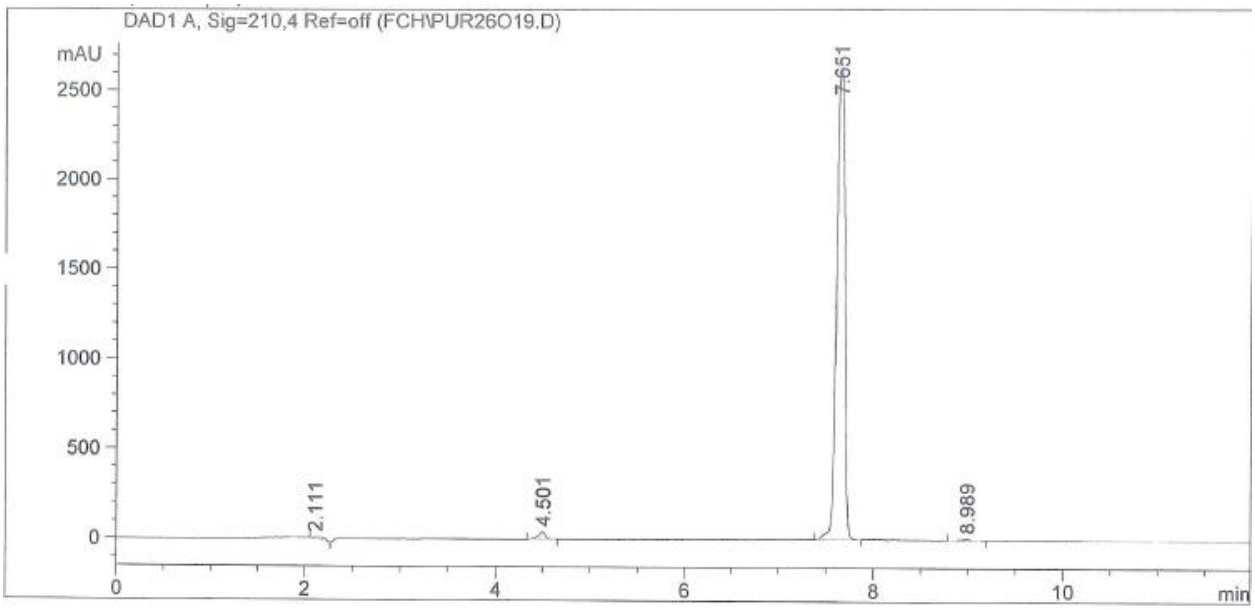
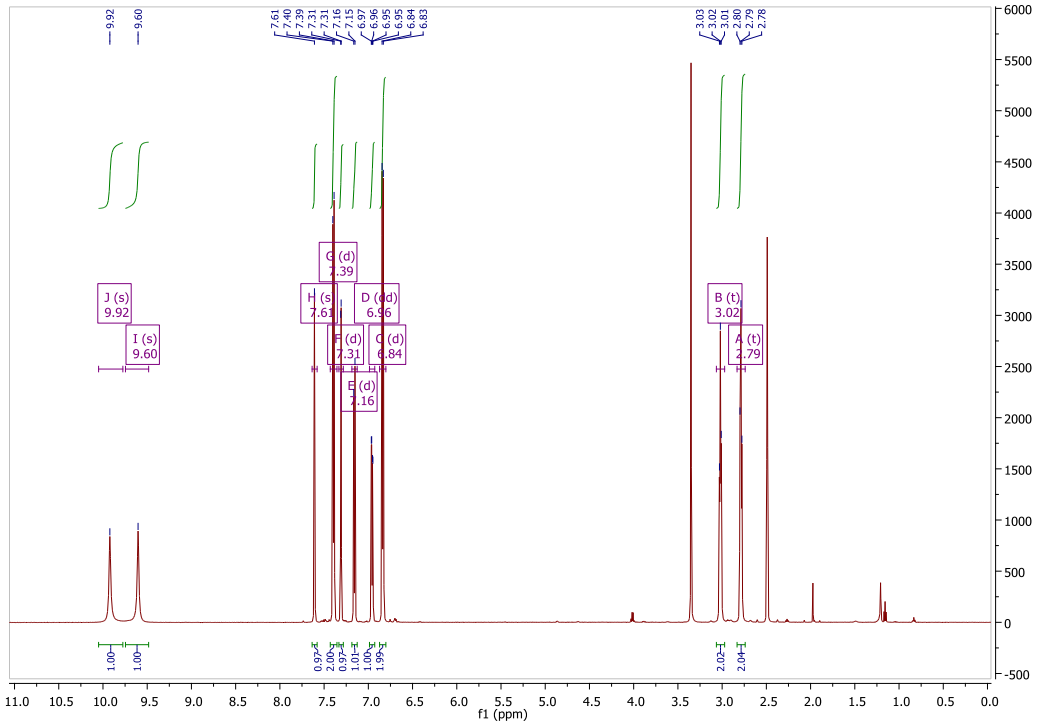
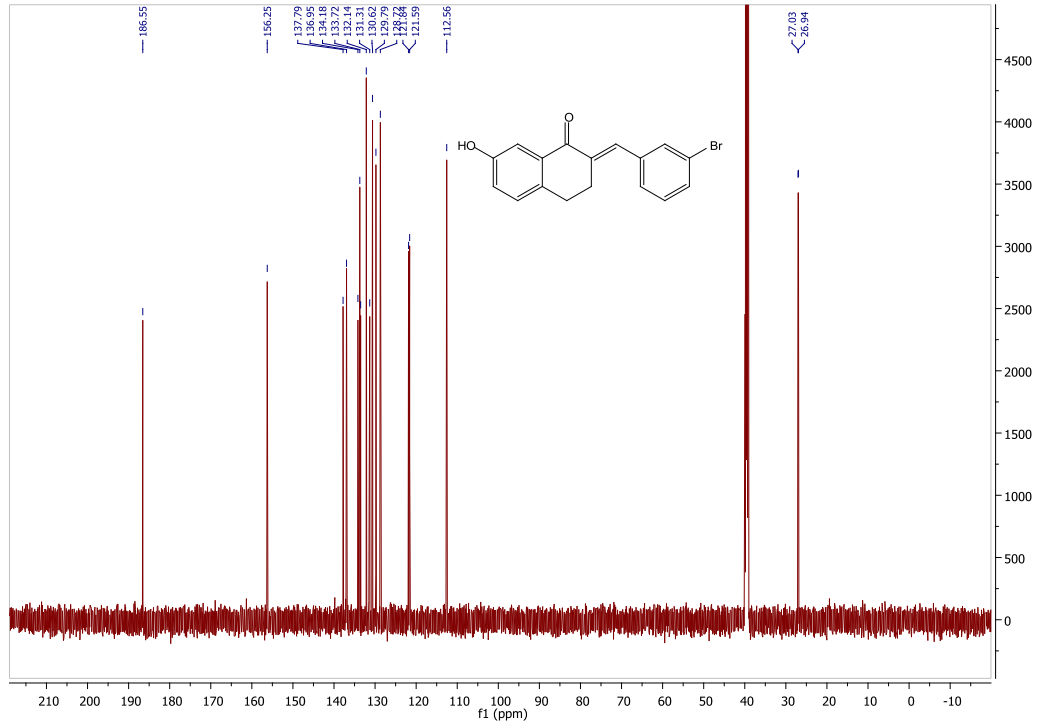


(2*E*)-7-Hydroxy-2-(4-hydroxybenzylidene)-3,4-dihydronaphthalen-1(2*H*)-on (2r)

^{1}H NMR



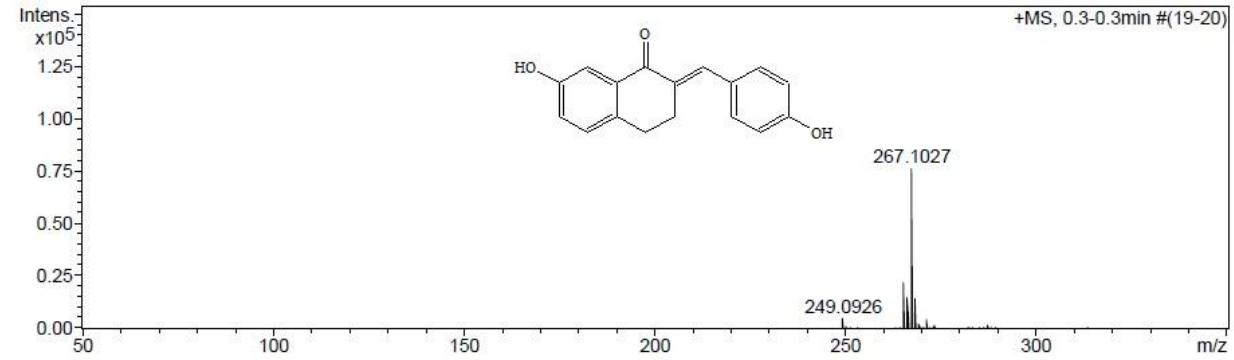
^{13}C NMR



HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste



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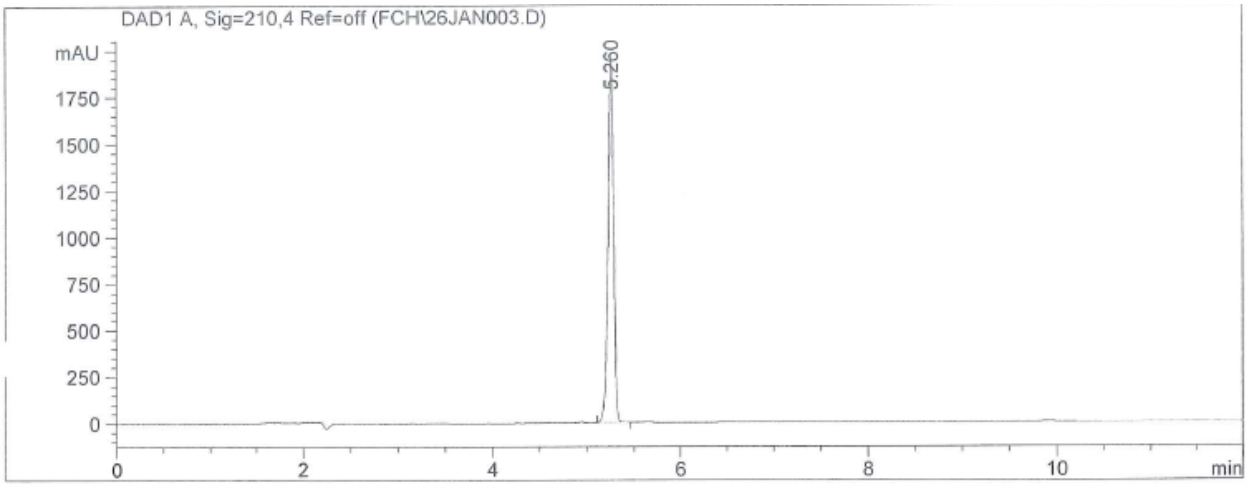
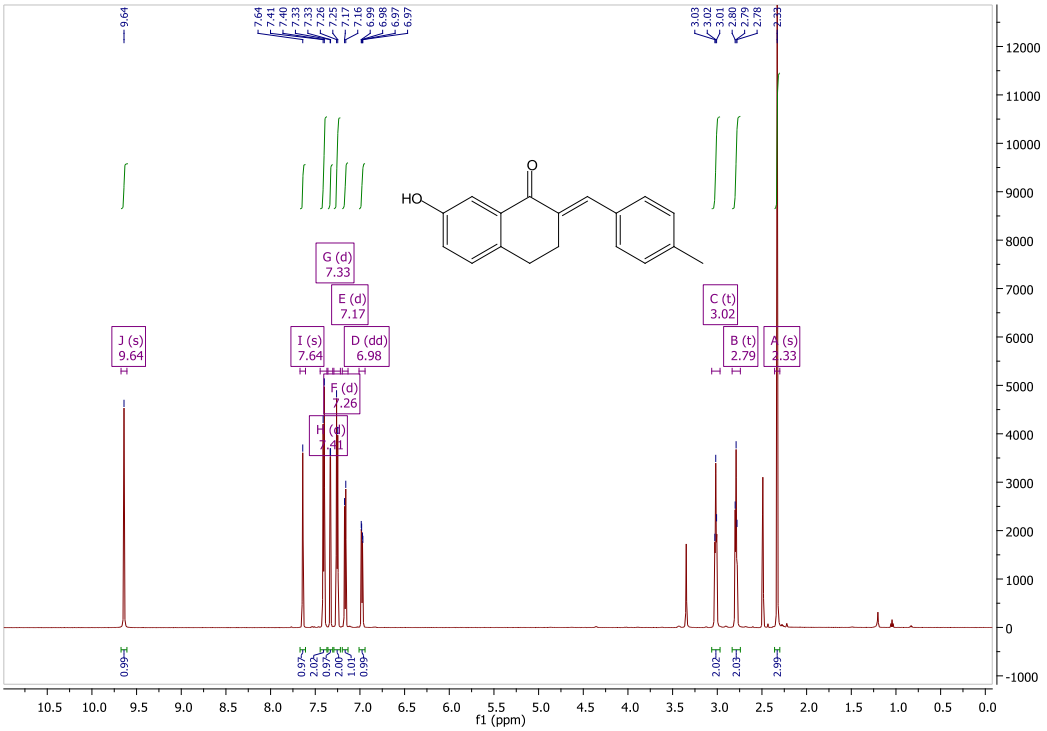
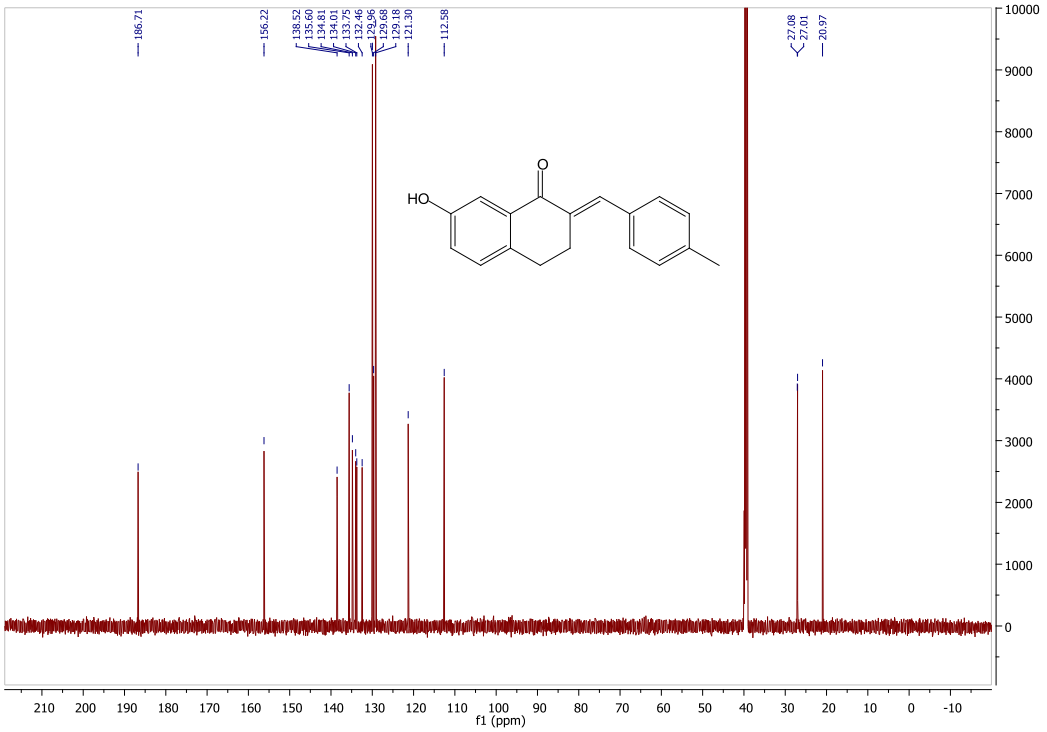


(2*E*)-7-Hydroxy-2-(4-methylbenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (2s)

^{1}H NMR



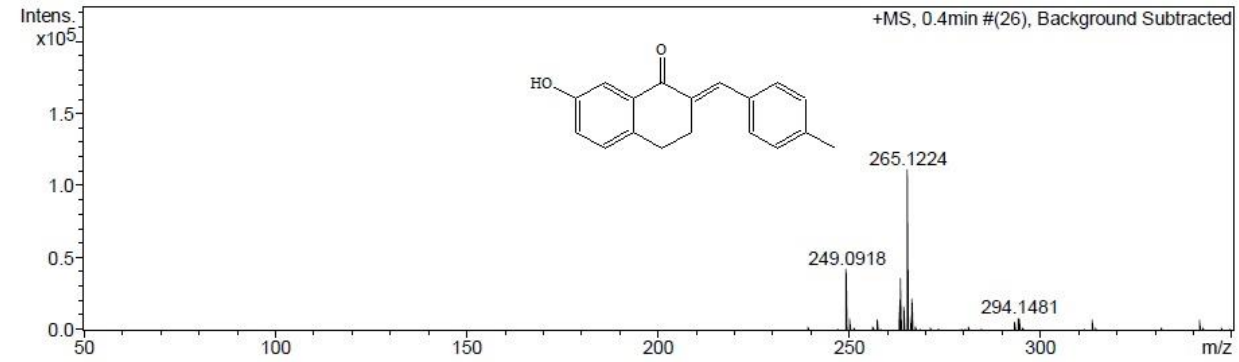
^{13}C NMR



HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste



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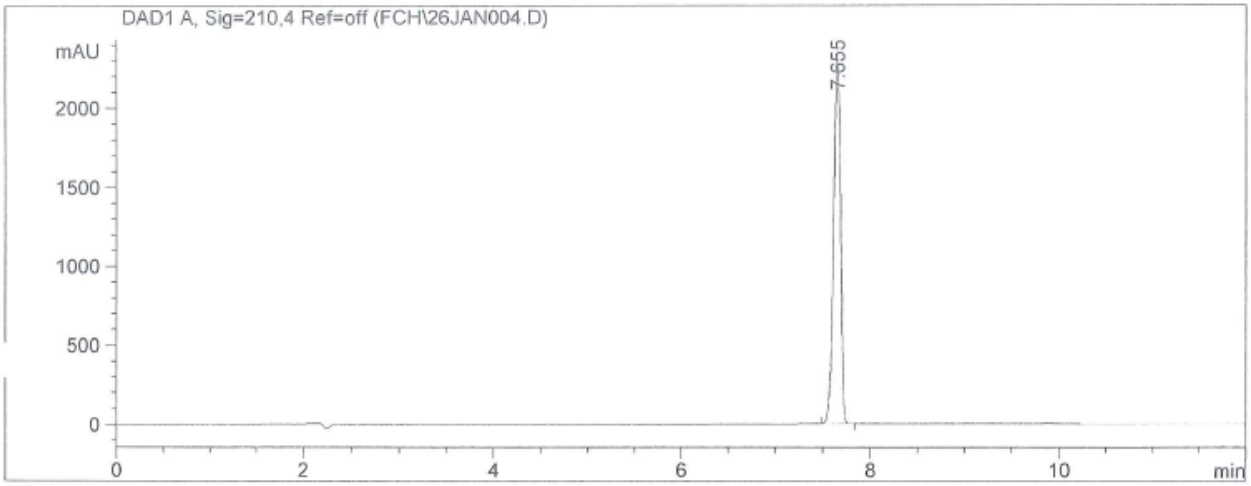
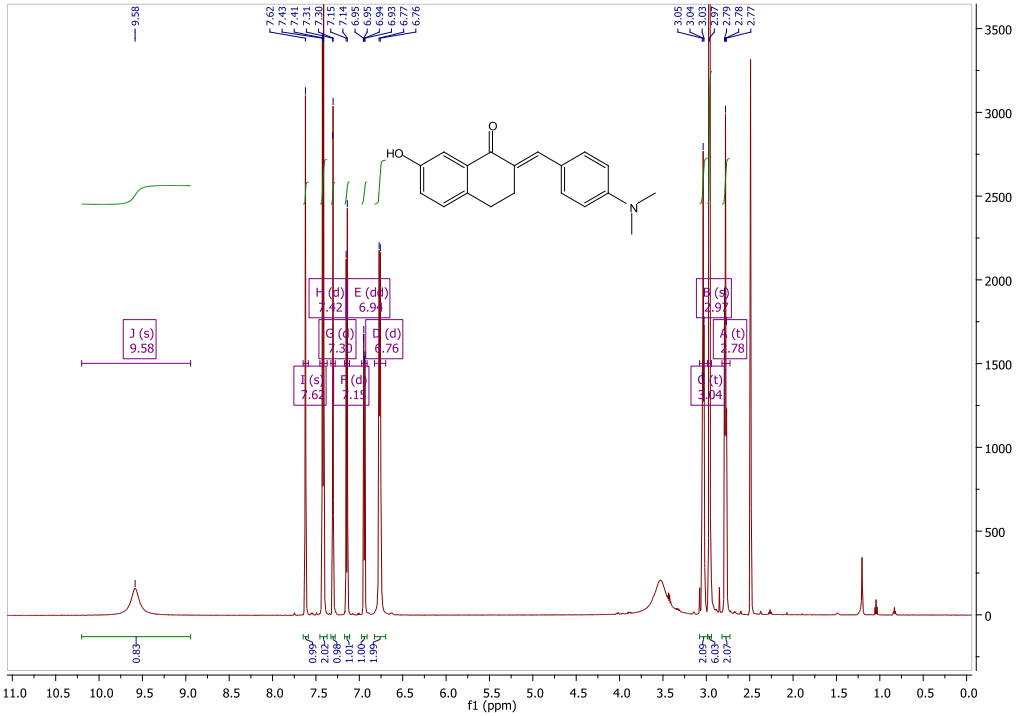
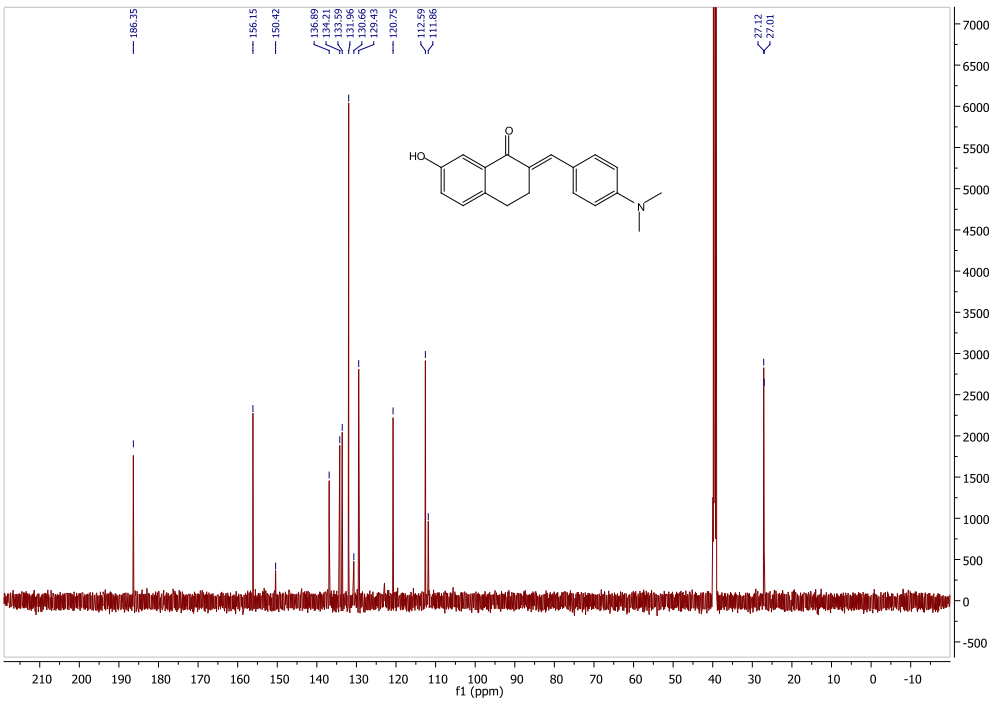


(2*E*)-2-[4-(Dimethylamino)benzylidene]-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2t)

^{1}H NMR



^{13}C NMR



HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
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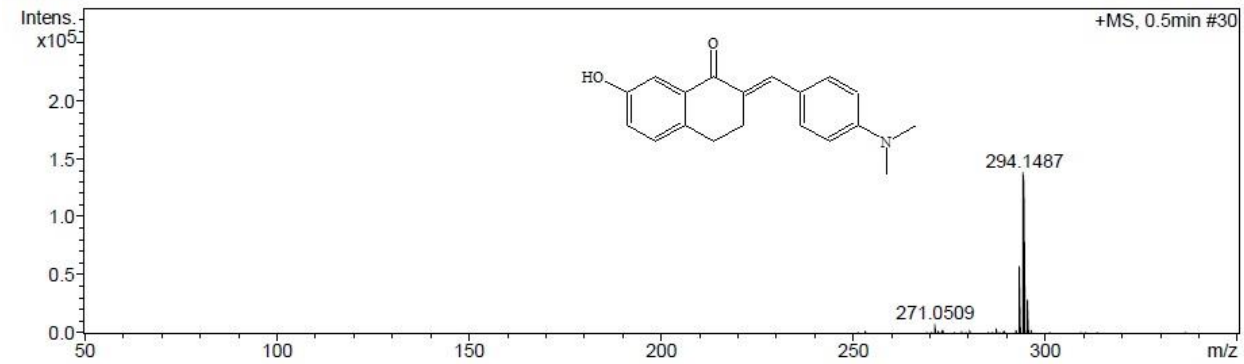

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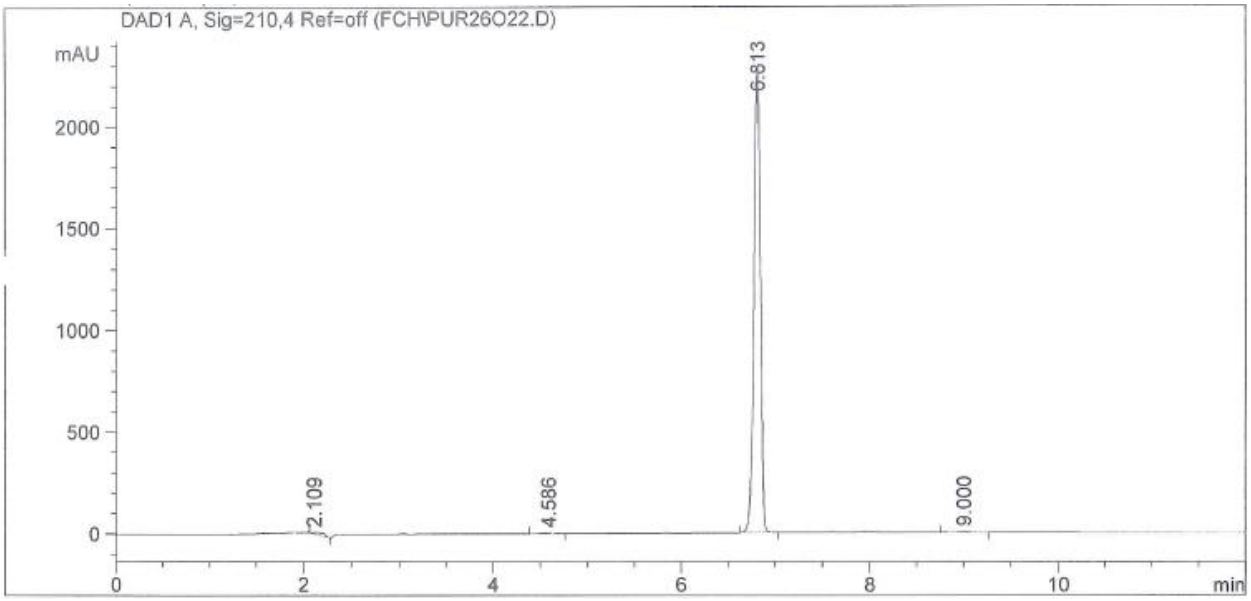
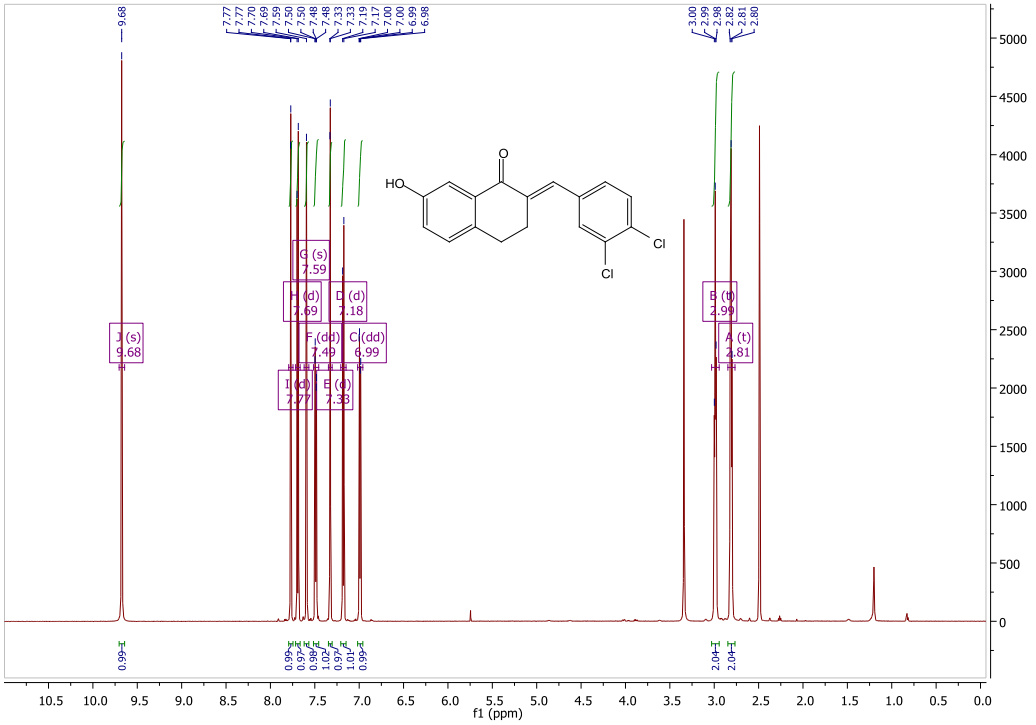
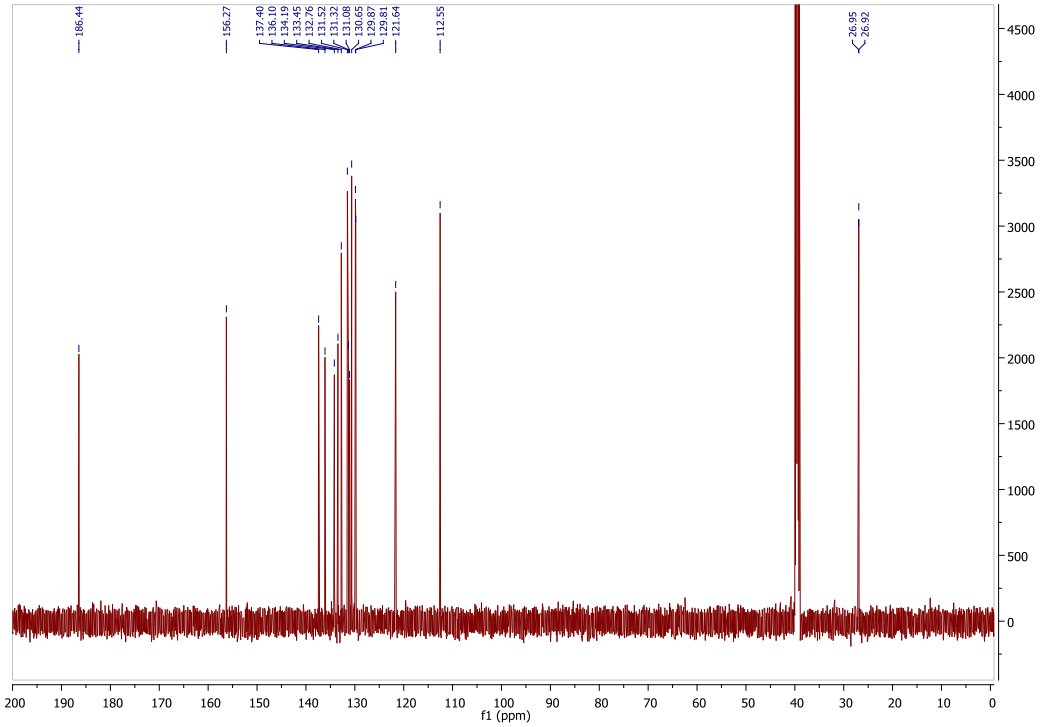


(2*E*)-2-(3,4-Dichlorobenzylidene)-7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one (2u)

^{1}H NMR



^{13}C NMR



HRMS

Acquisition Parameter

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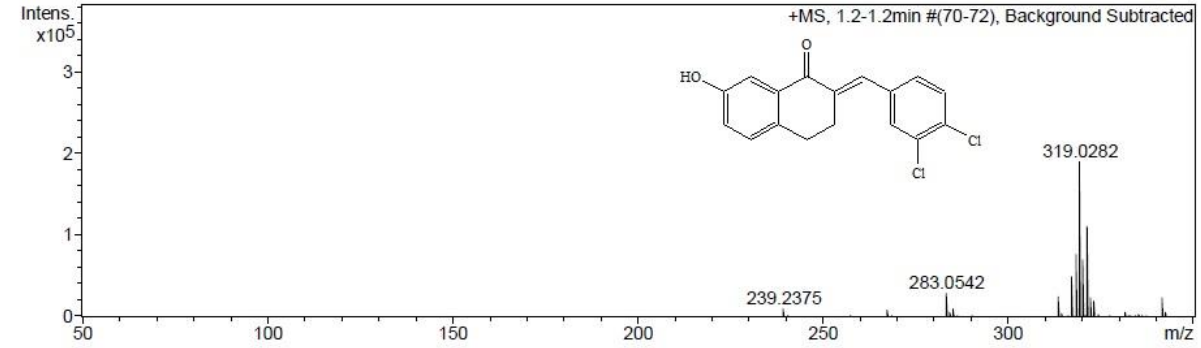

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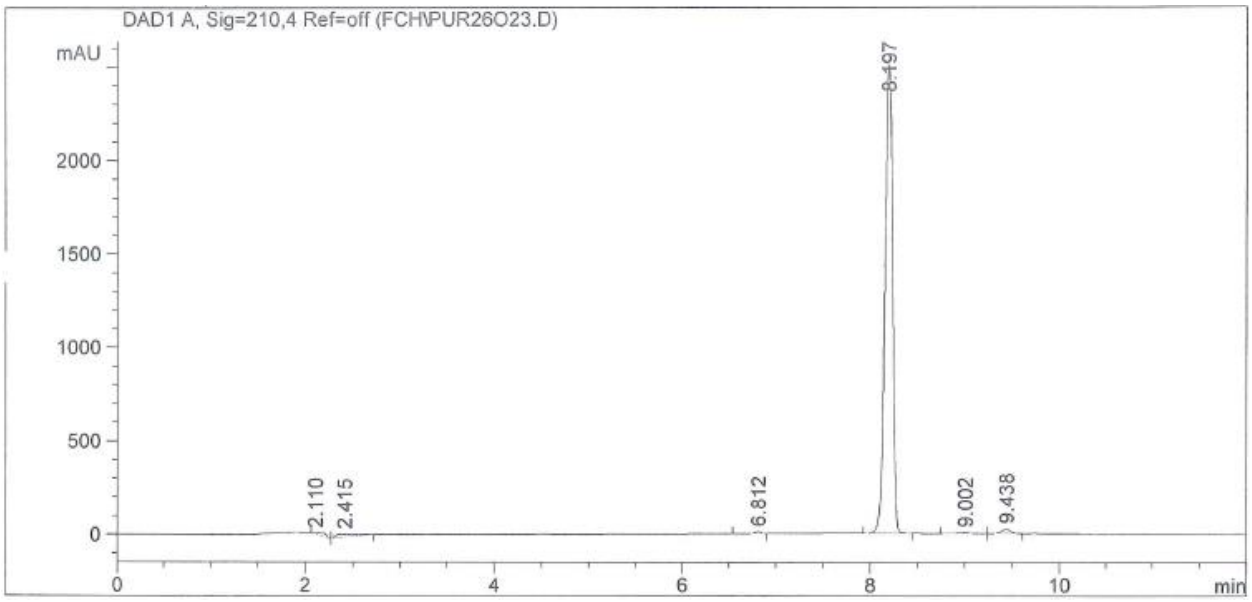
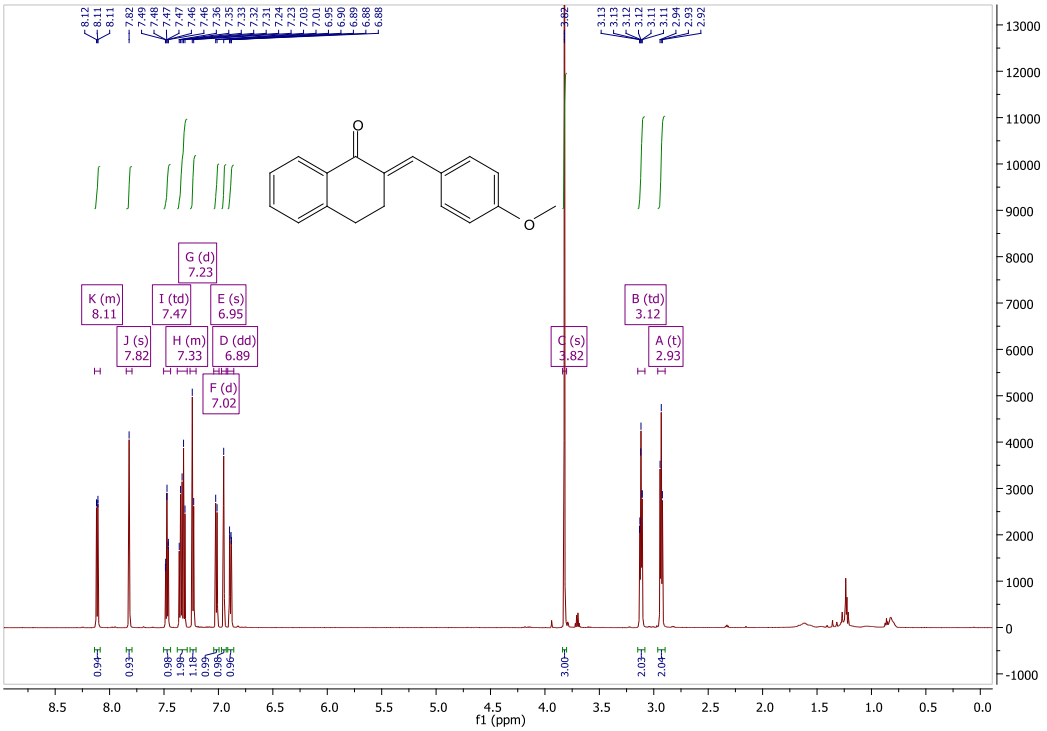
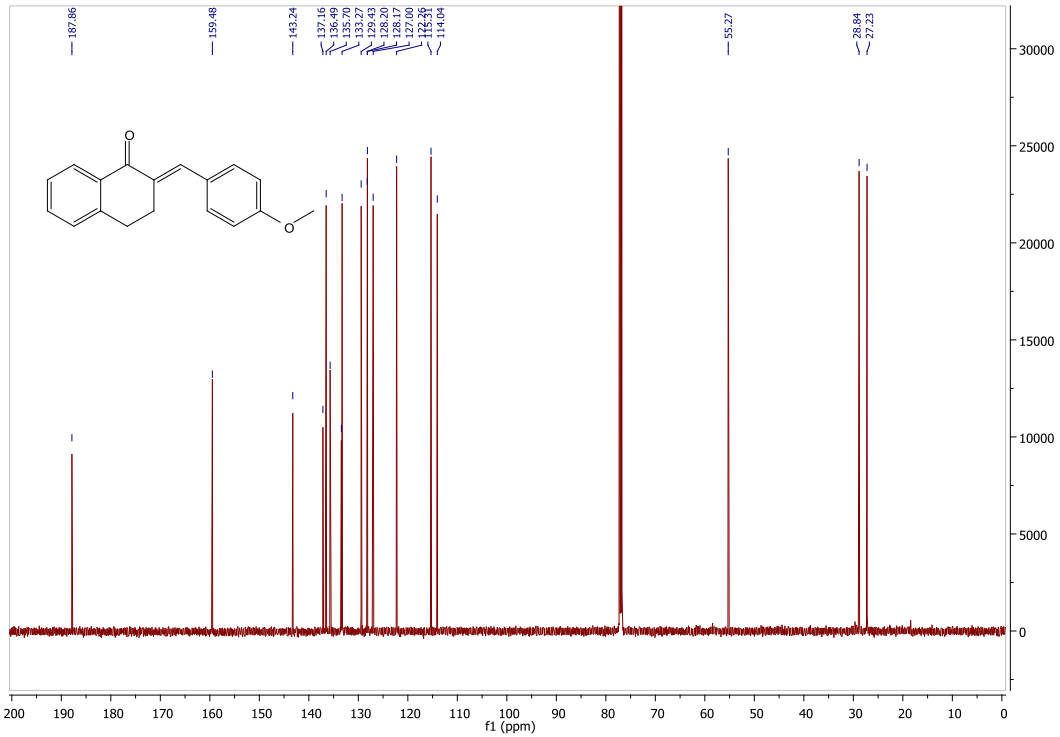


(2*E*)-2-(4-Methoxybenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (2v)

^{1}H NMR



^{13}C NMR



HRMS

Acquisition Parameter

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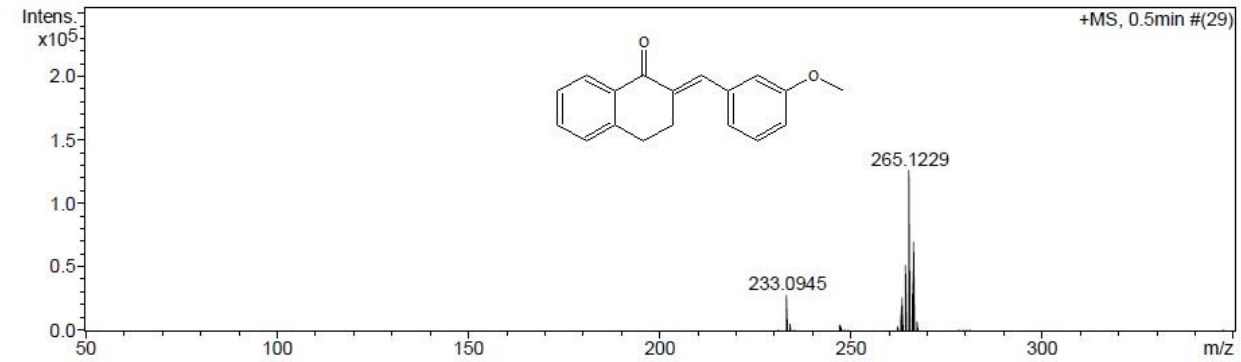
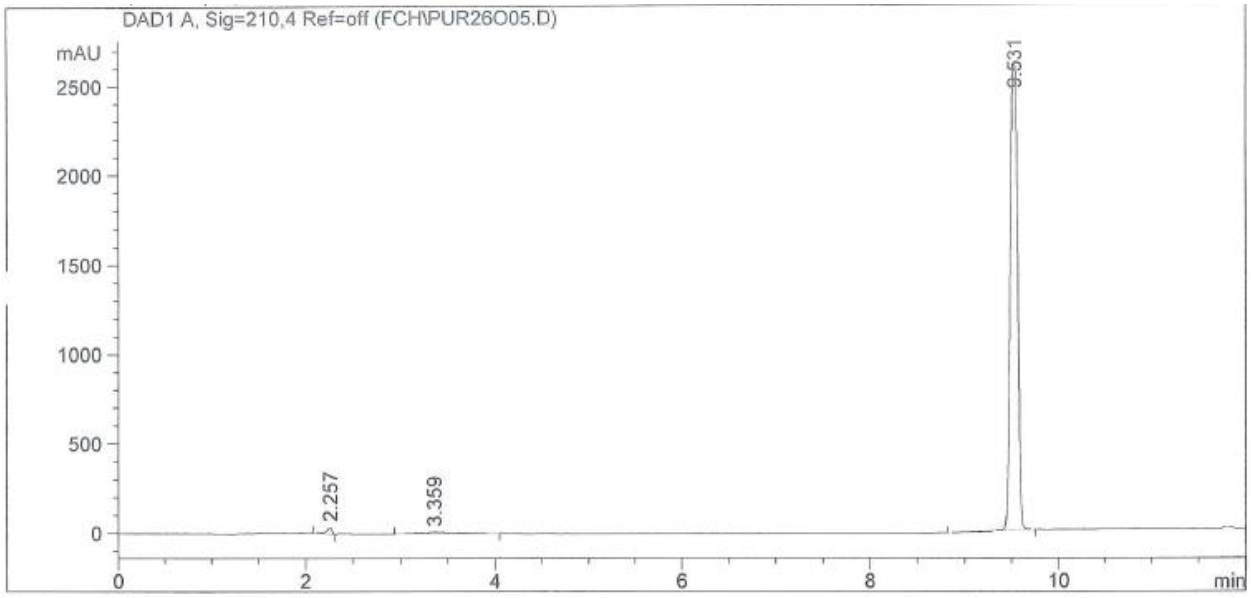
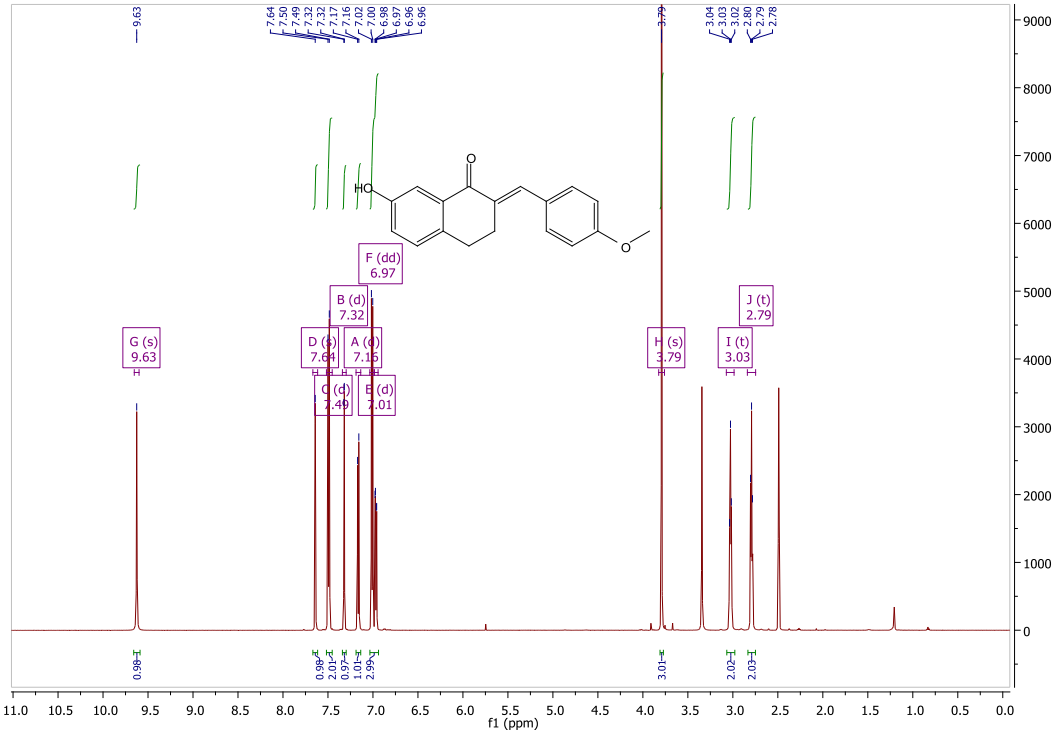


HPLC

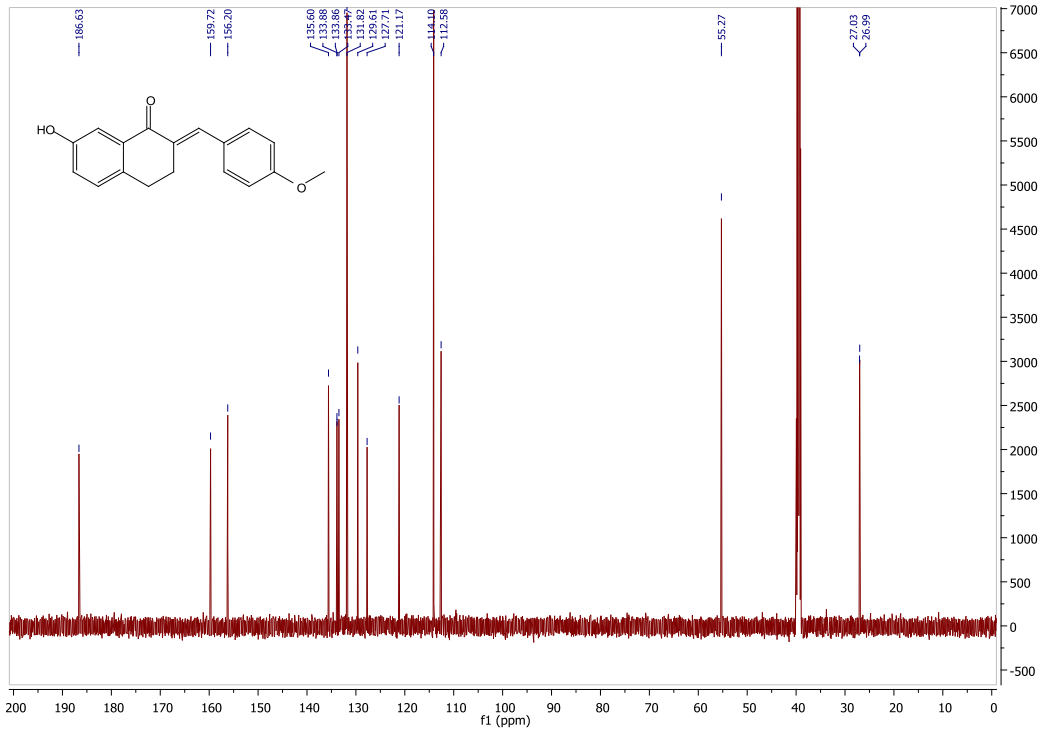


(2*E*)-7-Hydroxy-2-(4-methoxybenzylidene)-3,4-dihydronaphthalen-1(2*H*)-one (2w)

^{1}H NMR



^{13}C NMR



HRMS

Acquisition Parameter

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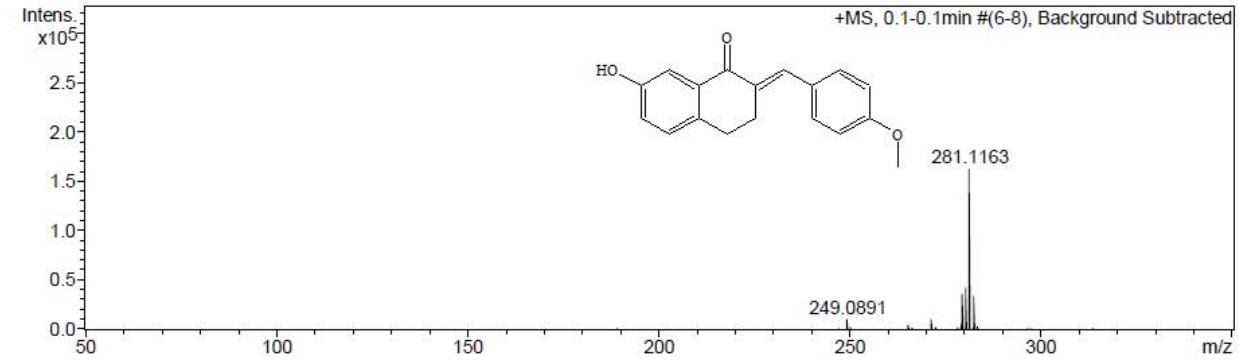

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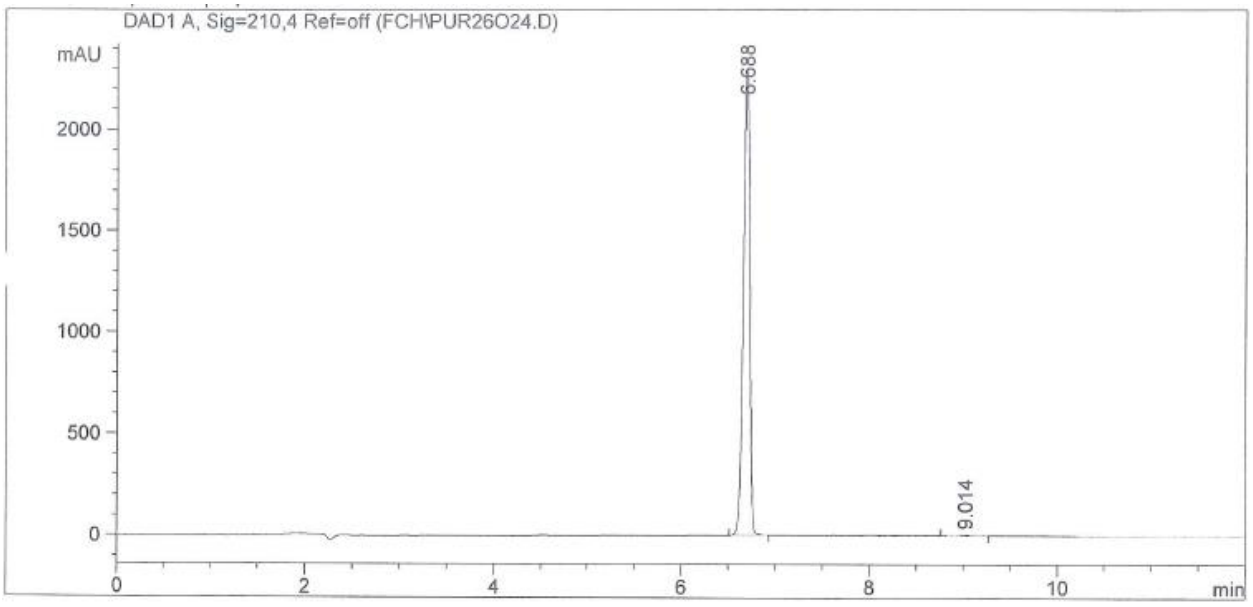

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CHAPTER 5: ARTICLE 3

Synthesis and *in vitro* evaluation of 2-heteroarylidene-1-tetralone derivatives as monoamine oxidase inhibitors

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ABSTRACT

The present study investigates the human monoamine oxidase (MAO) inhibition properties of a series of twelve 2-heteroarylidene-1-tetralone derivatives. Also included are the cyclohexylmethylidene cyclopentylmethylidene and benzylidene substituted 1-tetralones. These compounds are related to the 2-benzylidene-1-indanone class of compounds which has previously been shown to inhibit the MAOs, with specificity for the MAO-B isoform. The target compounds were synthesised by the Claisen-Schmidt condensation between 7-methoxy-1-tetralone or 1-tetralone and various aldehydes, employing KOH as base. The results of the MAO inhibition studies showed that the 2-heteroarylidene-1-tetralone derivatives are in most instances more selective inhibitors of the MAO-B isoform (compared to MAO-A). (2*E*)-2-(Cyclohexylmethylidene)-7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one (IC_{50} = 0.895 μM) was found to be the most potent MAO-B inhibitor, while the most potent MAO-A inhibitor was (2*E*)-2-[(2-chloropyridin-3-yl)methylidene]-7-methoxy-3,4-dihydronaphthalen-1(2*H*) (IC_{50} = 1.37 μM). The effect of the heteroaromatic substituent on MAO-B inhibition activity, in decreasing order was found to be: cyclohexyl, phenyl > thiophene > pyridine, furane, pyrrole, cyclopentyl. This study has identified 2-heteroarylidene-1-tetralone derivatives that may serve as lead compounds for the future design of MAO inhibitors, compounds that are used in the clinic as antiparkinsonian agents.

5.1 Introduction

The monoamine oxidases (MAOs) are enzymes responsible for the metabolism of monoamine neurotransmitters such as serotonin, norepinephrine and dopamine, thereby modulating their concentrations in the central and peripheral tissues. The MAOs exist as two isoforms, MAO-A and MAO-B, which are products of distinct genes. Although the amino acid sequences of the MAO isoforms are 70% identical, (Bach *et al.*, 1988) they differ significantly in their three-dimensional structures (Ferino *et al.*, 2013), tissue distributions (Grimsby *et al.*, 1990), inhibitor selectivities (Ma *et al.*, 2004) and substrate specificities (Shih *et al.*, 1999; Geha *et al.*, 2001). The MAOs also have different physiological roles. MAO-B is a key enzyme for the metabolism of dopamine in the human brain and represents a target for the development of drugs for the treatment of Parkinson's disease and Alzheimer's disease (Fernandez & Chen, 2007). Examples of MAO-B inhibitors that are used in the clinic for the treatment of Parkinson's disease are selegiline and rasagiline (Figure 5.1). MAO-A is a major serotonin metabolising

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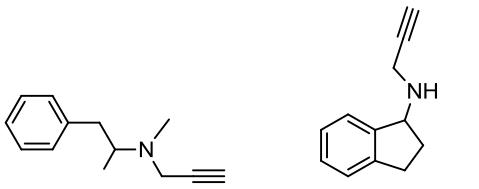
enzyme in the brain and is thus a drug target for the treatment of depression (Fowler *et al.*, 2002). MAO-A inhibitors that have been used for the treatment of depressive illness include tranylcypromine and moclobemide. An important consideration in MAO inhibitor design is the reversibility of inhibition. Irreversible MAO-A inhibitors are used with caution due to a potentially f;atal hypertensive event that may occur when these medications are combined with tyramine-containing food. This adverse effect is termed the “cheese reaction” and occurs when MAO-A is irreversibly inhibited. Since tyramine is metabolised by MAO-A in the gastrointestinal tract, irreversible inhibition of MAO-A leads to increased systemic concentrations of tyramine. Tyramine is a sympathomimetic amine and causes the release of noradrenaline from peripheral neurons leading to an increase in blood pressure. Reversible MAO-A inhibitors are considered to be safer in this regard and do not potentiate the sympathomimetic effects of tyramine. For the design of MAO inhibitors, a high degree of specificity for MAO-B and a reversible mode of MAO-A inhibition are therefore desirable characteristics.



Selegiline Rasagiline

Figure 5.1: Structures of selective MAO-B inhibitors in clinical use for the treatment of Parkinson’s disease.

Chalcones are *α,β*-unsaturated aromatic ketones that are essentially planar due to the presence of carbon atoms with sp^2 hybridization (de Oliveira *et al.*, 2012). Traditional medicinal use of chalcone-containing plants led to the discovery of their potential therapeutic applications (Devia *et al.*, 1999). Chalcones possess a broad spectrum of pharmacological activities including antimalarial, anticancer, anti-inflammatory, antifungal, antimicrobial, antioxidant and chemopreventive activities (Rahman, 2011; Mousavi *et al.*, 2014; Rozmer & Perjési, 2014). Of interest to us are reports that chalcones act as inhibitors of the MAO enzymes. For example, Chimenti and coworkers (2009) found that, with the appropriate substitution on the phenyl rings, synthetic chalcone derivatives may act as high potency inhibitors of the human MAOs. This is exemplified by chalcone (**1**), which inhibits human MAO-B with an IC_{50} value of 0.0044 µM (Figure.5.2) (Chimenti *et al*., 2009). This compound is highly specific for the MAO-B isoform and does inhibit MAO-A at concentrations up to 50 µM. Another study has recently investigated the human MAO inhibition properties of a series of furanochalcones and discovered potent MAO-B-selective inhibitors with the most active compound (**2**) exhibiting an IC_{50} value of 0.174 µM

(Robinson *et al.,*2013). Other heterocyclic chalcones have also been found to inhibit MAO-B potently and with high specificity as exemplified by **3**, which possesses an IC_{50} value of 0.067 µM (Minders *et al.*, 2015).

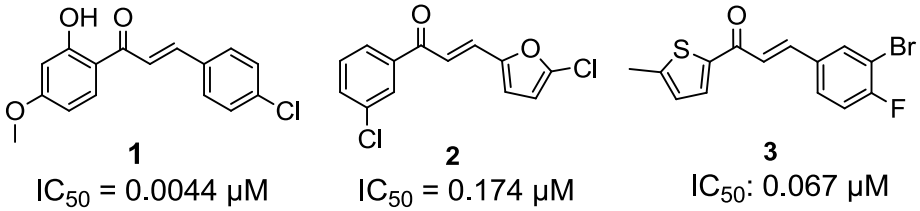


Figure.5.2: Some chalcone derivatives that inhibit the MAO-B enzymes.

The current study further explores the structure-activity relationships (SARs) for chalcones as MAO inhibitors by introducing conformational restriction and heteroaromatic substitution. This will be done by cyclising the structure of chalcone to yield a series of twelve 2-heteroarylidene-1-tetralone derivatives (**4a–l**) (Figure 5.3). These compounds are not only related to heterocyclic chalcones that have been reported to act as MAO-B specific inhibitors, but are also derivatives of the 2-benzylidene-1-indanone class of compounds which has previously been shown to inhibit the MAOs, also with specificity for the MAO-B isoform. This study will explore methoxy substitution on the tetralone phenyl ring (ring A) while a variety of heterocycles (pyridine, furan, pyrrole, thiophene) will be introduced as rings B. For comparison, the effect of non-heteroaromatic systems (cyclohexyl, cyclopentyl) and the phenyl ring as rings B will also be investigated.

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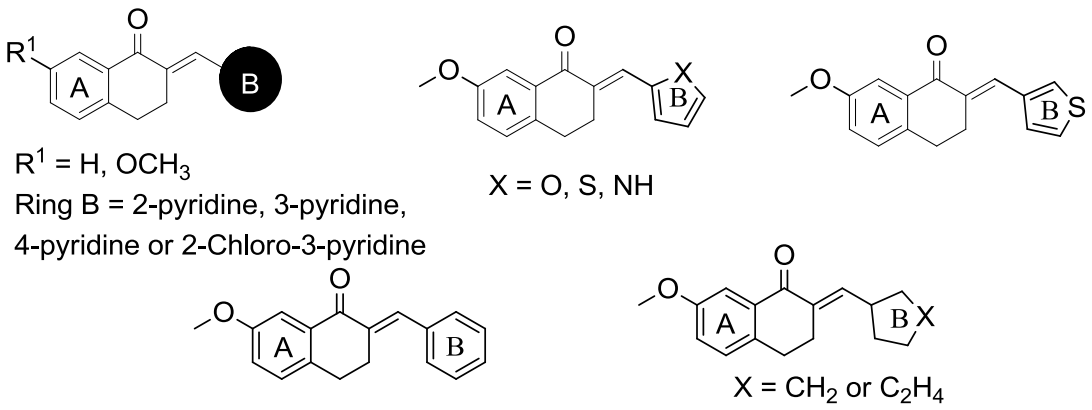


Figure 5.3: The 2-heteroarylidene-1-tetralone derivatives investigated in this study.

5.2 Results

5.2.1 Chemistry

The 2-heteroarylidene-1-tetralone derivatives were synthesised by the Claisen-Schmidt condensation between 1-tetralone (**5**) or 7-methoxy-1-tetralone (**6**) and various aldehydes (**7**) in reactions catalysed by KOH and HCl with methanol serving as solvent. The structures of the products were verified by ^{1}H NMR, ^{13}C NMR and mass spectrometry while the purities were estimated by HPLC. These data are cited in the supplementary information. On the ^{1}H NMR spectra of the 2-heteroarylidene-1-tetralone derivatives the anticipated signal of the vinylic proton of the *α,β*-unsaturated moiety was observed at approximately 7.6–8.0 ppm. Where appropriate the methoxy CH_3 group was evident as a sharp singlet in the region of δ 3.78–3.85 ppm. On the ^{13}C NMR spectra signals in the regions δ 185.6–188.2 ppm and δ 55.5–55.6 ppm represent the carbonyl and methoxy carbons, respectively.

5.2.2 MAO inhibition studies

For the inhibition studies, the catalytic activities of MAO-A and MAO-B were determined by measuring the production of 4-hydroquinoline (4-HQ) from the oxidation of kynuramine by the MAOs. 4-HQ is fluorescent in basic media and can thus be measured by fluorescence spectrophotometry at endpoint, after alkalinisation of the enzyme reactions. Recombinant human MAO-A and MAO-B served as enzyme sources (Novaroli *et al.,* 2005). By measuring MAO activities in the presence of the 2-heteroarylidene-1-tetralone derivatives, sigmoidal graphs (rate of enzyme catalysis versus logarithm of inhibitor concentration) were constructed with the Prism 5 software package (GraphPad) from which IC_{50} values were estimated.

The potencies by which the 2-heteroarylidene-1-tetralone derivatives inhibit MAO-A and MAO-B are expressed as the IC_{50} values and are given in Table.5-1. The inhibitors exhibit moderate inhibitory activities for MAO-A and MAO-B with the IC_{50} values mostly in the micromolar range. With the exception of **4g**, the 2-heteroarylidene-1-tetralone derivatives display little isoform selectivity. Compound **4g** is a MAO-A selective inhibitor and does not inhibit MAO-B at a maximal tested concentration of 100 µM. This compound also was the most potent MAO-A inhibitor of the series. The most potent MAO-B inhibitor of the series was the phenyl substituted derivative, (**4l)**. Although the IC_{50} values are for the most part spaced closely together, some preliminary SARs may be derived: (1) Among the derivatives evaluated, phenyl substitution (**4l**) is most optimal for MAO-B inhibition while the 2-chloro-3-pyridine moiety (**4g**) yields weakest MAO-B inhibition. 2-Chloro-3-pyridine and phenyl substitution, however, are most optimal for MAO-A inhibition; (2) The thiophene substituted derivatives (**4j**, **4k**) are more potent MAO-B

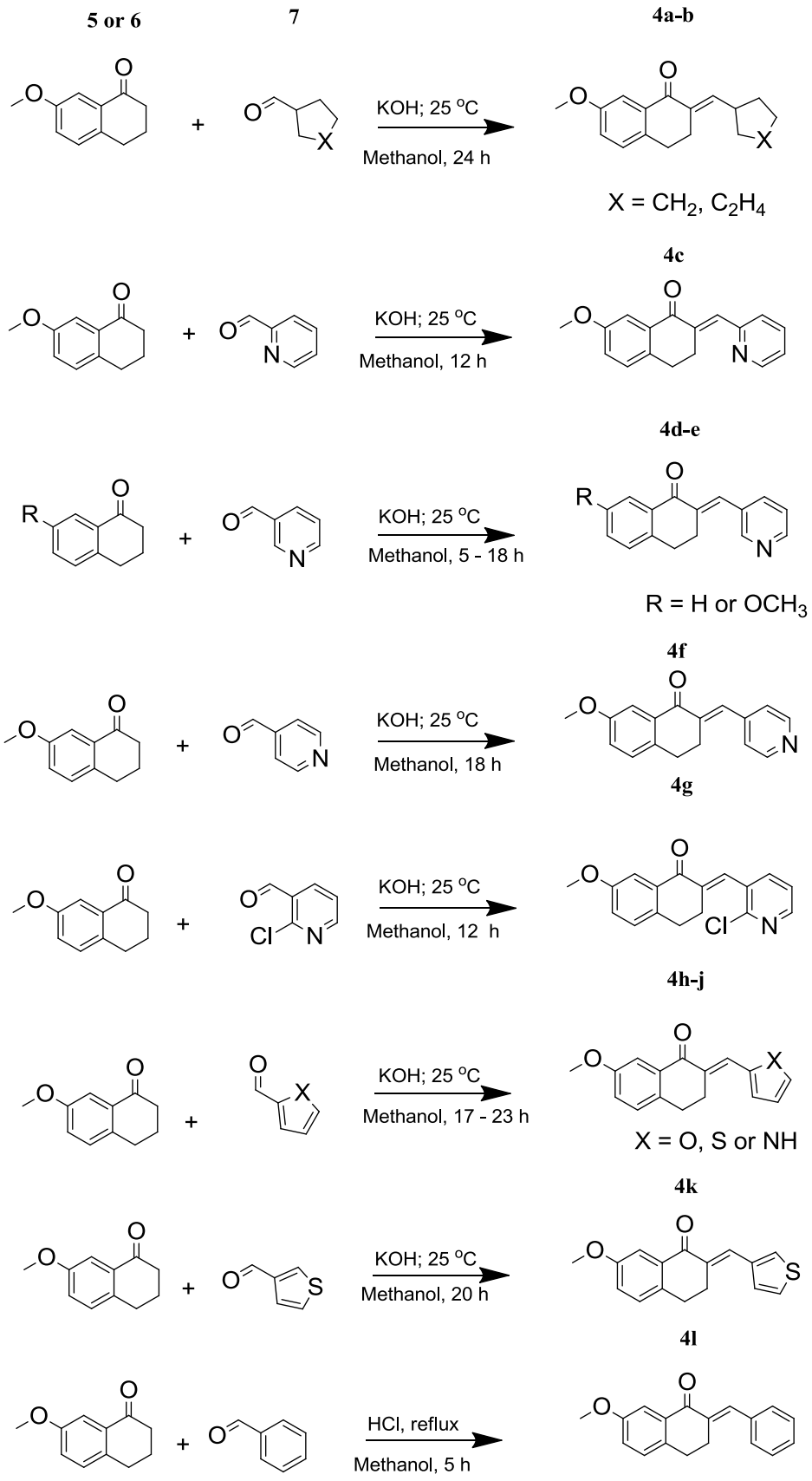


Figure 5.4: Synthetic route to the 2-heteroarylidene-1-tetralone derivatives.

inhibitors than the pyridine (**4c**–**g**) substituted compounds; (3) Substitution with the non-aromatic cyclohexyl ring (**4a**) also yields relatively potent MAO-B inhibition. Interestingly, the cyclopentane derivative (**4b**) is comparatively weaker as a MAO-B inhibitor; (5) In general the effect of substitution on MAO-B inhibition potency in decreasing order is: cyclohexyl, phenyl > thiophene > pyridine, furane, pyrrole, cyclopentyl; (6) Methoxy substitution on ring A (**4e**) yields more potent MAO inhibition compared to the unsubstituted homologue (**4d**).

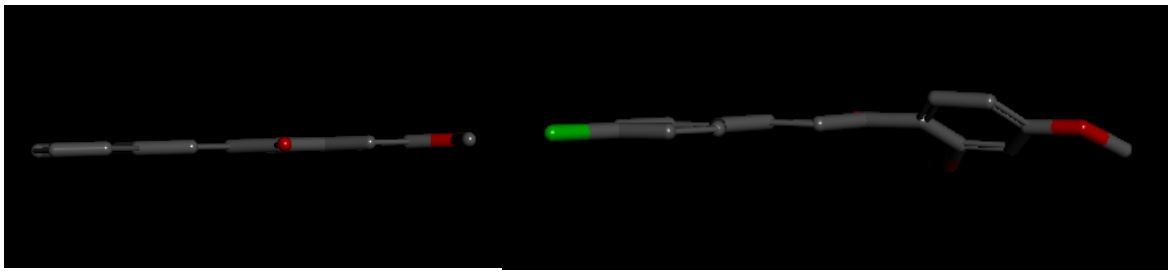
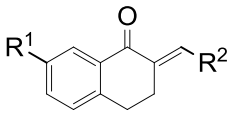
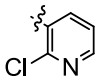


Figure 5.5: 3-Dimensional structures (MMFF94) of chalcone derivatives 4l (left) and 1 (right).

Table.5-1: IC_{50} values for the inhibition of human MAO-A and MAO-B by 2-heteroarylidene-1-tetralone derivatives.



Compound	cLogP[a]	MAO-A (IC_{50} µM ± SD)[b]	MAO-B (IC_{50} µM ± SD)[b]	SI[c]	R^1	(R^2)
4a	5.20 ± 0.84	4.59 ± 2.11	0.895 ± 0.066	5.1	7-OMe	
4b	4.63 ± 0.84	32.3 ± 4.85	5.46 ± 0.978	5.9	7-OMe	
4c	3.40 ± 0.86	8.85 ± 0.981	3.37 ± 0.580	2.6	7-OMe	
4d	3.61 ± 0.45	23.4 ± 0.055	6.26 ± 0.241	3.7	7-H	
4e	3.27 ± 0.84	8.22 ± 0.402	2.69 ± 0.373	3.1	7-OMe	
4f	3.52 ± 0.84	5.82 ± 0.541	6.04 ± 0.355	0.96	7-OMe	
4g	3.98 ± 0.85	1.37 ± 0.209	No Inhibition	-	7-OMe	
4h	4.39 ± 0.85	6.31 ± 0.349	4.53 ± 0.629	1.4	7-OMe	
4i	3.30 ± 0.86	14.5 ± 1.60	4.69 ± 0.245	3.1	7-OMe	
4j	4.58 ± 0.95	6.03 ± 0.062	1.75 ± 0.126	3.4	7-OMe	
4k	4.56 ± 0.95	3.86 ± 0.608	1.03 ± 0.144	3.7	7-OMe	
4l	3.57 ± 0.83	1.96 ± 0.064	0.707 ± 0.088	2.8	7-OMe	
Lazabemide	-	202[d]	0.091[d]			
Toloxatone	-	3.92[d]	-			

[a]Calculated by Chemsketch. [b]All values are reported as mean ± standard deviation (SD) of triplicate experiments. [c]Selectivity index (SI) = IC_{50}(MAO -A)/IC_{50}(MAO-B). [d]Inhibition values for the reference inhibitors were obtained from literature (Petzer *et al.*, 2013).

5.3 Discussion

The chalcone class of compounds is well known for inhibitors of the MAOs, with specificity for the MAO-B isoform. This is exemplified by the study of Chimenti *et al.* (2009) in which a series of chalcone derivatives substituted on both phenyl rings exhibited very good specificity for MAO-B with potencies in the micro to nanomolar range. The most potent MAO-B inhibitor, compound (**1**) (IC_{50} = 0.0044 μM) is considerably better than the derivatives of the current study. Furanochalcones (such as (**2**), IC_{50} = 0.174 μM) and other heterocyclic chalcones (such as (**3**), IC_{50} = 0.067 μM) (Robinson et al., 2013; Minders et al., 2015) also are more potent MAO-B inhibitors than the 2-heteroarylidene-1-tetralone derivatives of this study. This suggests that introducing conformational restriction and heteroaromatic substitution such as with derivatives (**4**) do not improve MAO inhibition compared to open chain chalcones and heterocyclic chalcones. Although the molecular basis for this behaviour is not clear, it may be suggested that conformational freedom and rotation of the *α,β*-unsaturated ketone moiety of chalcone, although limited, is important for establishing productive interactions with the MAO-B active site. Alternatively, the 2-heteroarylidene-1-tetralone derivatives "freezes" the structures in a conformation that is not optimal for MAO-B binding compared to the open chain chalcones. To illustrate this, the calculated three-dimensional structures (MMFF94) of chalcone derivative (**1**) and a 2-heteroarylidene-1-tetralone derivative (**4l**) are shown in (Figure 5.4). As shown (**1**) significantly deviates from planarity compared to chalcone (**4l**), which is frozen in a coplanar conformation. The flexibility of (**1**) compared to (**4l**) may, in part, explain its ability to better bind to the MAO-B active site.

5.4 Experimental section

5.4.1 Chemicals and instrumentation

All reagents and solvents were from Sigma-Aldrich and were used without further purification. Column chromatography was carried out with silica gel 60 (Fluka, particle size 0.063–0.200 mm) while thin-layer chromatography (TLC) was performed on 0.20 mm thick aluminium sheets coated with silica gel 60 (Macherey-Nagel). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III 600 spectrophotometer at 600 MHz and 151 MHz, respectively, and $CDCl_3$ or DMSO-*d6* served as NMR solvent. Chemical shifts are reported in parts per million (*∂*) and were referenced to the residual solvent signal ($CDCl_3$: 7.26 and 77.16 ppm for ^{1}H and ^{13}C, respectively; DMSO-*d6*: 2.50 and 39.52 ppm for ^{1}H and ^{13}C, respectively). Spin multiplicities are given as s (singlet), d (doublet), t (triplet), dd (doublet of doublets), dt (doublet of triplets), tt (triplet of triplets), q (quartet), qn (quintet), m (multiplet) or brs (broad singlet). Melting points (mp) were determined on a Buchi B-545 melting point apparatus and were not corrected.

Chemical purity was determined by high performance liquid chromatography (HPLC). HPLC analyses were performed using an Agilent 1200 series HPLC system equipped with a quaternary pump and an Agilent 1200 series diode array detector. A Venusil XBP C18 column (4.60 × 150 mm, 5 µm) was used for separation and the mobile phase consisted initially of 30% acetonitrile and 70% MilliQ water at a flow rate of 1 mL/min. At the start of each HPLC run a solvent gradient program was initiated by linearly increasing the composition of the acetonitrile in the mobile phase to 85% acetonitrile over a period of 5 min. Each HPLC run lasted 15 min and a time period of 5 min was allowed for equilibration between runs. A volume of 20 µL of solutions of the test compounds in acetonitrile (1 mM) was injected into the HPLC system and the eluent was monitored at wavelengths of 210, 254 and 300 nm.

5.4.2 Synthesis and characterisation of the inhibitors (4a–l)

The 2-heteroarylidene-1-tetralone derivatives (**4a**–**k**) were synthesised via the base-catalysed Claisen-Schmidt condensation reaction of 1-tetralone or 7-methoxy-1-tetralone (2.27 mmol) and appropriately aldehydes (2.497 mmol). The reactants and potassium hydroxide (4.54 mmol) were dissolved in 5 mL methanol and stirred for 10–18 h at room temperature. Compound **4l** on the other hand was synthesised using concentrated hydrochloric acid (22 mL) as a catalyst also via the Claisen-Schmidt condensation reaction. The products were precipitated by addition of a minimum of 20 mL water and the crude products were collected by filtration, and recrystallised from a suitable solvent (ethanol).

(2*E*)-2-(Cyclohexylmethylidene)-7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one (4a)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one and cyclohexanecarbaldehyde in a yield of 10.6%: light brown crystals, mp 94.0–95.1 ^{o}C (ethyl acetate). ^{1}H NMR ($CDCl_3$) δ ppm 7.56 (d, *J* = 2.7 Hz, 1H), 7.13 (d, *J* = 8.3 Hz, 1H), 7.02 (dd, *J* = 8.3, 2.8 Hz, 1H), 6.74 (d, *J* = 9.7 Hz, 1H), 3.83 (s, 3H), 2.89 – 2.82 (m, 2H), 2.75 (t, *J* = 6.0 Hz, 2H), 2.42 – 2.31 (m, 1H), 1.80 – 1.50 (m, 5H), 1.37 – 1.16 (m, 5H); ^{13}C NMR ($CDCl_3$) δ ppm 187.86, 158.49, 145.13, 136.40, 134.50, 133.24, 129.40, 121.14, 110.20, 55.51, 37.27, 32.24, 28.46, 25.95, 25.82, 25.63. APCI-MS (m/z) calcd for $C_{18}H_{22}O_2$. $[M+H]^+$, 271.1693, found 271.1723. Purity (HPLC): 99.5%.

(2*E*)-2-(Cyclopentylmethylidene)-7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one (4b)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one and cyclopentanecarbaldehyde in a yield of 24.6%: yellow oil (ethyl acetate). ^{1}H NMR ($CDCl_3$) δ ppm 7.56 (d, J = 2.7 Hz, 1H), 7.13 (d, J = 8.3 Hz, 1H), 7.02 (dd, J = 8.3, 2.8 Hz, 1H), 6.84 (d, J = 9.8

Hz, 1H), 3.82 (d, J = 4.2 Hz, 3H), 2.92 – 2.73 (m, 5H), 1.96 – 1.08 (m, 8H); ^{13}C NMR ($CDCl_3$) δ ppm 187.67, 158.50, 145.29, 136.40, 134.50, 133.54, 129.40, 121.12, 110.22, 55.51, 38.90, 33.34, 28.36, 25.96, 25.61. APCI-MS (m/z) calcd for $C_{17}H_{20}O_2$ $[M+H]^+$, 257.1536, found 257.1552. Purity (HPLC): 97.1%.

(2*E*)-7-Methoxy-2-(pyridin-2-ylmethylidene)-3,4-dihydronaphthalen-1(2*H*)-one (4c)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one and picolinaldehyde in a yield of 45.3%: yellow crystals, mp 86.8–89.1 ^{o}C (ethanol). ^{1}H NMR ($CDCl_3$) δ 8.71 – 8.65 (m, 1H), 7.75 – 7.67 (m, 2H), 7.60 (d, J = 2.7 Hz, 1H), 7.42 (d, J = 7.8 Hz, 1H), 7.22 – 7.14 (m, 2H), 7.06 (dd, J = 8.3, 2.8 Hz, 1H), 3.85 (s, 3H), 3.59 – 3.52 (m, 2H), 2.95 – 2.88 (m, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 188.16, 158.54, 155.36, 149.43, 139.18, 136.75, 136.34, 134.01, 133.53, 129.54, 126.96, 122.64, 121.67, 110.21, 55.52, 27.93, 27.01. APCI-MS (m/z) calcd for $C_{17}H_{15}NO_2$ $[M+H]^+$, 266.1176, found 266.1166. Purity (HPLC): 100%.

(2*E*)-2-(Pyridin-3-ylmethylidene)-3,4-dihydronaphthalen-1(2*H*)-one (4d)

The title compound is a product of 3, 4-dihydronaphthalen-1(2*H*)-one and nicotinaldehyde in a yield of 29.7%: yellow crystals, mp 77.5–77.7 ^{o}C (petroleum ether). ^{1}H NMR ($CDCl_3$) δ ppm 8.67 (s, 1H), 8.59 – 8.49 (m, 1H), 8.11 (d, J = 7.8 Hz, 1H), 7.81 – 7.66 (m, 2H), 7.48 (td, J = 7.5, 1.1 Hz, 1H), 7.30 (ddd, J = 56.7, 10.9, 6.5 Hz, 3H), 3.08 (dd, J = 9.1, 3.8 Hz, 2H), 2.95 (t, J = 6.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 187.27, 150.49, 149.13, 143.08, 137.46, 136.77, 133.54, 133.11, 132.50, 131.70, 128.28, 128.24, 127.14, 123.30, 28.71, 27.15. APCI-MS (m/z) calcd for $C_{16}H_{13}NO$ $[M+H]^+$, 236.1070, found 236.1062. Purity (HPLC): 98.5%

(2*E*)-7-Methoxy-2-(pyridin-4-ylmethylidene)-3,4-dihydronaphthalen-1(2*H*)-one (4e)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one and isonicotinaldehyde in a yield of 66.9%: yellow crystals, mp 136.9–137.6 ^{o}C (ethanol). ^{1}H NMR ($CDCl_3$) δ ppm 8.64 (d, J = 5.5 Hz, 2H), 7.68 (s, 1H), 7.58 (d, J = 2.7 Hz, 1H), 7.25 (t, J = 5.7 Hz, 2H), 7.15 (d, J = 8.4 Hz, 1H), 7.07 (dd, J = 8.3, 2.8 Hz, 1H), 3.84 (s, 3H), 3.03 (dd, J = 9.0, 3.7 Hz, 2H), 2.88 (t, J = 6.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 187.14, 158.72, 149.97, 143.49, 138.84, 135.92, 133.77, 133.11, 129.57, 123.78, 121.97, 110.25, 55.52, 27.87, 27.32. APCI-MS (m/z) calcd for $C_{17}H_{15}NO_2$ $[M+H]^+$, 266.1176, found 266.1189. Purity (HPLC): 100%.

(2*E*)-7-Methoxy-2-(pyridin-3-ylmethylidene)-3,4-dihydronaphthalen-1(2*H*)-one (4f)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one and nicotinaldehyde in a yield of 72.2%: peach crystals, mp 105.4–107.1 ^{o}C (ethanol). ^{1}H NMR ($CDCl_3$) δ ppm 8.68 (s, 1H), 8.56 (d, J = 2.9 Hz, 1H), 7.76 (s, 1H), 7.71 (d, J = 7.9 Hz, 1H), 7.59 (d, J = 2.7 Hz, 1H), 7.34 (dd, J = 7.7, 4.9 Hz, 1H), 7.15 (d, J = 8.4 Hz, 1H), 7.07 (dd, J = 8.3, 2.8 Hz, 1H), 3.85 (s, 3H), 3.06 (dd, J = 9.1, 3.7 Hz, 2H), 2.89 (t, J = 6.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 187.22, 158.71, 150.50, 149.12, 137.49, 136.76, 135.83, 133.91, 132.54, 129.53, 123.31, 121.83, 110.28, 55.54, 27.92, 27.36. APCI-MS (m/z) calcd for $C_{17}H_{15}NO_2$, $[M+H]^+$, 266.1176, found 266.1176. Purity (HPLC): 98.6%.

(2*E*)-2-[(2-Chloropyridin-3-yl)methylidene]-7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one (4g)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one and 2-chloronicotinaldehyde in a yield of 75%: yellow crystals, mp 131.0–132.0 ^{o}C (ethanol). ^{1}H NMR ($CDCl_3$) δ ppm 8.36 (dd, J = 4.7, 1.5 Hz, 1H), 7.75 (s, 1H), 7.65 – 7.59 (m, 2H), 7.28 (dd, J = 7.5, 4.8 Hz, 1H), 7.15 (d, J = 8.4 Hz, 1H), 7.07 (dd, J = 8.4, 2.8 Hz, 1H), 3.85 (s, 3H), 2.89 (s, 4H); ^{13}C NMR ($CDCl_3$) δ ppm 187.00, 158.73, 151.37, 149.05, 138.76, 138.61, 135.96, 133.77, 131.12, 131.04, 129.62, 122.01, 121.99, 110.25, 55.54, 28.00, 27.49. APCI-MS (m/z) calcd for $C_{17}H_{14}NO_2Cl$ $[M+H]^+$, 300.0786, found 300.0803. Purity (HPLC): 99.4%

(2*E*)-2-(Furan-2-ylmethylidene)-7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one (4h)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one and furan-2-carbaldehyde in a yield of 85.5%: yellow crystals, mp 90.1–90.9 ^{o}C (petroleum ether). ^{1}H NMR ($CDCl_3$) δ ppm 7.60 – 7.52 (m, 3H), 7.15 (d, J = 8.3 Hz, 1H), 7.04 (dd, J = 8.3, 2.8 Hz, 1H), 6.69 (d, J = 3.4 Hz, 1H), 6.50 (dd, J = 3.3, 1.8 Hz, 1H), 3.84 (s, 3H), 3.32 – 3.25 (m, 2H), 2.95 – 2.89 (m, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 187.27, 158.57, 152.48, 144.30, 136.22, 134.29, 131.80, 129.36, 122.85, 121.27, 116.53, 112.19, 110.20, 55.51, 27.49, 26.84. APCI-MS (m/z) calcd for $C_{16}H_{14}O_3$ $[M+H]^+$, 255.1016, found 255.1011. Purity (HPLC): 100%.

(2*E*)-7-Methoxy-2-(1H-pyrrol-2-ylmethylidene)-3,4-dihydronaphthalen-1(2*H*)-one (4i)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one and 1*H*-pyrrole-2-carbaldehyde in a yield of 35%: yellow crystals, mp 186.1–190.2 ^{o}C (ethanol). ^{1}H NMR (DMSO) δ ppm 11.53 (s, 1H), 7.70 (s, 1H), 7.39 (d, J = 2.7 Hz, 1H), 7.27 (d, J = 8.4 Hz, 1H), 7.14 – 7.05 (m, 2H), 6.64 (d, J = 2.7 Hz, 1H), 6.27 (s, 1H), 3.78 (s, 3H), 3.00 (t, J = 6.2 Hz, 2H), 2.87 (t, J = 6.6 Hz, 2H); ^{13}C NMR (DMSO) δ ppm 185.60, 158.10, 135.34, 134.36, 129.59,

128.55, 127.42, 126.95, 122.60, 120.02, 113.46, 110.90, 110.08, 55.23, 26.56, 26.40. APCI-MS (m/z) calcd for $C_{16}H_{15}NO_2$ $[M+H]^+$, 254.1176, found 254.1182. Purity (HPLC): 99.1%.

(2*E*)-7-Methoxy-2-(thiophen-2-ylmethylidene)-3,4-dihydronaphthalen-1(2*H*)-one (4j)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one and thiphene-2-carbaaldehyde in a yield of 86.2%: yellow crystals, mp 106.5–116.9 ^oC (ethanol). ^1H NMR ($CDCl_3$) δ ppm 8.02 (s, 1H), 7.58 (d, J = 2.7 Hz, 1H), 7.49 (d, J = 5.0 Hz, 1H), 7.38 (d, J = 3.4 Hz, 1H), 7.19 – 7.08 (m, 2H), 7.05 (dd, J = 8.3, 2.8 Hz, 1H), 3.85 (s, 3H), 3.16 (dd, J = 9.3, 3.8 Hz, 2H), 2.95 (t, J = 6.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 187.05, 158.63, 139.13, 135.67, 134.42, 133.23, 131.76, 129.47, 129.33, 129.31, 127.54, 121.26, 110.27, 55.52, 27.27, 27.19. APCI-MS (m/z) calcd for $C_{16}H_{14}O_2S$ $[M+H]^+$, 271.0787, found 271.0765. Purity (HPLC): 100%.

(2*E*)-7-Methoxy-2-(thiophen-3-ylmethylidene)-3,4-dihydronaphthalen-1(2*H*)-one (4k)

The title compound is a product of 3,4-dihydronaphthalen-1(2*H*)-one and thiophene-3-carbaldehyde in a yield of 83.0%: cream crystals, mp 135.4–136.9 ^oC (ethanol). ^1H NMR ($CDCl_3$) δ ppm 7.82 (s, 1H), 7.58 (d, J = 2.8 Hz, 1H), 7.50 (d, J = 2.5 Hz, 1H), 7.36 (dd, J = 4.9, 3.0 Hz, 1H), 7.28 (dd, J = 5.0, 0.7 Hz, 1H), 7.15 (d, J = 8.3 Hz, 1H), 7.05 (dd, J = 8.3, 2.8 Hz, 1H), 3.85 (s, 3H), 3.15 – 3.08 (m, 2H), 2.90 (t, J = 6.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 187.55, 158.62, 137.36, 135.76, 134.38, 133.76, 130.52, 129.35, 128.96, 127.71, 125.83, 121.35, 110.25, 55.52, 27.59, 27.39. APCI-MS (m/z) calcd for $C_{16}H_{14}O_2S$ $[M+H]^+$, 271.0787, found 271.0805. Purity (HPLC): 99.5%.

(2*E*)-2-Benzylidene-7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one (4l)

The title compound is a product of 7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one and benzaldehyde in a yield of 51.2%: pale yellow crystals, mp 121.5–122.7 ^oC (dichloromethane). ^1H NMR ($CDCl_3$) δ ppm 7.85 (s, 1H), 7.61 (d, J = 2.8 Hz, 1H), 7.41 (dt, J = 15.2, 7.4 Hz, 4H), 7.33 (t, J = 7.0 Hz, 1H), 7.15 (d, J = 8.3 Hz, 1H), 7.06 (dd, J = 8.3, 2.8 Hz, 1H), 3.85 (s, 3H), 3.09 (td, J = 6.7, 1.6 Hz, 2H), 2.90 – 2.84 (m, 2H); ^{13}C NMR ($CDCl_3$) δ ppm 187.80, 158.62, 136.68, 135.92, 135.85, 135.43, 134.23, 129.84, 129.42, 128.49, 128.40, 121.50, 110.26, 55.52, 28.02, 27.34. APCI-HRMS m/z calcd for $C_{18}H_{16}O_2$ $[M+1]^+$, 265.1223, found 265.1212. Purity (HPLC): 99.2%.

5.4.3 Determinations of IC_{50} values

The IC_{50} values for the inhibition of human MAO-A and MAO-B were determined as described in literature (Strydom *et al.*, 2011). All enzymatic reactions were carried out in 96-well microtiter plates in potassium phosphate buffer (100 mM, pH 7.4) to a final volume of 200 µL. The reactions contained MAO-A (0.0075 mg protein/mL) or MAO-B (0.015 mg protein/mL), the mixed MAO-A/B substrate kynuramine (50 µM) and the test inhibitors (0.003–100 µM). DMSO was added to the reactions to yield a final concentration of 4% (v/v) DMSO. The reactions were incubated at 37 °C for 20 min and terminated with the addition of 80 µL NaOH (2N). The concentrations of the MAO generated 4-hydroxyquinoline in the reactions were measured by fluorescence spectrophotometry (λ_{ex} = 310 nm, λ_{em} = 400 nm) (Novaroli *et al.,* 2005). To quantify 4-hydroxyquinoline, a linear calibration curve was constructed from solutions of authentic 4-hydroxyquinoline (0.0469–1.5 µM). The rates of kynuramine oxidation were calculated and the rate data were fitted to the one site competition model incorporated into the Prism software package (GraphPad). From the resulting sigmoidal curves (catalytic rate versus the logarithm of the inhibitor concentration), the IC_{50} values were estimated. All experiments were carried out in triplicate and the IC_{50} values are expressed as mean ± standard deviation (SD).

5.5 Acknowledgements

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REFERENCES

Bach, A.W.J., Lan, N.C., Johnson, D.L., Abell, C.W., Bembenek, M.E., Kwa, S., Seeburg, P.H. & Shih, J.C. 1988. cDNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymatic properties. *Proceedings of the national academy of sciences*, 85(13):4934-4938.

Chimenti, F., Fioravanti, R., Bolasco, A., Chimenti, P., Secci, D., Rossi, F., Matilde, Y.n.e., Orallo, F., Ortuso, F. & Alcaro, S. 2009. Chalcones: a valid scaffold for monoamine oxidases inhibitor. *Journal of medicinal chemistry*, 52(9):2818-2824.

de Oliveira, G.R., de Oliveira, H.C.B., Silva, W.A., da Silva, V.H.C., Sabino, J.R. & Martins, F.T. 2012. Structure and theoretical approaches to a chalcone deriative. *Structural chemistry journal*, 2012(23):1667-1676.

Devia, A.C., Ferretti, F.H., Ponce, C.A. & Tomás, F. 1999. Conformational equilibrium and intramolecular hydrogen bond of 4′X and 4X substituted 2′(OH)chalcones. *Journal of molecular structure: THEOCHEM*, 493(1–3):187-197.

Ferino, G., Cadoni, E., Matos, M.J., Quezada, E., Uriarte, E., Santana, L., Vilar, S., Tatonetti, N.P., Yanez, M., Vina, D., Picciau, C., Serra, S. & Delogu, G. 2013. Monoamine inhibitory activity of arylbenzofurans versus 3-aryl coumarins: synthesis, in vitro study and docking calculations. *International journal of medicinal chemistry and pharmaceutical chemistry*, 8(6):956-966.

Fernandez, H.H. & Chen, J.J. 2007. Monoamine oxidase-B inhibition in the treatment of Parkinson's disease. *Pharmacotherapy*, 27(12 Pt 2):174S-185S.

Fowler, J.S., Logan, J., Wang, G.J., Volkow, N.D., Zhu, W., Franceschi, D., Pappas, N., Ferrieri, R., Shea, C., Garza, V., Xu, Y., MacGregor, R.R., Schlyer, D., Gatley, S.J., Ding, Y. & Alexoff, D. 2002. PET imaging of monoamine oxidase B in peripheral organs in humans. *Journal of nuclear medicine*, 43(10):1331-1338.

Geha, R.M., Rebrin, I., Chen, K. & Shih, J.C. 2001. Substrate and inhibitor specificities for human monoamine oxidase A and B are influenced by a single amino acid. *Biological chemistry*, 276(13):9877-9882.

Grimsby, J., Lan, N.C., Neve, R., Chen, K. & Shih, J.C. 1990. Tissue distribution of human monoamine oxidase A and B mRNA. *Journal of neurochemistry*, 55(4):1166-1169.

Ma, J., Yoshimura, M., Yamashita, E., Nakagawa, A., Ito, A. & Tsukihara, T. 2004. Structure of rat monoamine oxidase A and its specific recognitions for substrates and inhibitors. *Journal of Molecular Biology*, 338(1):103-114.

Minders, C., Petzer, J.P., Petzer, A. & Lourens, A.C. 2015. Monoamine oxidase inhibitory activities of heterocyclic chalcones. *Bioorganic & medicinal chemistry letters*, 25(22):5270-5276.

Mousavi, M.R., Maghsoodlou, M.T. & Habibi-khorassani, S.M. 2014. One-pot diastereoselective synthesis of highly functionalized cyclohexenones: 2-oxo--N,4,6-triarylycyclohex-3-ene carboxamides. *Molecular diversity*, 2014(18):821-828.

Novaroli, L., Reist, M., Favre, E., Carotti, A., Catto, M. & Carrupt, P.-A. 2005. Human recombinant monoamine oxidase B as reliable and efficient enzyme source for inhibitor screening. *Bioorganic & medicinal chemistry*, 13(22):6212-6217.

Petzer, A., Grobler, P., Bergh, J.J. & Petzer, J.P. 2013. Inhibition of monoamine oxidase by selected phenylalkylcaffeine analogues. *Journal of Pharmacy and Pharmacology*(66):677-687.

Rahman, M.A. 2011. Chalcone: a valuable insight into the recent advances and potential pharmacological activities. *Chemical sciences journal*, 2011(29):1-16.

Robinson, S.J., Petzer, J.P., Petzer, A., Bergh, J.J. & Lourens, A.C.U. 2013. Selected furanochalcones as inhibitors of monoamine oxidase. *Bioorganic & medicinal chemistry letters*, 23(17):4985-4989.

Rozmer, Z. & Perjési, P. 2016. Naturally occurring chalcones and their biological activities. *Phytochemistry reviews*, 15(1):87-120.

Shih, J.C., Chen, K. & Ridd, M.J. 1999. Monoaminde oxidase: from genes to behavior. *Annual reviews neuroscience*, 22(1):197-217.

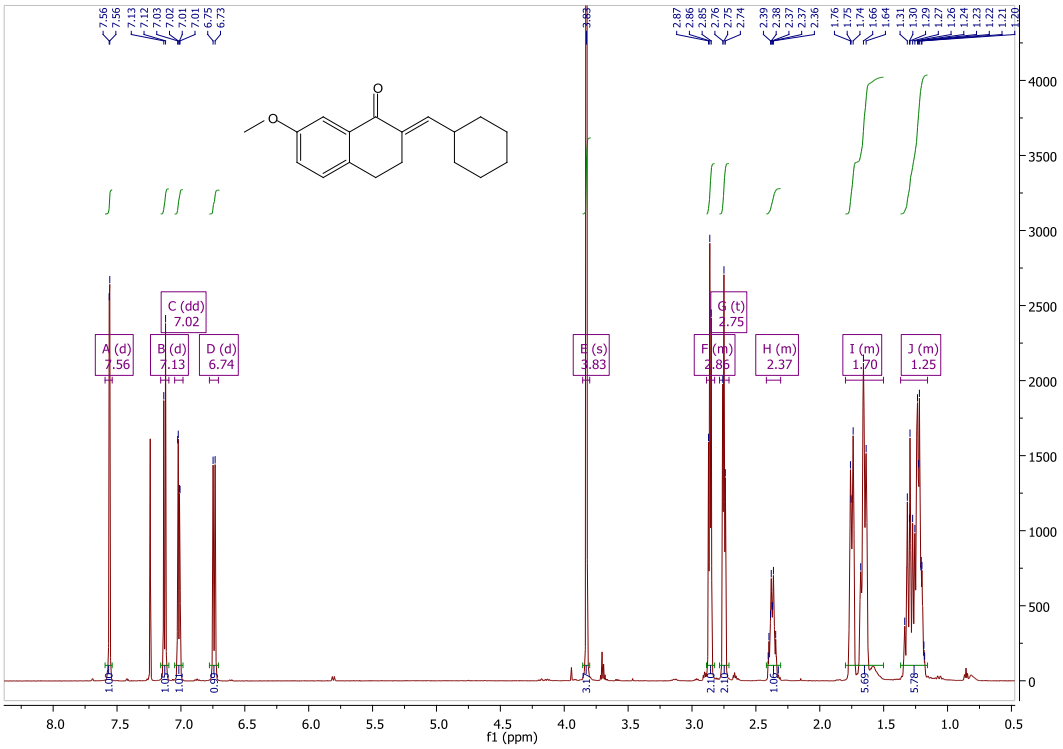
Strydom, B., Bergh, J.J. & Petzer, J.P. 2011. 8-Aryl- and alkyloxycaffeine analogues as inhibitors of monoamine oxidase. *European journal of medicinal chemistry*, 46(8):3474-3485.

APPENDIX C: SPECTRA

^{1}H NMR, ^{13}C NMR, HRMS and HPLC

(2*E*)-2-(Cyclohexylmethylidene)-7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one (4a)

^{1}H NMR



APCI-MS

Acquisition Parameter

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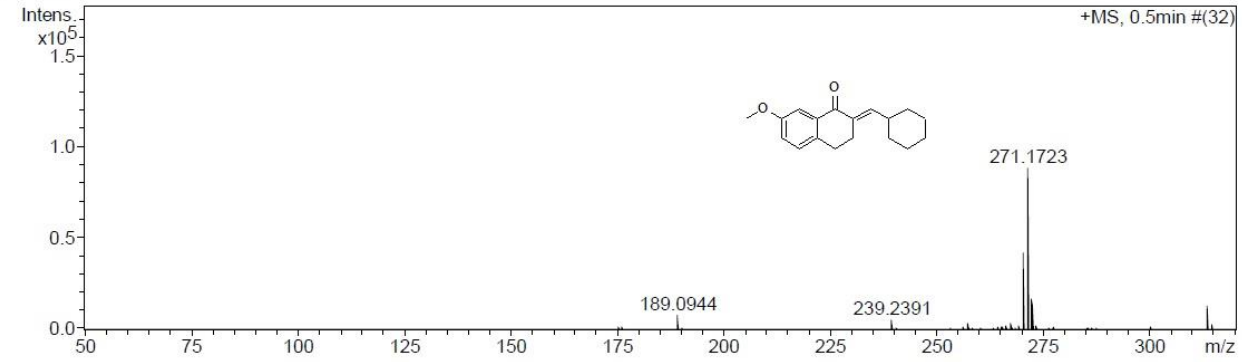
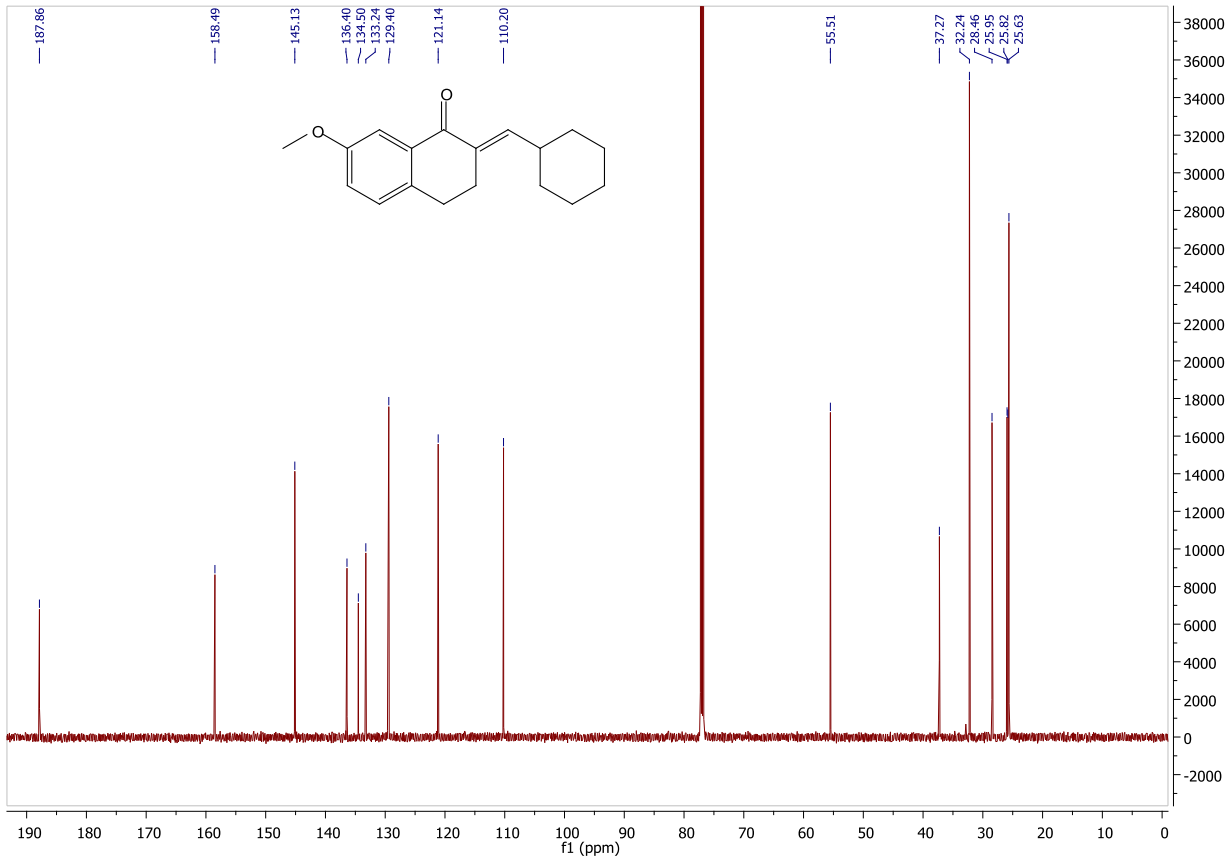


^{13}C NMR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136.40
134.50
133.24
129.40
121.14
110.20
55.51
37.27
32.24
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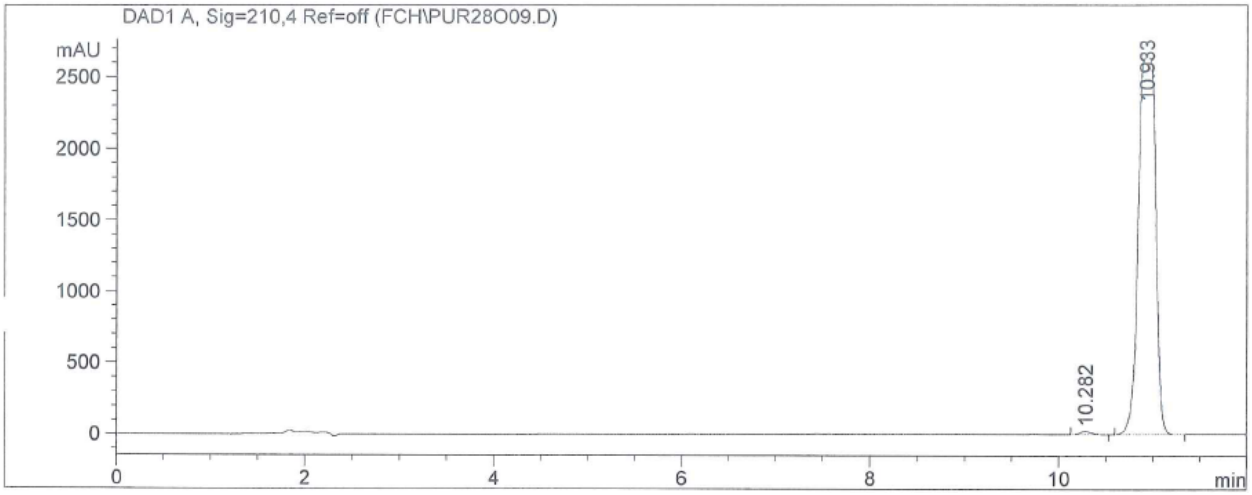
25.95
25.82
25.63
O
O
f1 (ppm)

HPLC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mAU
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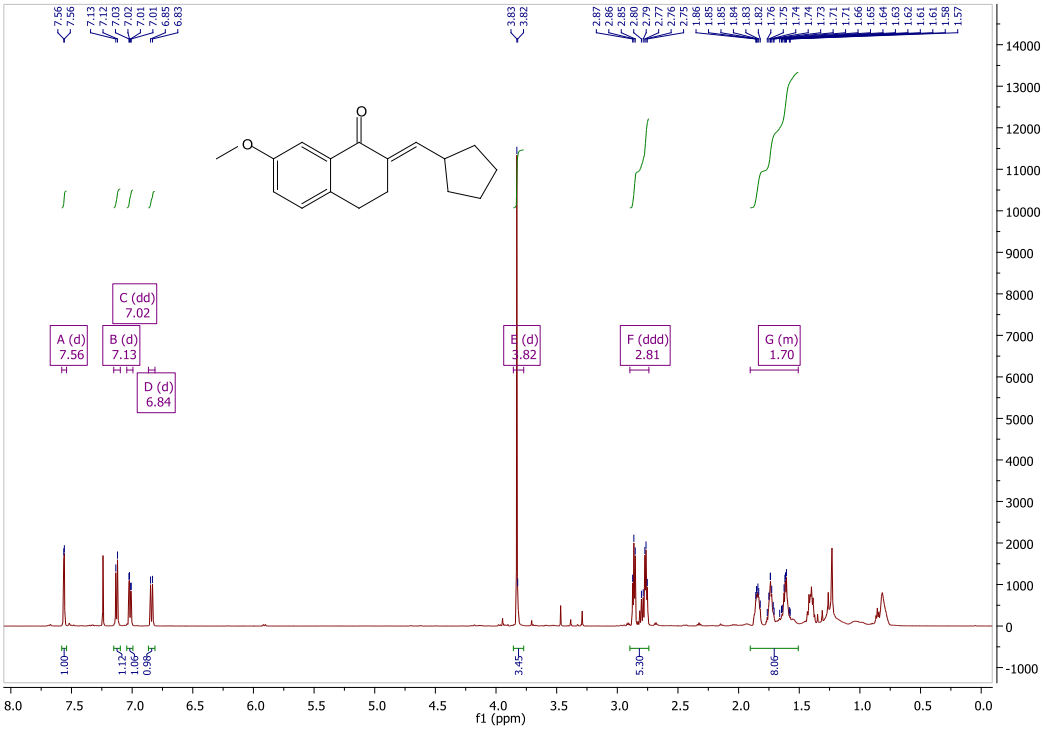
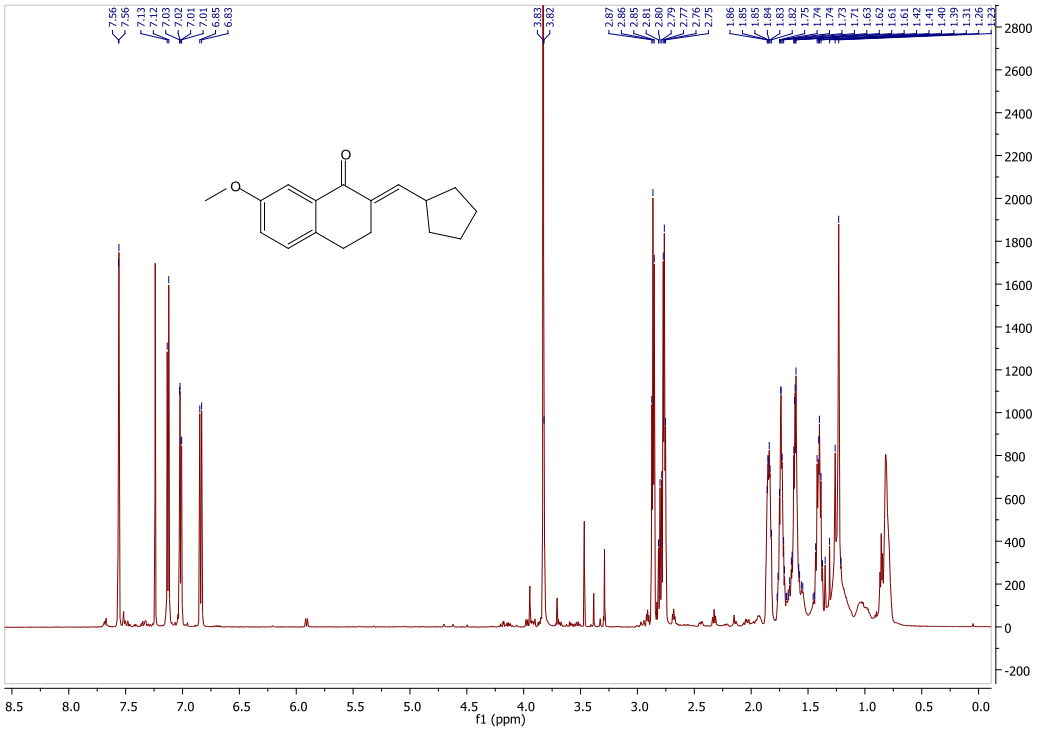
min

(2*E*)-2-(Cyclopentylmethylidene)-7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one (4b)

^{1}H NMR



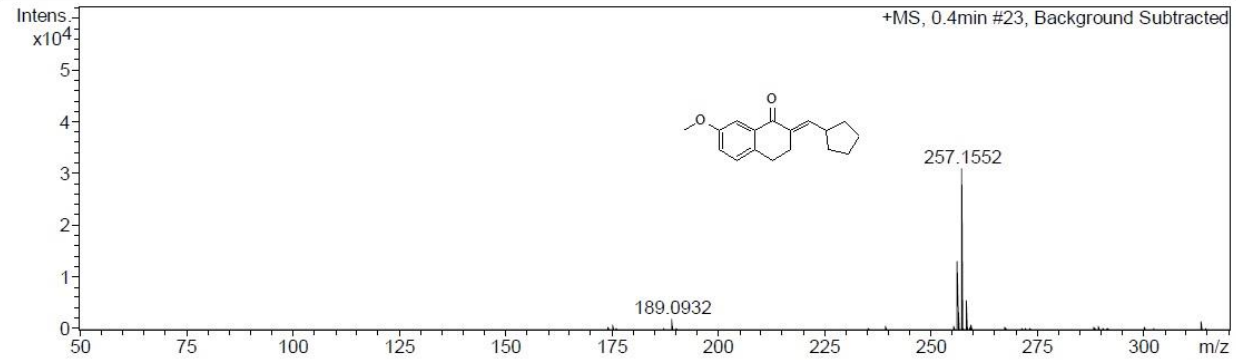
^{13}C NMR



APCI-MS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste



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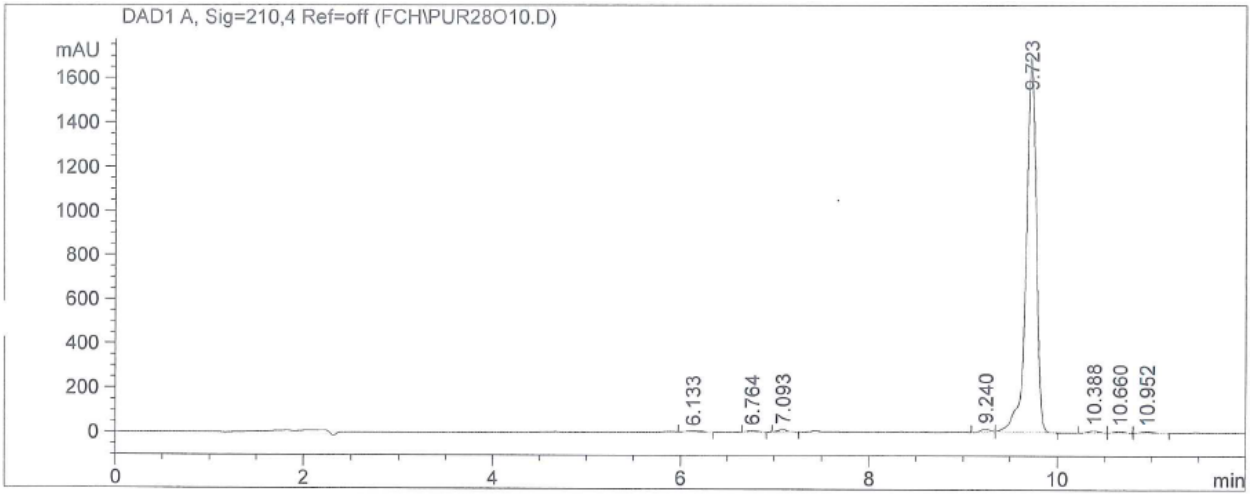
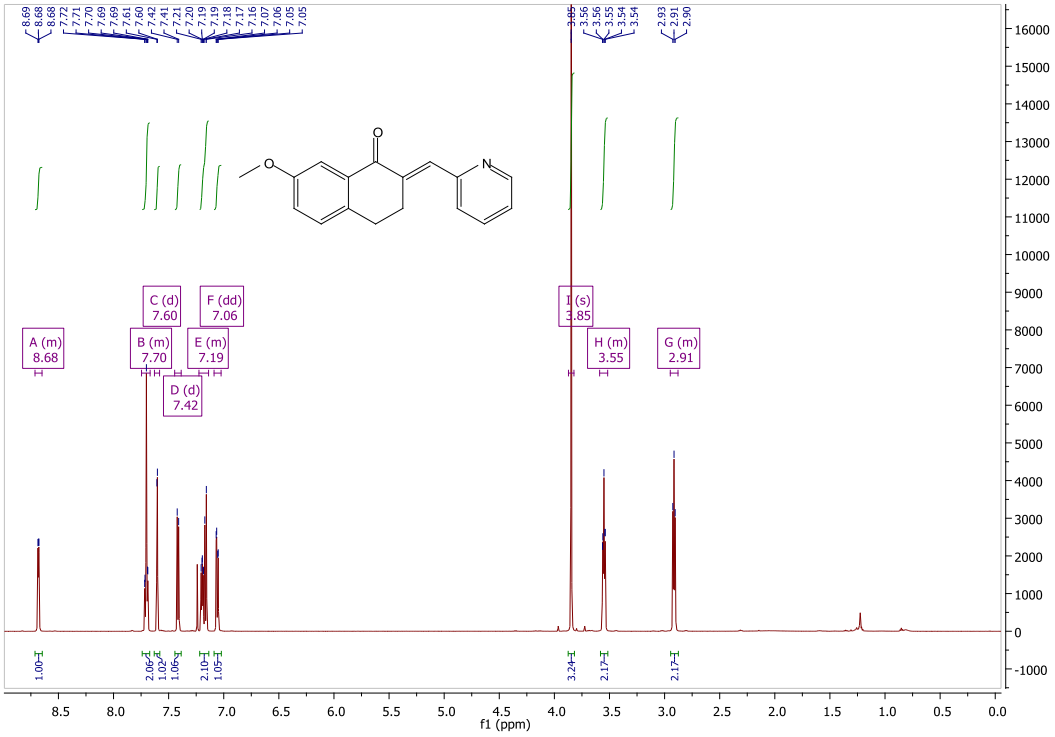
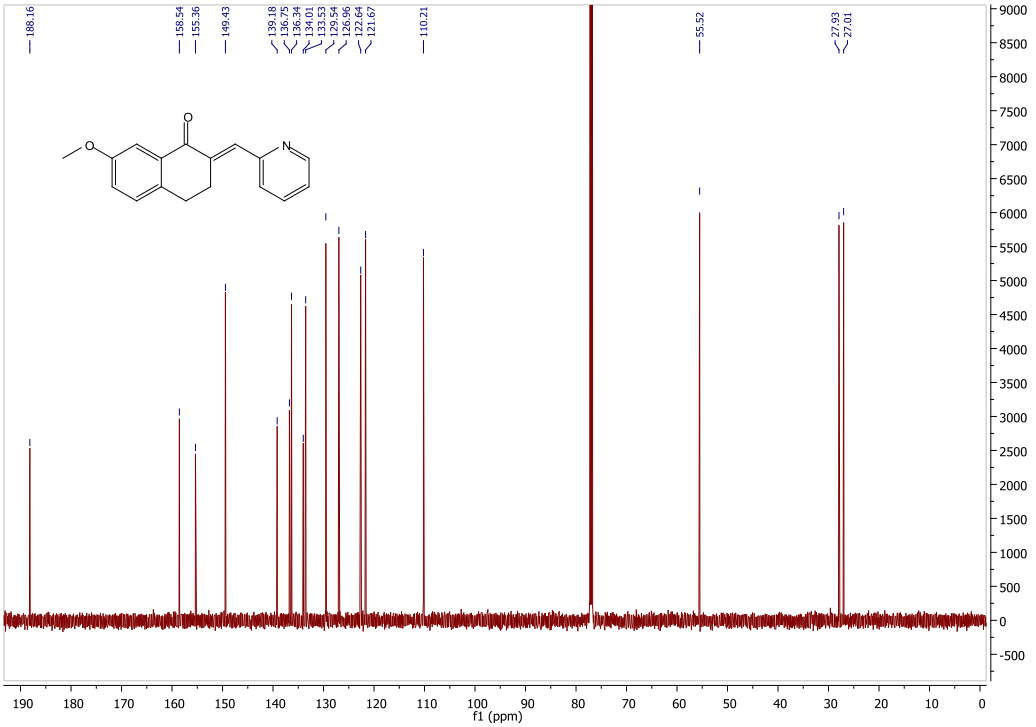


(2*E*)-7-Methoxy-2-(pyridin-2-ylmethylidene)-3,4-dihydronaphthalen-1(2*H*)-one (4c)

^{1}H NMR



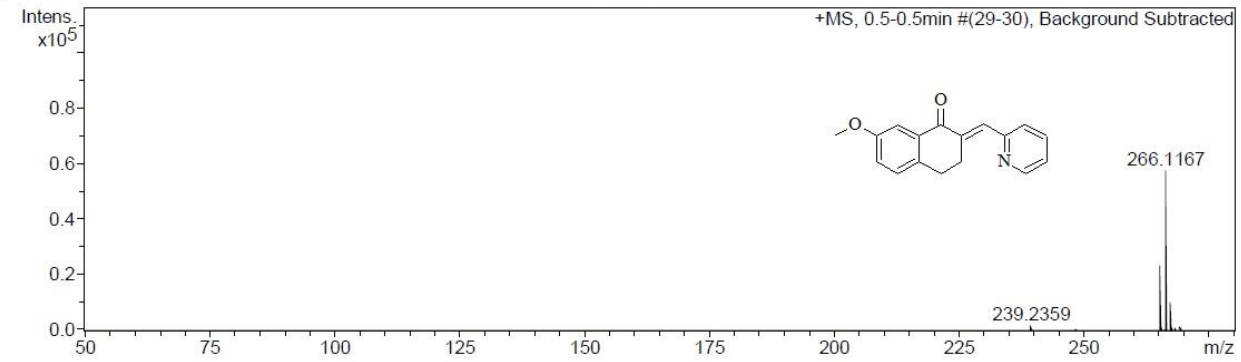
^{13}C NMR



APCI-MS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
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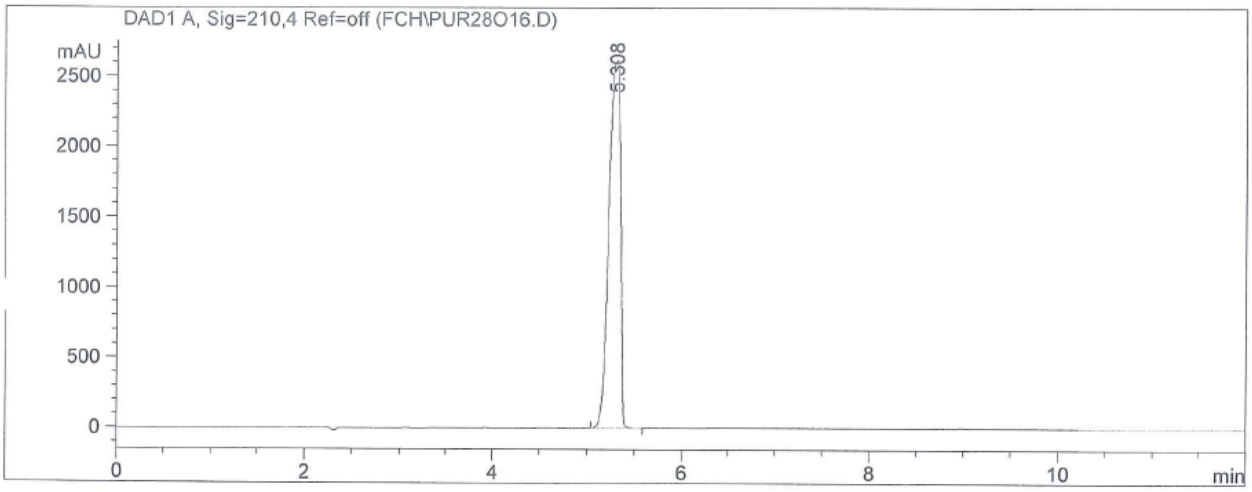
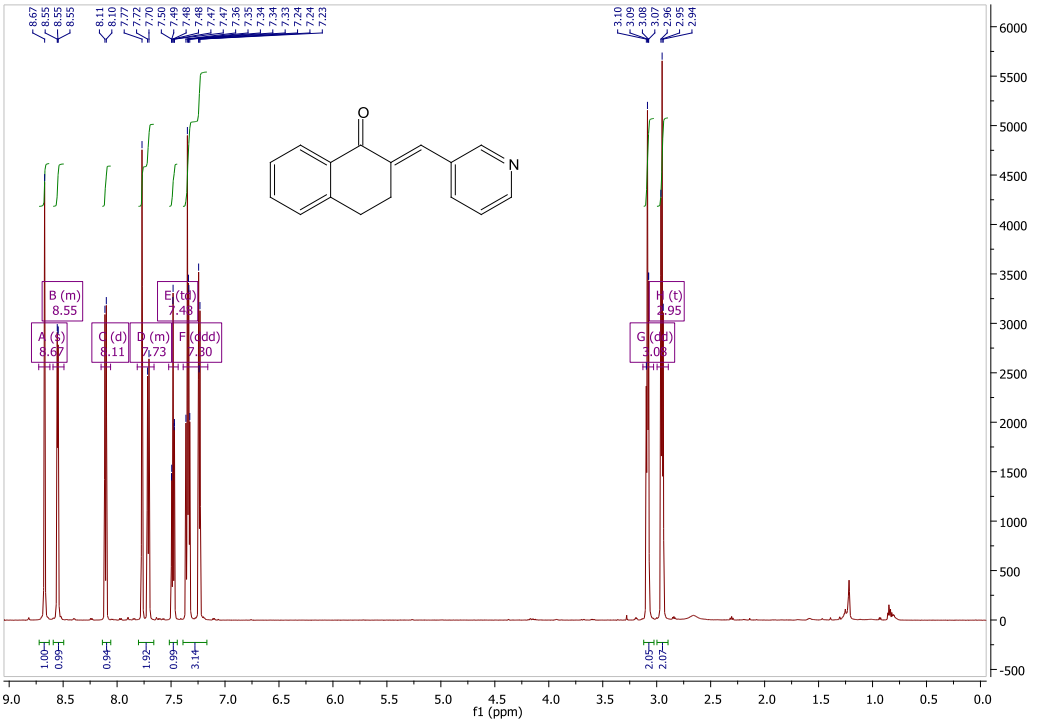
HPLC

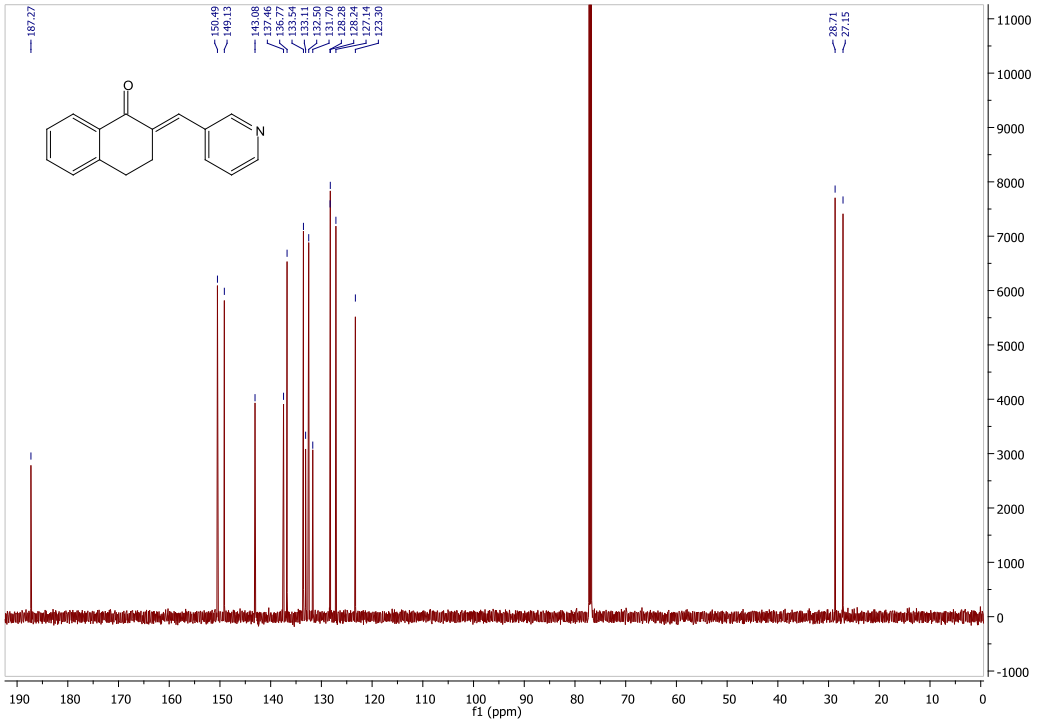


(2*E*)-2-(Pyridin-3-ylmethylidene)-3,4-dihydronaphthalen-1(2*H*)-one (4d)

^{1}H NMR



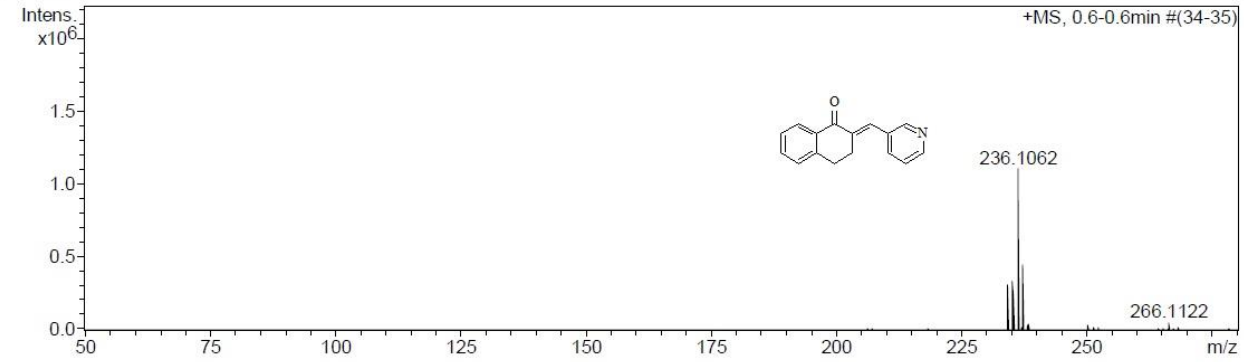
^{13}C NMR



APCI-MS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste



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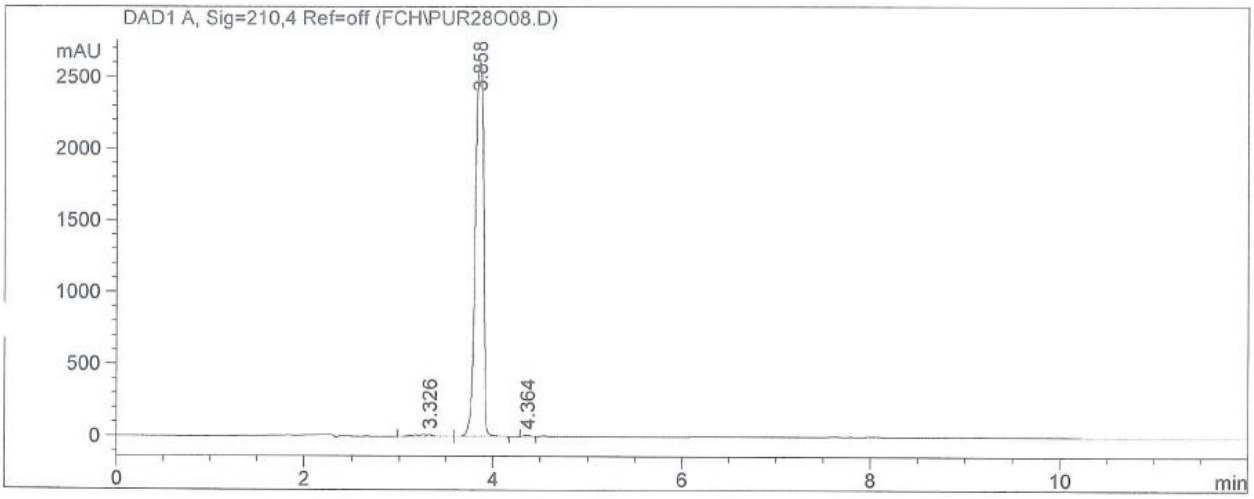
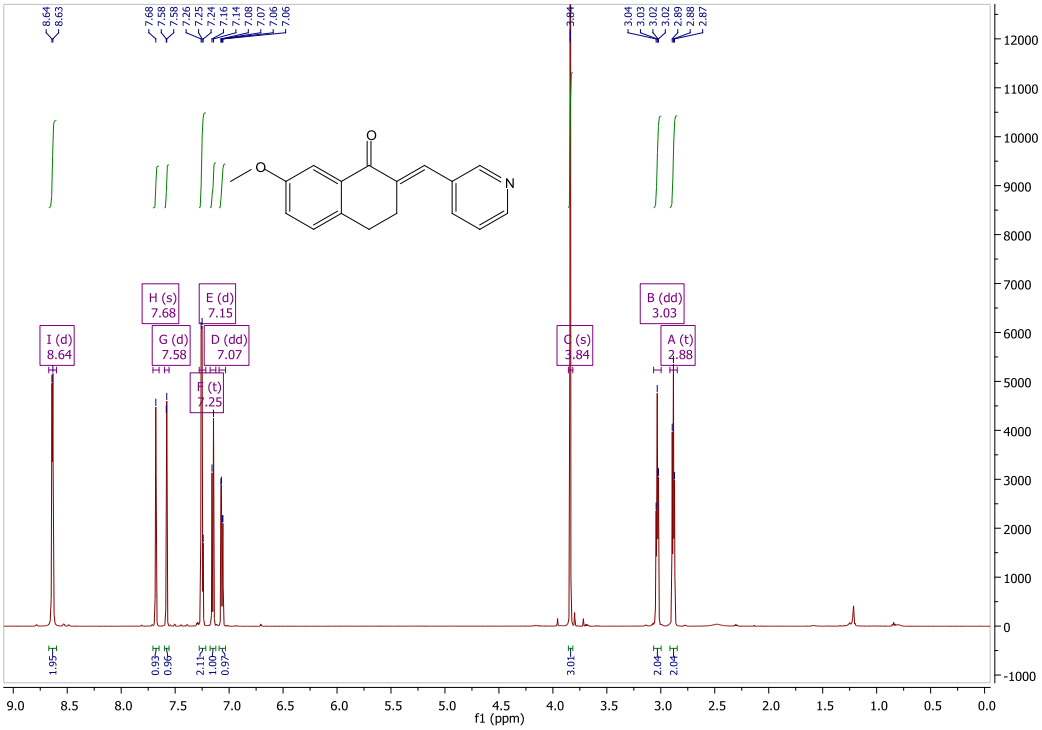
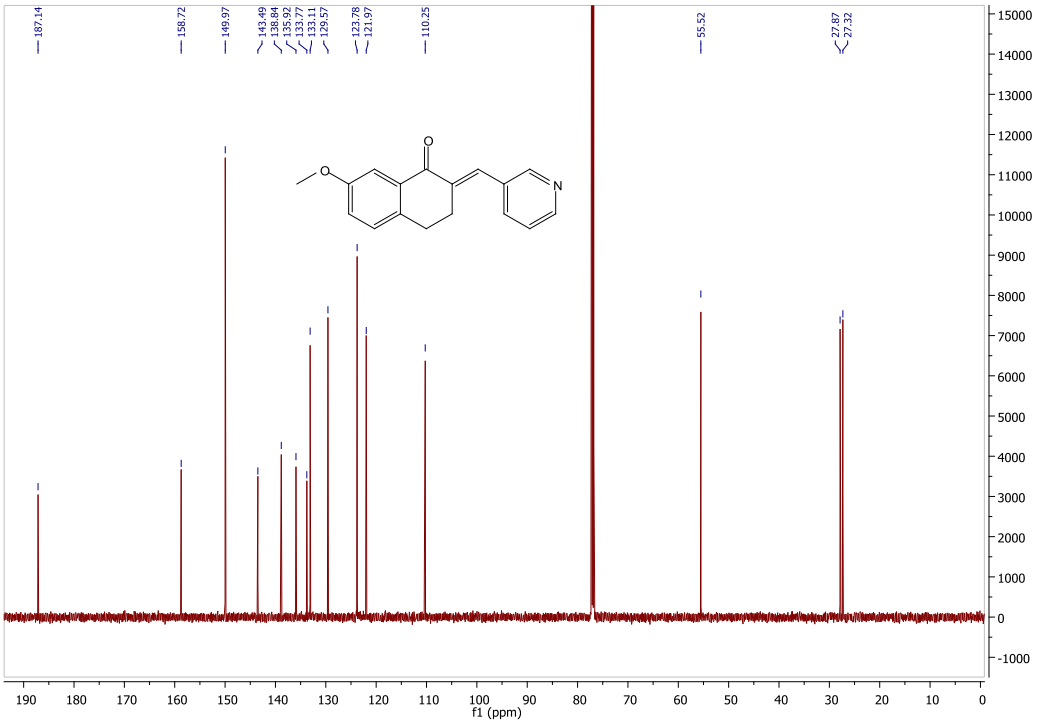


(2*E*)-7-Methoxy-2-(pyridin-4-ylmethylidene)-3,4-dihydronaphthalen-1(2*H*)-one (4e)

^{1}H NMR



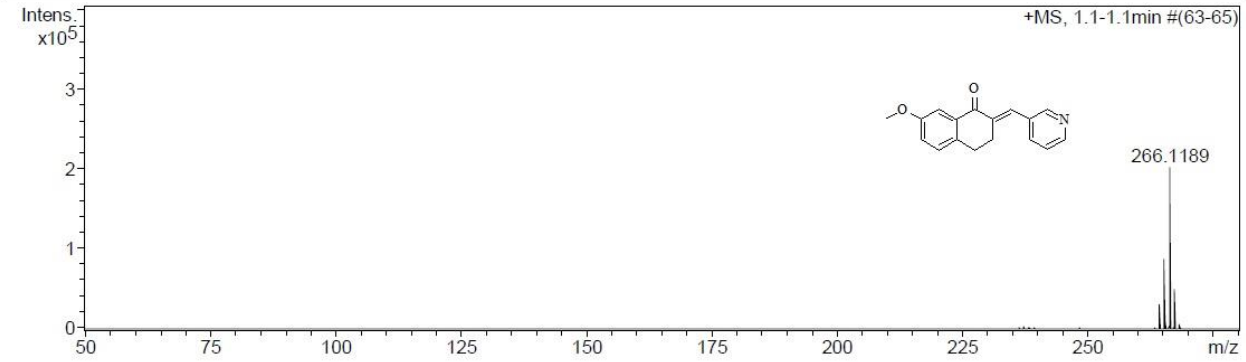
^{13}C NMR



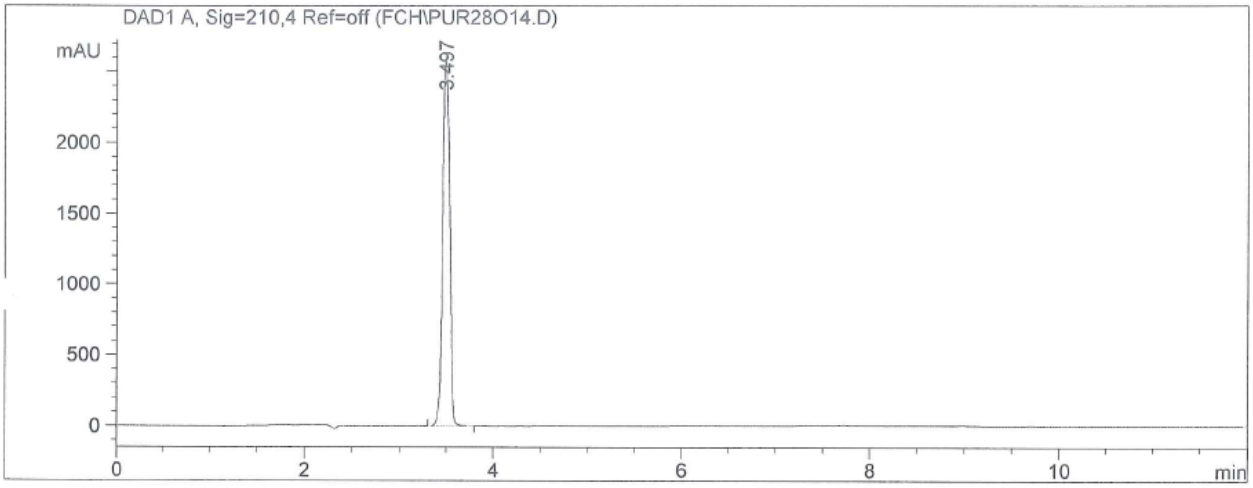
APCI-MS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste

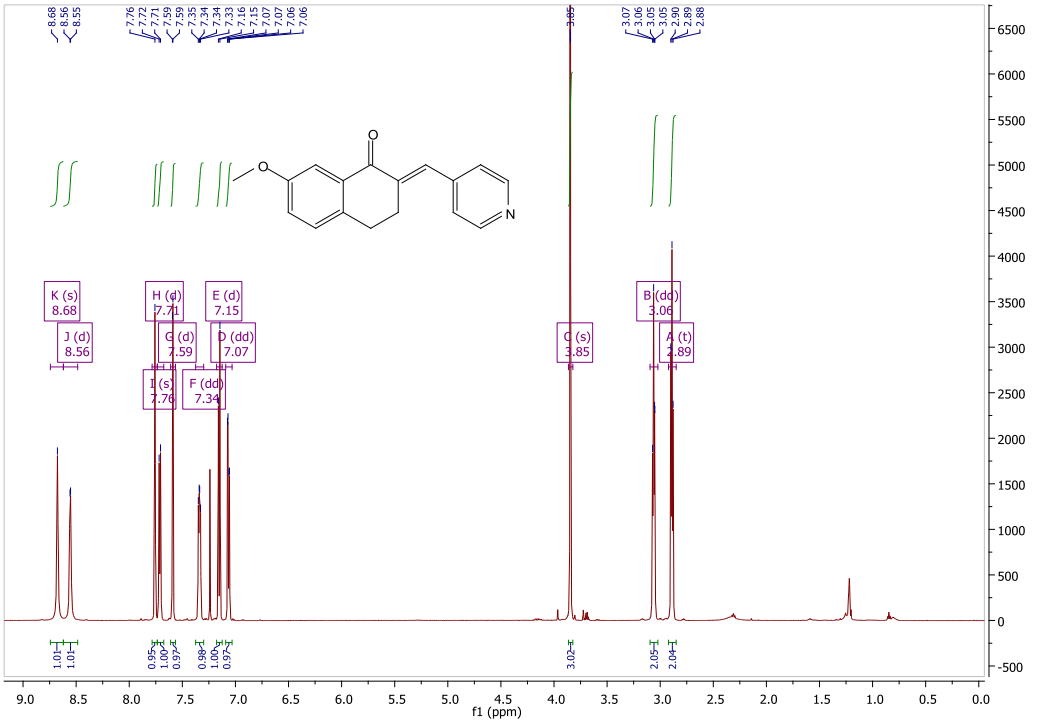


HPLC

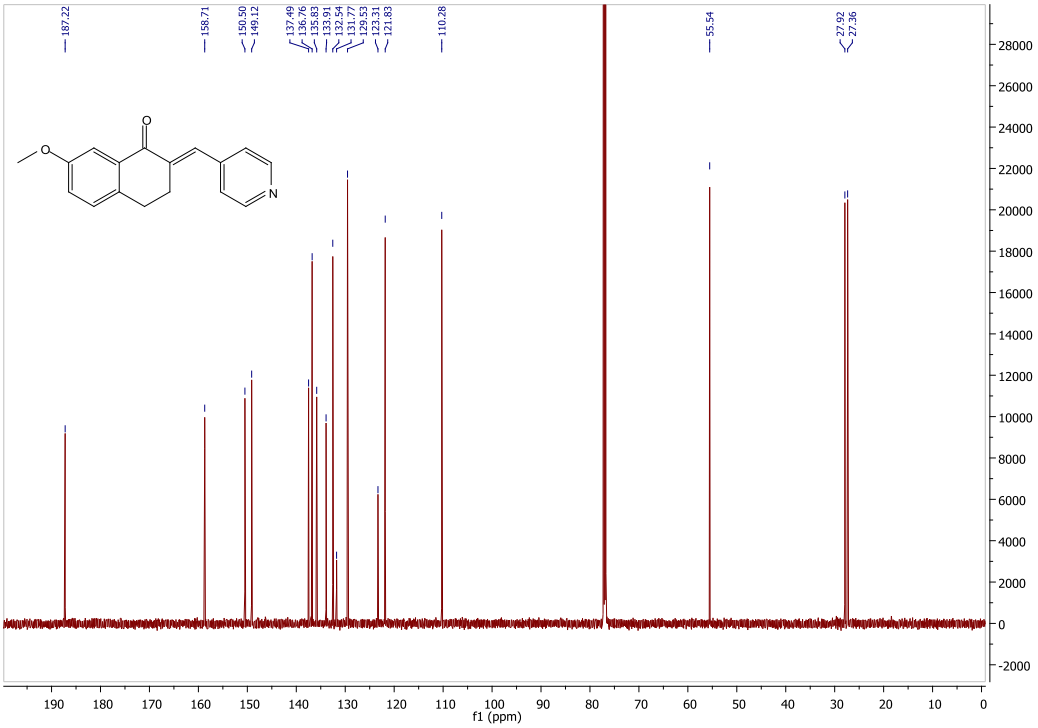


(2*E*)-7-Methoxy-2-(pyridin-3-ylmethylidene)-3,4-dihydronaphthalen-1(2*H*)-one (4f)

^{1}H NMR



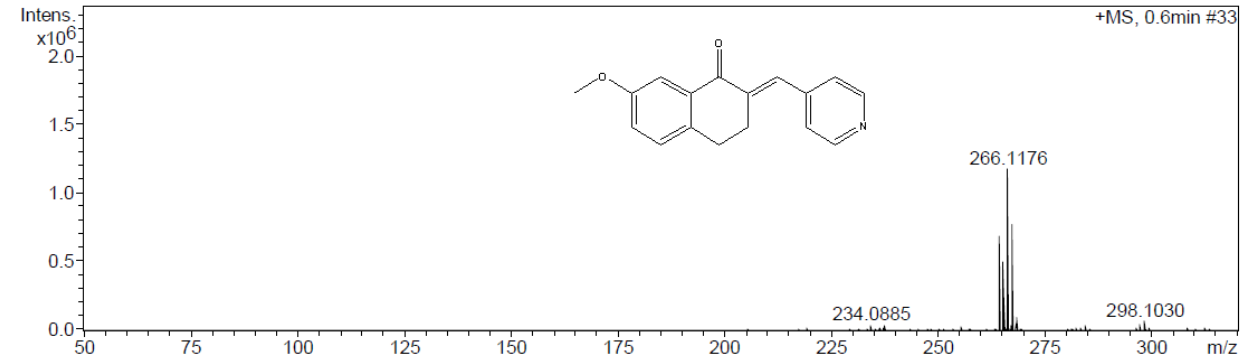
^{13}C NMR



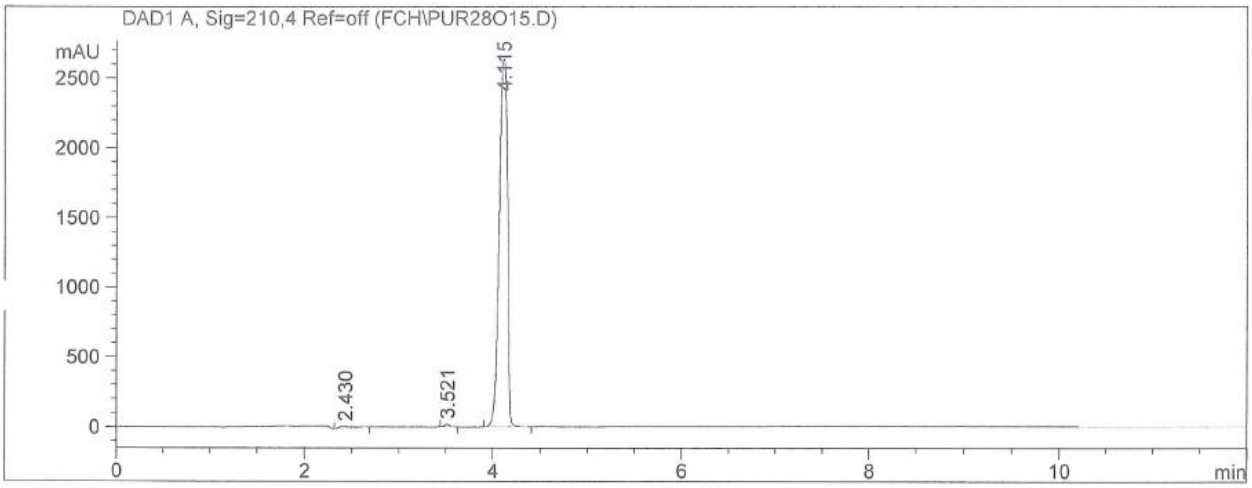
APCI-MS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste

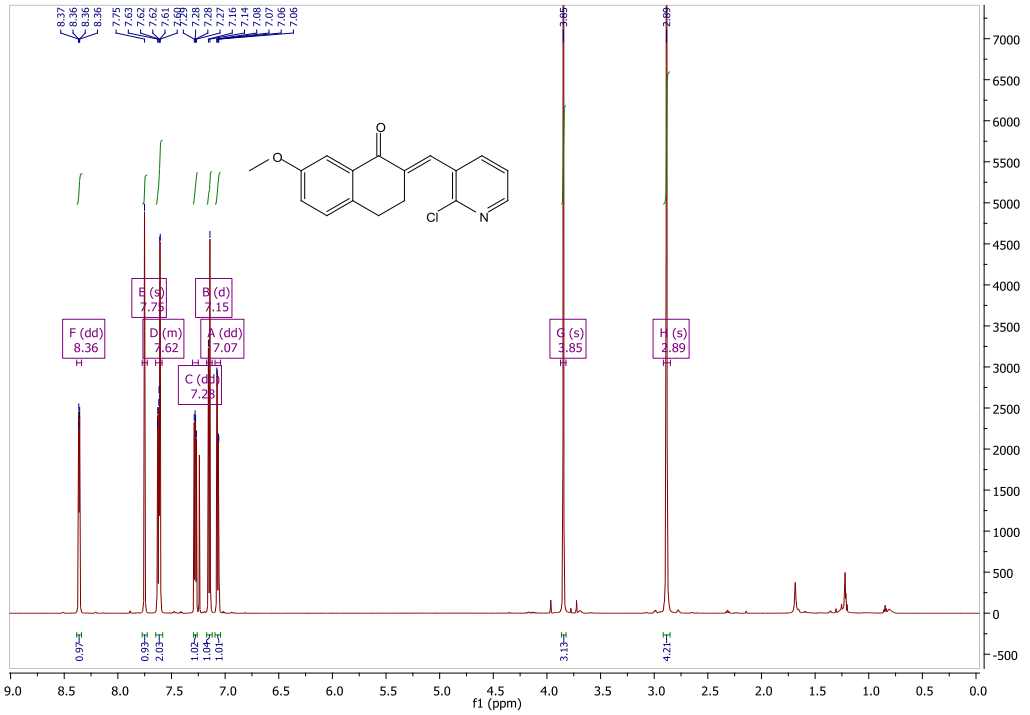


HPLC

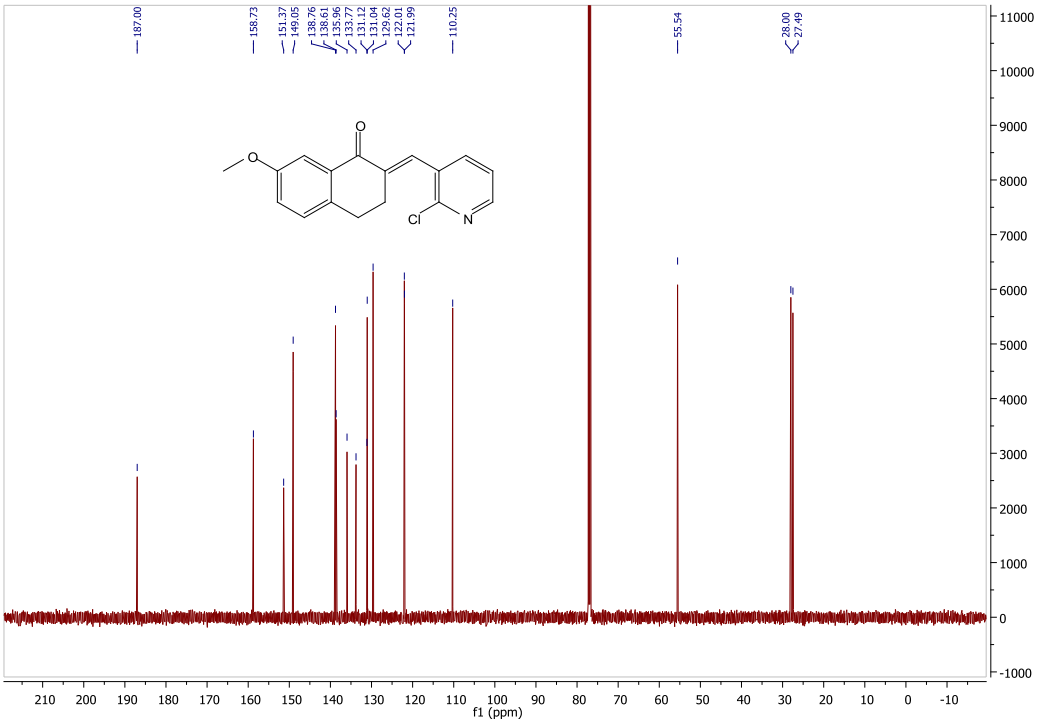


(2*E*)-2-[(2-Chloropyridin-3-yl) methylidene]-7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one (4g)

^{1}H NMR



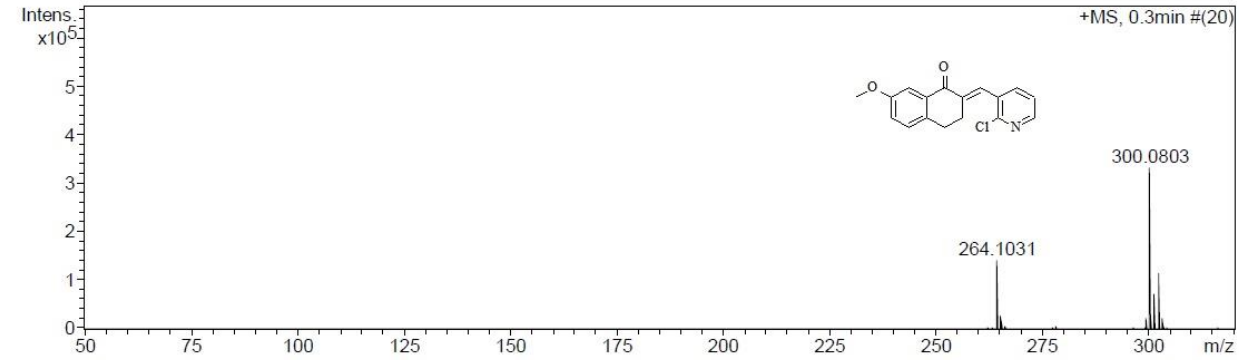
^{13}C NMR



APCI-MS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste



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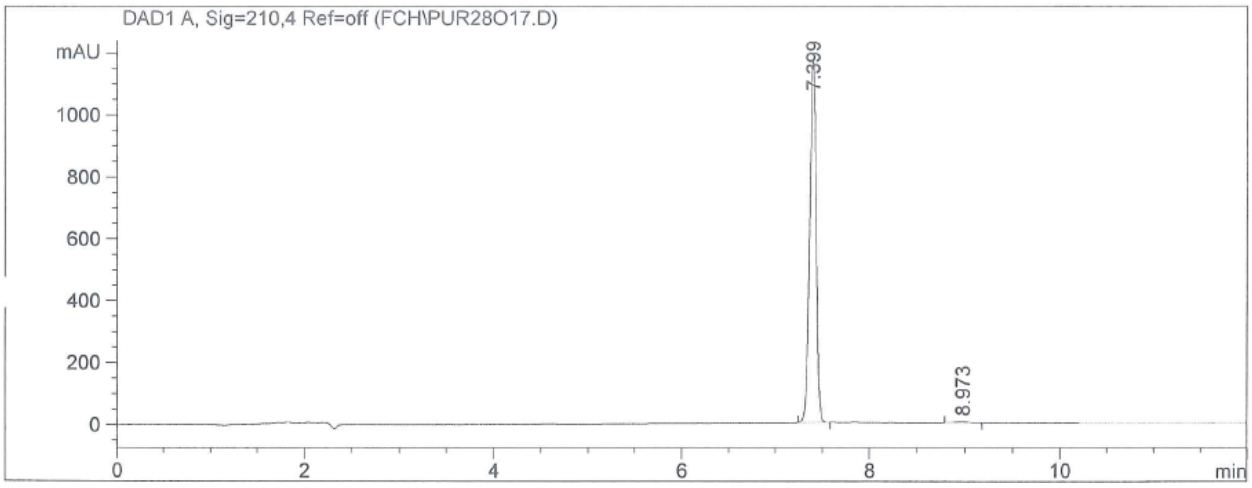
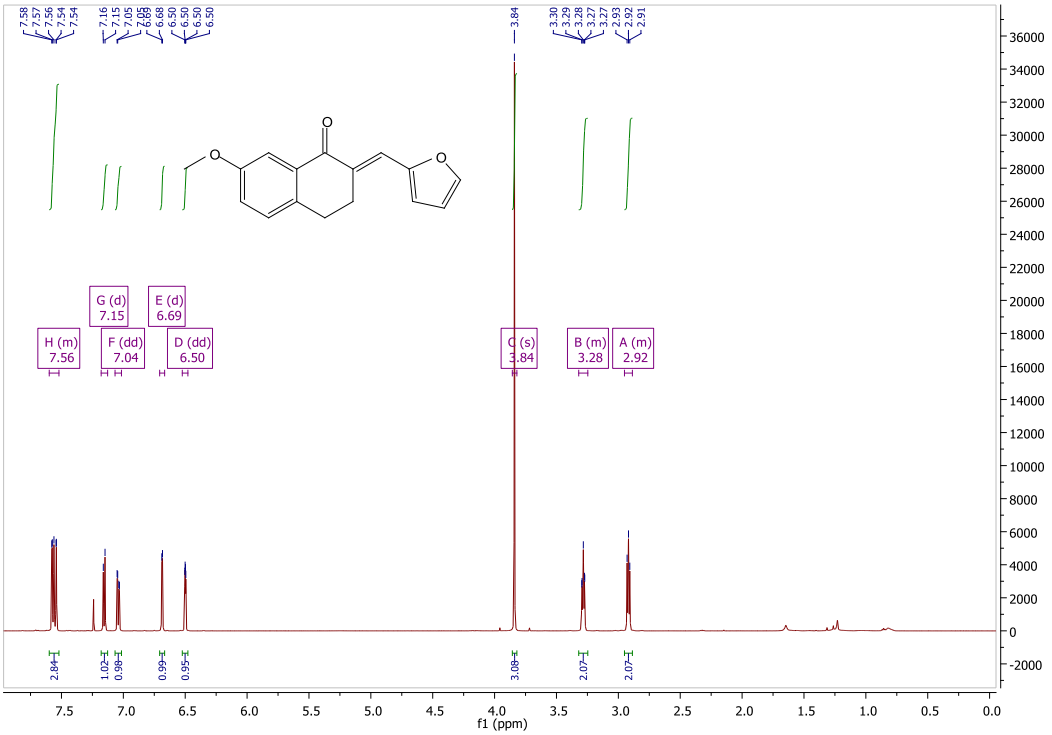
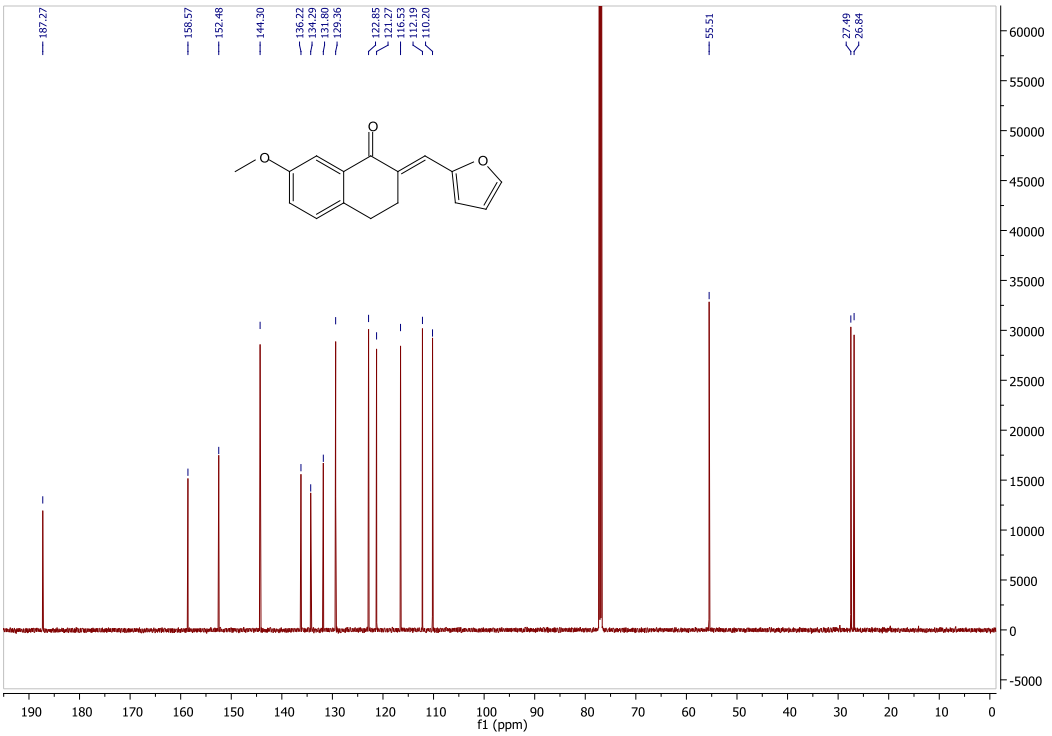


(2*E*)-2-(Furan-2-ylmethylidene)-7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one (4h)

^{1}H NMR



^{13}C NMR



APCI-MS

Acquisition Parameter

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Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
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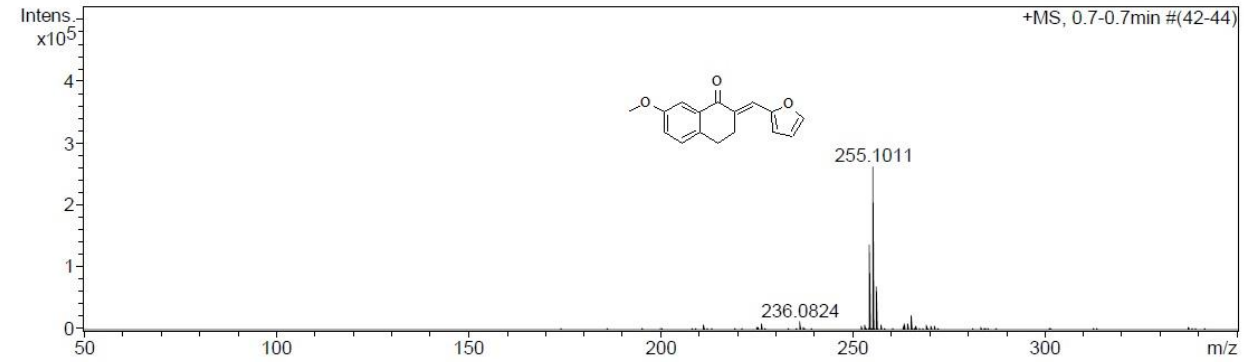
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste



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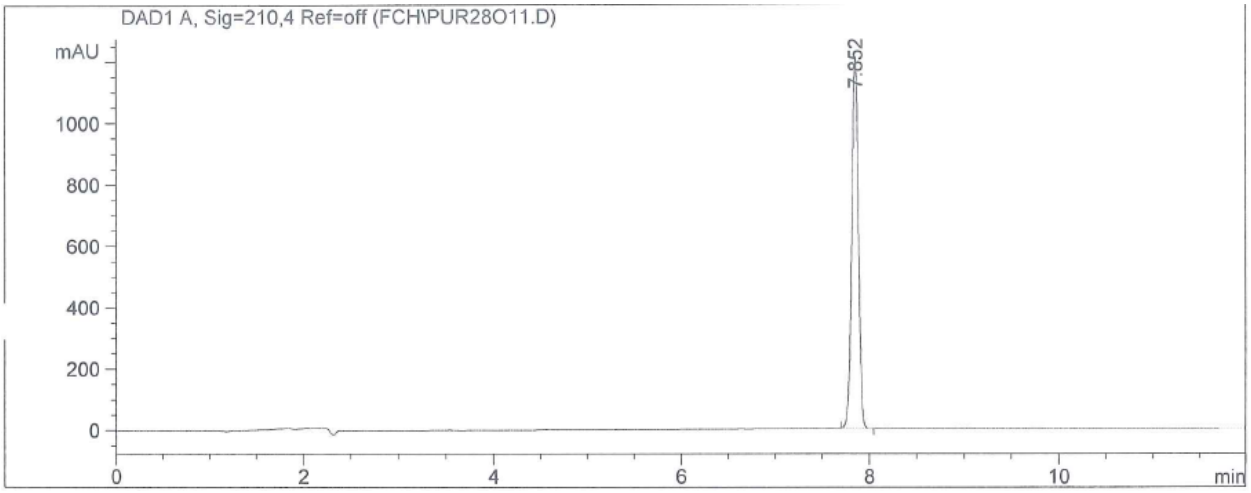
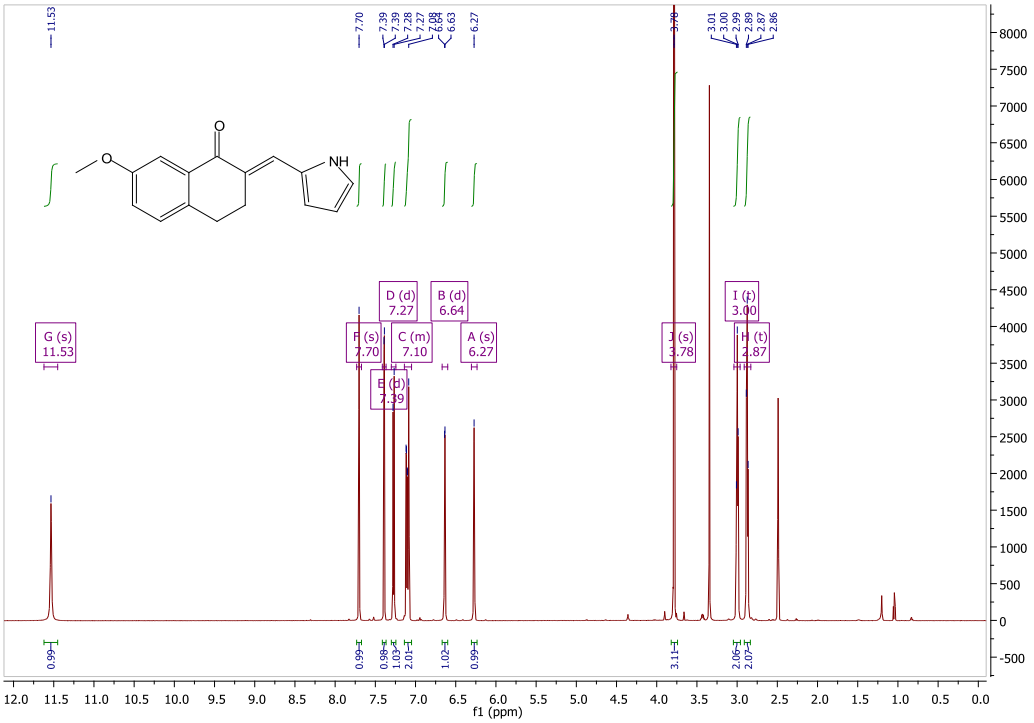
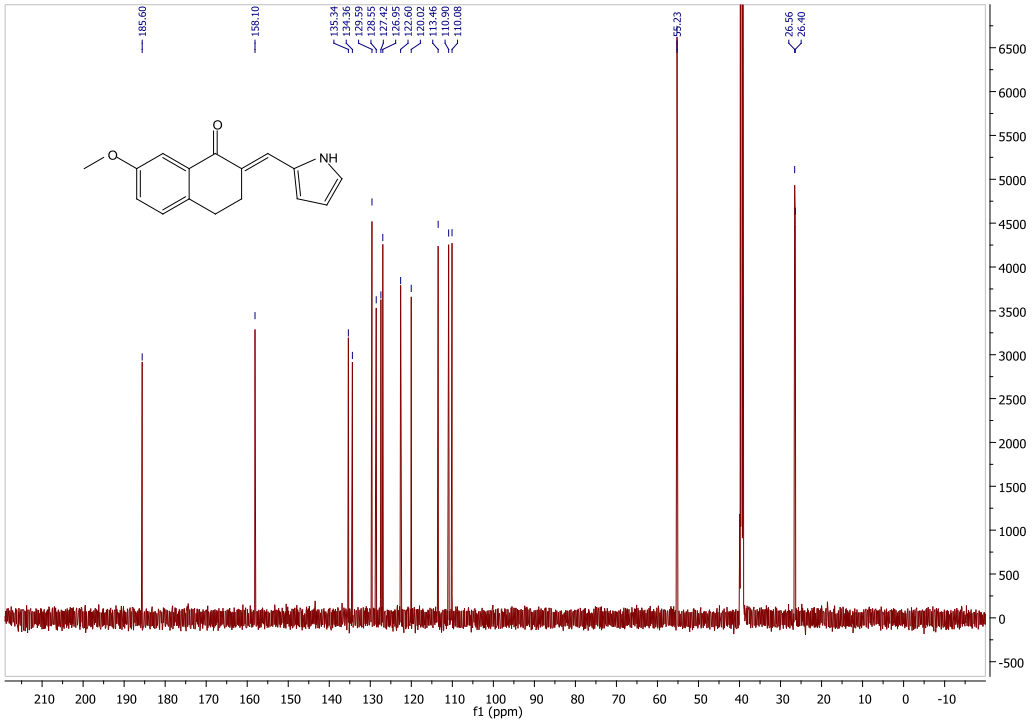


(2*E*)-7-Methoxy-2-(1H-pyrrol-2-ylmethylidene)-3,4-dihydronaphthalen-1(2*H*)-one (4i)

^{1}H NMR



^{13}C NMR



APCI-MS

Acquisition Parameter

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Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
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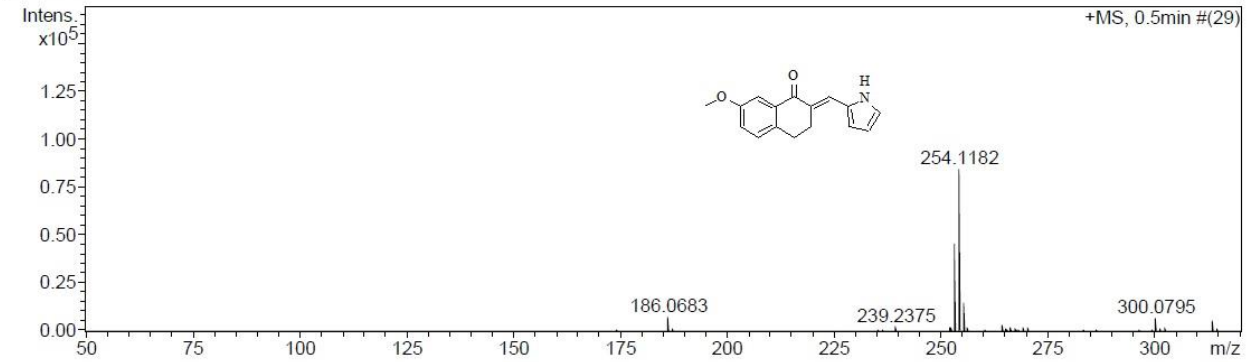
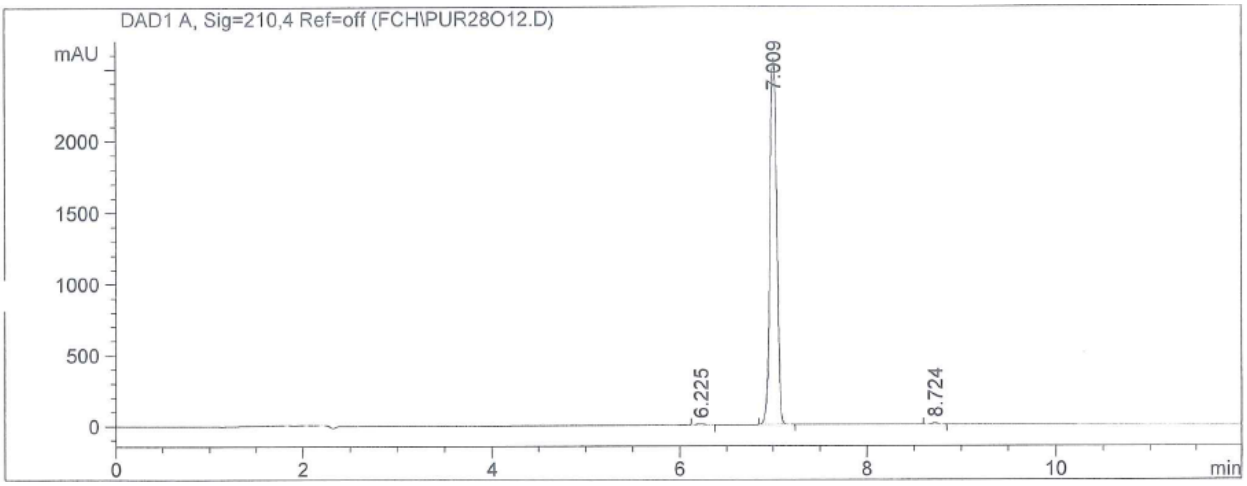
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste

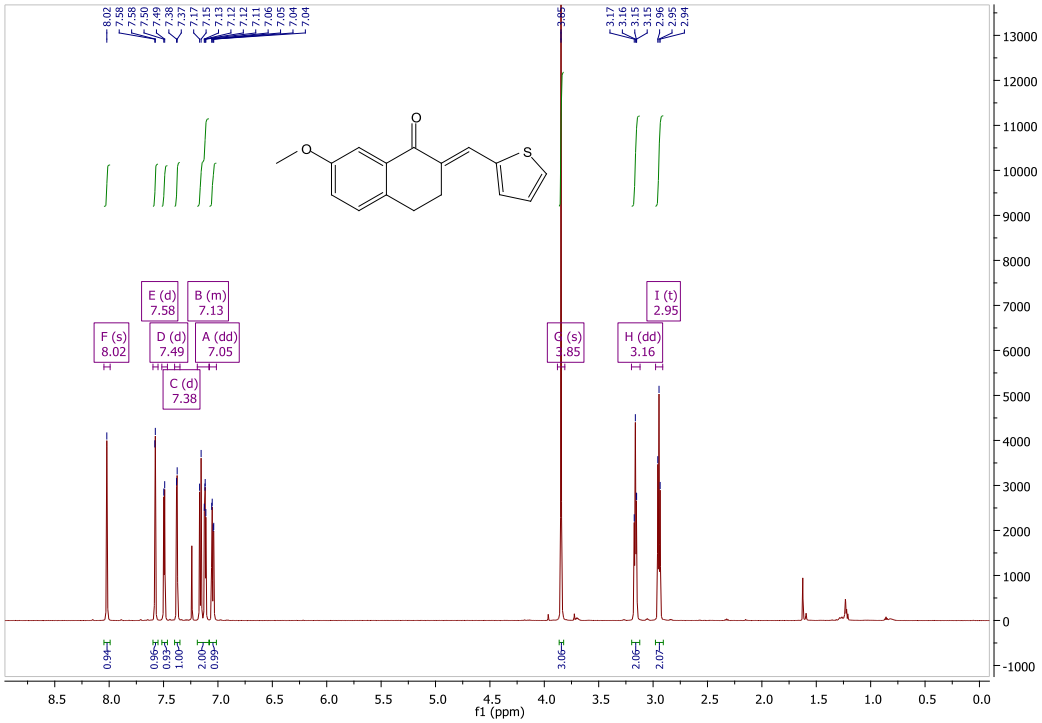


HPLC

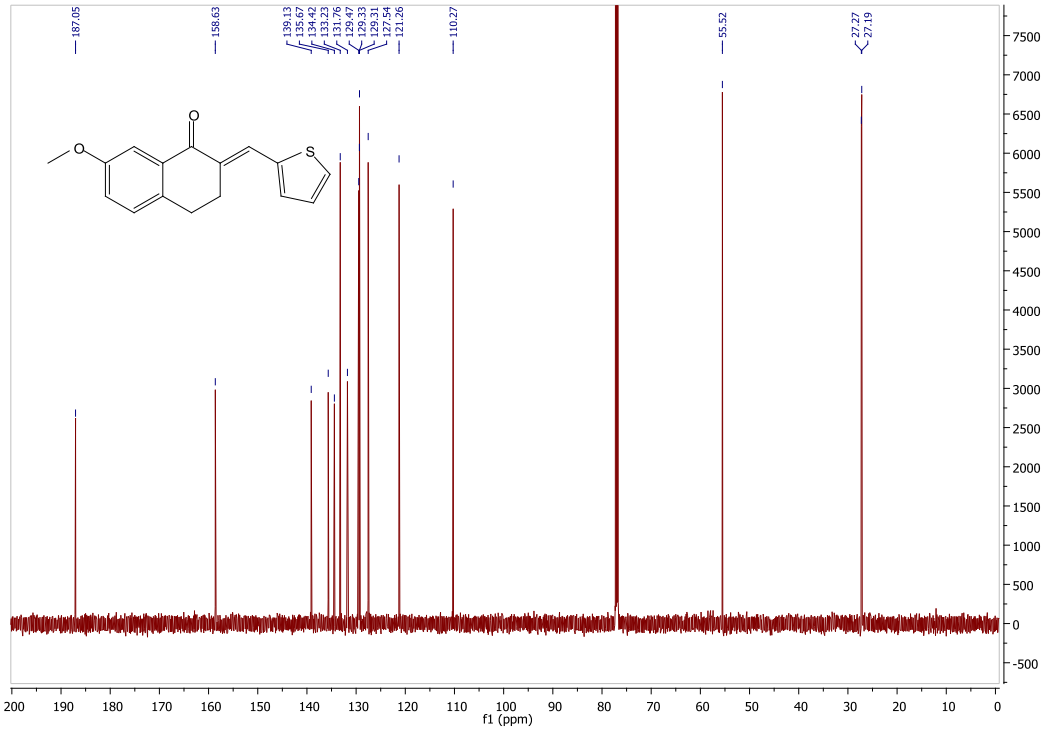


(2*E*)-7-Methoxy-2-(thiophen-2-ylmethylidene)-3,4-dihydronaphthalen-1(2*H*)-one (4j)

^{1}H NMR



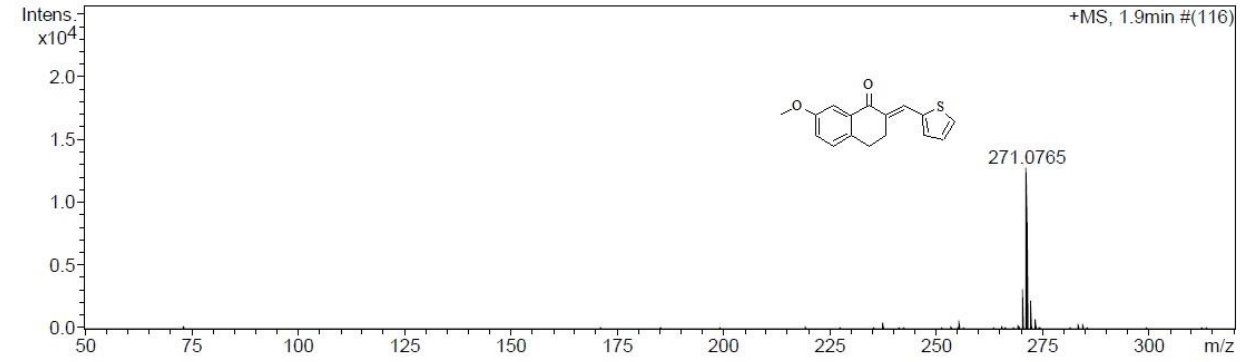
^{13}C NMR



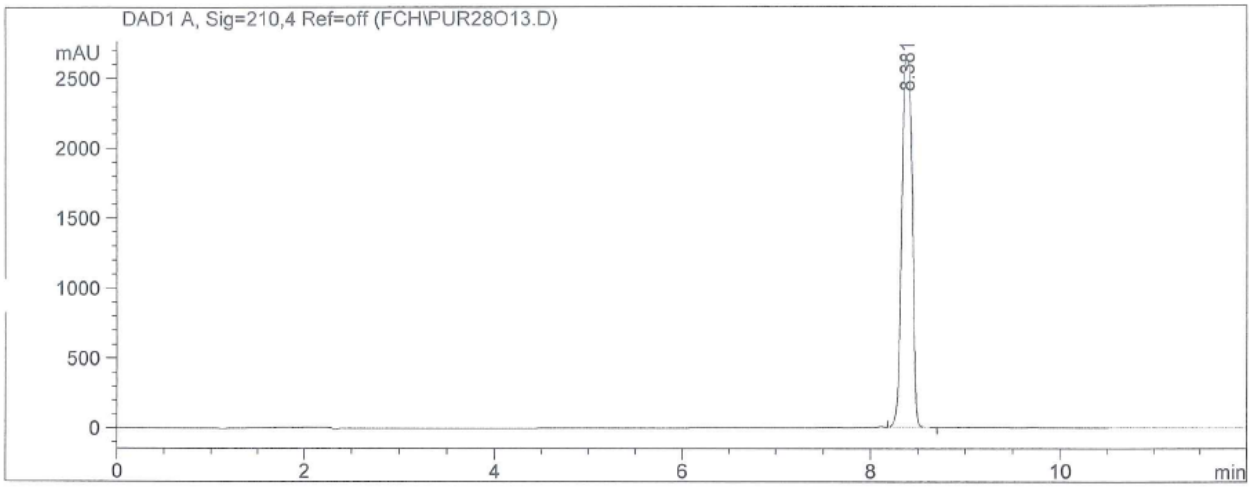
APCI-MS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste

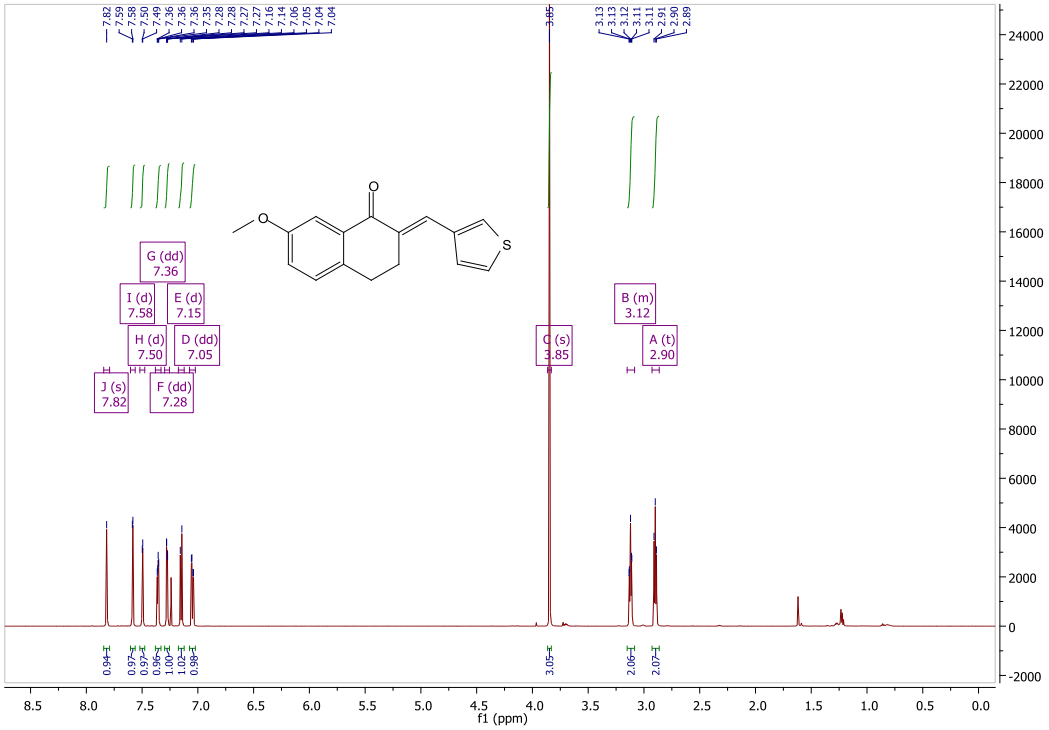


HPLC

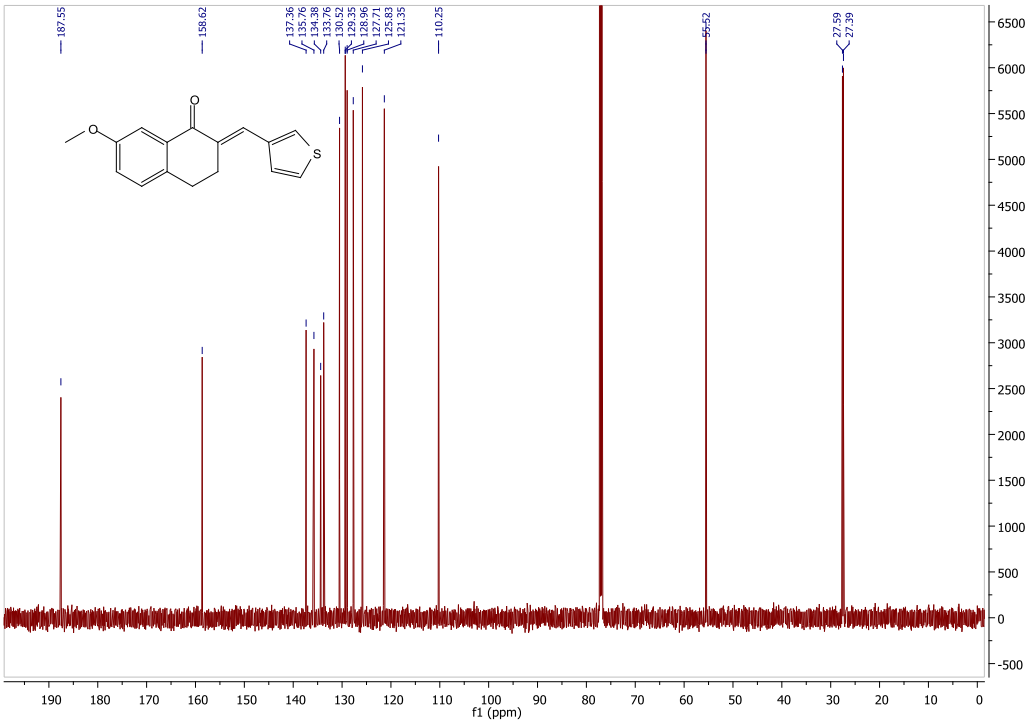


(2*E*)-7-Methoxy-2-(thiophen-3-ylmethylidene)-3,4-dihydronaphthalen-1(2*H*)-one (4k)

^{1}H NMR



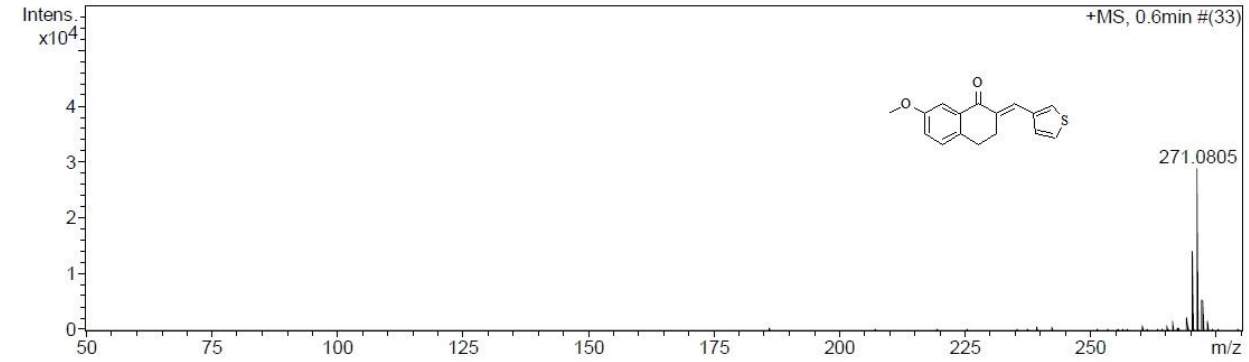
^{13}C NMR



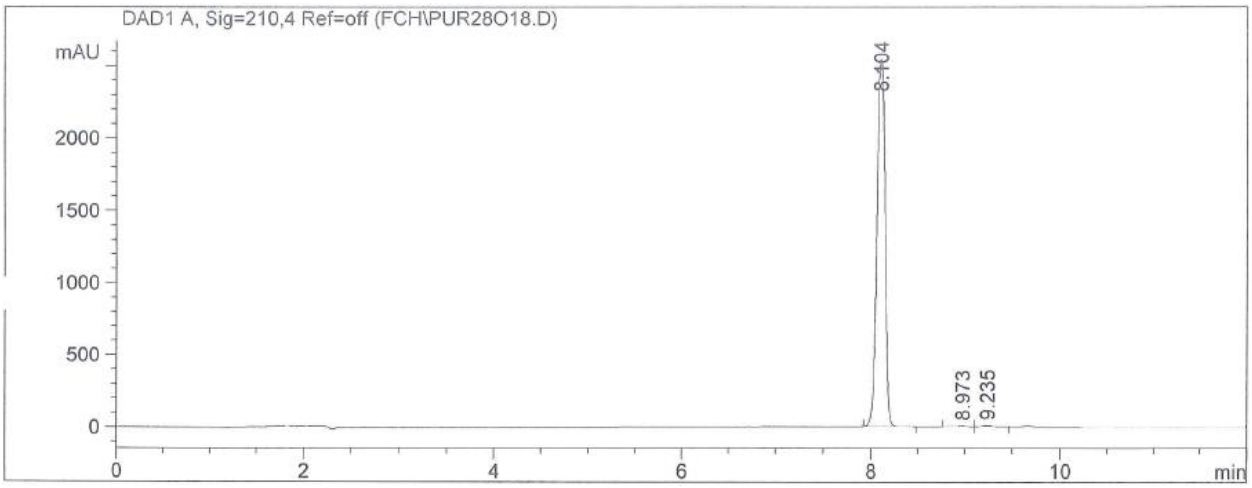
IAPCI-MS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste

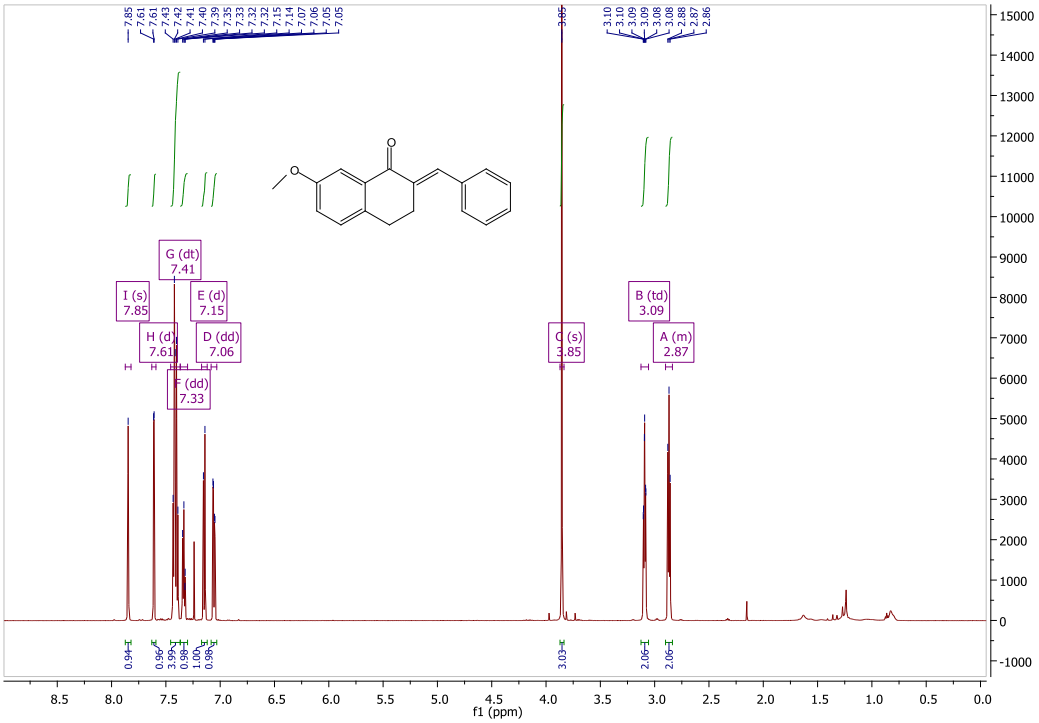


HPLC

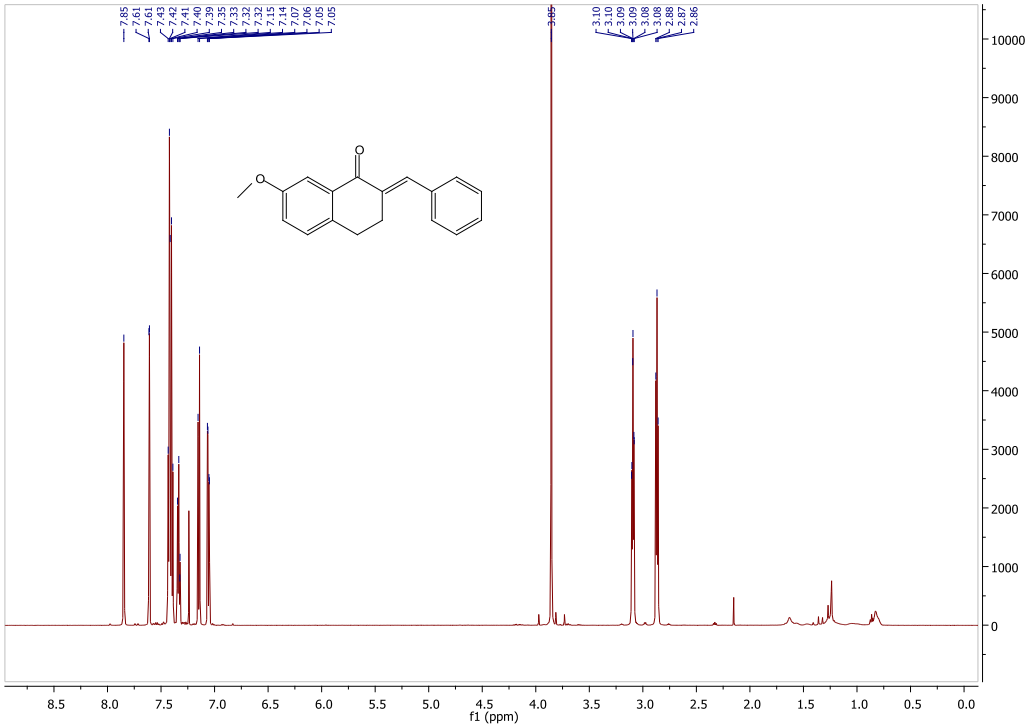


(2*E*)-2-Benzylidene-7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one (4l)

^{1}H NMR



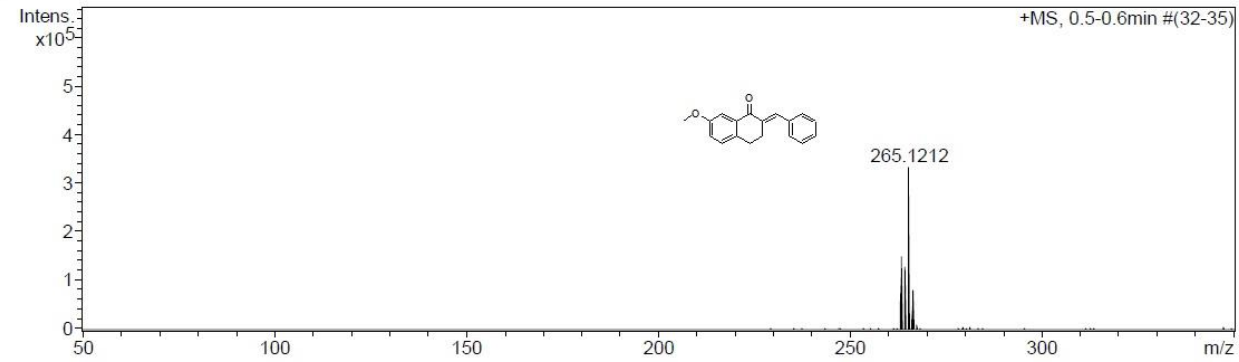
^{13}C NMR



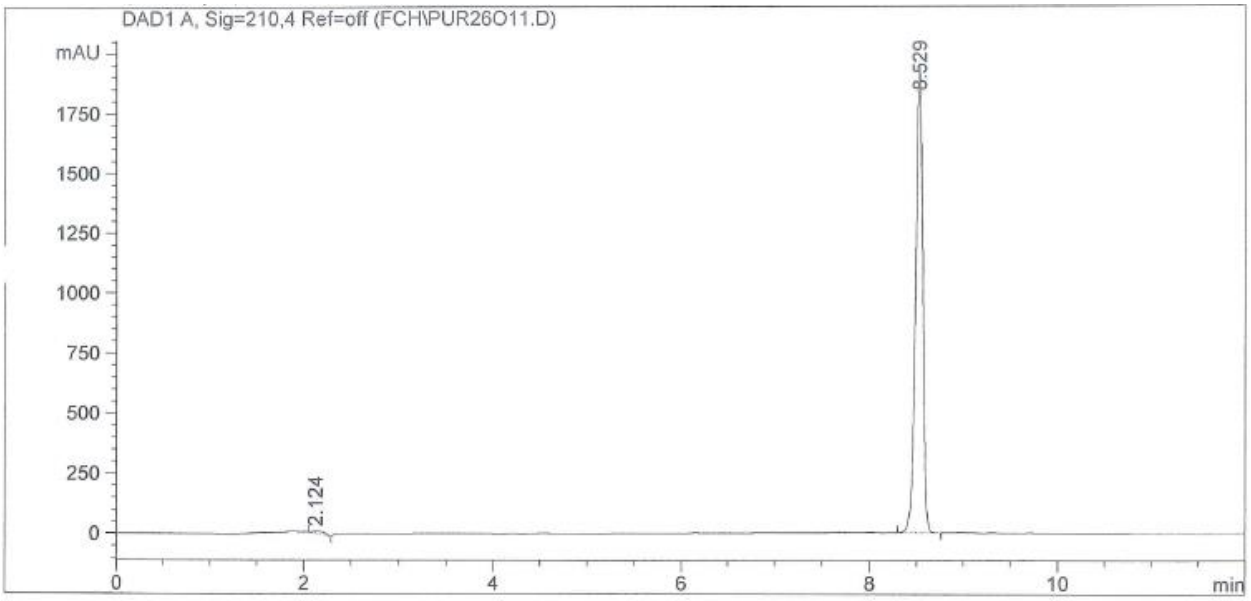
APCI-MS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.6 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	350.0 Vpp	Set Divert Valve	Waste



HPLC



CHAPTER 6: SUMMARY AND CONCLUSION

The MAO enzymes are important targets for the treatment of PD. PD is a disorder found mainly in the aged population and is characterised by the depigmentation of the SNpc which is caused by the selective and progressive degeneration of dopaminergic neurons (Lees *et al.*, 2009). The subsequent depletion of dopamine in the central regions leads to the abnormal regulation of the motor circuit and the clinical manifestation of PD (Dauer & Przedborski, 2003).

Since the discovery of levodopa as therapy for PD 45 years ago, levodopa has been the most effective therapy for the symptomatic treatment of PD. Levodopa constitutes dopamine replacement therapy (Thanvi & Lo, 2004). MAO exists as two distinct isoforms, MAO-A and MAO-B (Bach *et al.*, 1988). In the brain MAO-B predominates and represents a key metabolic pathway for DA. Thus, MAO-B inhibitors are currently used as adjuncts to levodopa in PD therapy (Fernandez & Chen, 2007). An important consideration of MAO inhibitors is the reversibility and selectivity of inhibition. Irreversible MAO-A inhibitors are used with caution in the clinic since these compounds may potentiate the sympathomimetic effects of dietary tyramine (Youdim & Bakhle, 2006). Tyramine, present in certain food such as cheese and wine, is metabolised by MAO-A in the gut and peripheral organs, which limits its entry into the systemic circulation. Irreversible MAO-A inhibitors prevent the peripheral breakdown of tyramine leading to excessive systemic concentrations and a potentially fatal increase in blood pressure termed the “cheese reaction” (Hubalek *et al.*, 2005; Finberg, 2014).These adverse effects can be as disabling as the disease itself and are often inevitable. This led to the application of other drugs as replacement and/or adjunct therapies to levodopa which are aimed at preventing or treating chronic levodopa complications. The drugs include amongst others dopamine agonists, peripheral dopa decarboxylase inhibitors, catechol-O-methyl-transferase inhibitors and MAO-B inhibitors. Unfortunately, some of these alternative therapies may also result in complications and therefore much research is aimed at discovering new potent and safe drugs for PD.

Chalcone (1,3-diphenyl-2-propen-1-one) derivatives have recently been shown to act as potent and selective inhibitors of MAO-B. Chalcones evolve naturally in edible plants and are the precursors of flavonoids and isoflavonoids (Patil *et al.*, 2009; Prashar *et al.*, 2012). This study further explored the SARs for chalcones as MAO inhibitors by introducing conformational restriction and heteroaromatic substitution. This was done by cyclising the structure of chalcone to yield three series of 2-benzylidene-1-tetralones as well as 2-heteroarylidene-1-tetralone derivatives as potential new classes of MAO inhibitors. It was hypothesised that cyclic chalcones would also act as inhibitors of MAO. The development of analogues with restricted or

rigid conformations may result in the selective binding to target sites, which could result in very active drugs with reduced unwanted adverse effects (Gareth, 2007).

The three series of chalcones studied were synthesised using the Claisen-Schmidt reaction. Reactions were catalysed by either acid (HCl) or base (NaOH/KOH/piperidine) and were conducted at room temperature. In most instances, methanol served as solvent (except the solvent-less piperidine catalysed reactions). Under these conditions the appropriately substituted cyclic ketones and aldehydes were reacted to yield characteristic 2-benzylidene-1-tetralone as well as 2-heteroarylidene-1-tetralone derivatives. The target compounds were characterised by ^{1}H NMR and ^{13}C NMR, APCI-MS. HPLC was used to estimate the chemical purities of all compounds which were mostly in the range 96−100%.

For the inhibition studies, the catalytic activities of MAO-A and MAO-B were determined by measuring the production of 4-hydroquinoline (4-HQ) from the oxidation of kynuramine by the MAOs. 4-HQ is fluorescent in basic media and can thus be measured by fluorescence spectrophotometry at endpoint, after alkalinisation of the enzyme reactions. The IC_{50} values from the three series studied indicate that the compounds are generally moderate inhibitors of MAO.

Series 1 explored the MAO inhibition properties of benzylidene-substituted indanones, tetralones, benzosuberones, chromones, chromanones and thiochromanones. Compound **1f**, a 1-indanone derivative, exhibited good MAO inhibitory activities with IC_{50} values of 0.346 µM and 0.420 µM for the inhibition of MAO-A and MAO-B, respectively. Since the 1-indanone derivative is the most potent MAO-A inhibitor, it was concluded that ring expansion is not desirable for MAO-A inhibition by cyclic chalcones. The IC_{50} values for the inhibition of MAO-B, shows that compound **1b**, the 4-chromanone derivative, is the most potent MAO-B inhibitor of the series with an IC_{50} value of 0.156 µM**.** It may thus be concluded that among the cyclic chalcones evaluated, **1b** is the most appropriate lead for the discovery of PD therapy.

The second series of the study further explored the MAO inhibition properties of 2-benzylidene-1-tetralone derivatives. In this respect, ten derivatives exhibited IC_{50} values for the inhibition of MAO-B <0.1 µM while only two derivatives, **2m** and **2p**, possess IC_{50} values for the inhibition for MAO-A <1 µM. The most potent MAO-B inhibitor, derivative **2u**, exhibited an IC_{50} value of 0.0064 µM. From this study it may be concluded that 7-hydroxy substitution on the A-ring combined with substitution on the B-ring with a variety of substituents (F, Cl, Br, CH_3, $N(CH_3)_2$,

OCH_3) yield particularly potent MAO-B inhibitors. In fact all 2-benzylidene-1-tetralone derivatives with this substitution pattern exhibited IC_{50} < 0.109 µM.

Finally the 2-heteroarylidene-1-tetralone derivatives examined in series 3 with the exception of **4g** display little isoform selectivity. Compound **4g** is a MAO-A selective inhibitor and it was also the most potent MAO-A inhibitor. The phenyl substituted derivative, **4l**, was the most potent MAO-B inhibitor of the series. In general the effect of substitution on MAO-B inhibition potency in decreasing order was: cyclohexyl, phenyl > thiophene > pyridine, furane, pyrrole, cyclopentyl. In addition, methoxy substitution on ring A (**4e**) yielded more potent MAO inhibition compared to the unsubstituted homologue (**4d**). This supports the notion that a combination of polar and lipophilic substituents on rings A and B, respectively, facilitate MAO inhibition.

Previous studies have confirmed that the chalcone class of compounds act as inhibitors of the MAOs, with specificity for the MAO-B isoform. Open-chain chalcones are considerably more potent MAO inhibitors than the derivatives of the current study. This suggests that introducing conformational restriction does not improve MAO inhibition compared to open-chain chalcones. Although the molecular basis for this behaviour is not clear, it may be suggested that conformational freedom and rotation of the *α,β*-unsaturated ketone moiety of chalcone, although limited, is important for establishing productive interactions with the MAO-B active site. Alternatively, the restricted derivatives are “frozen” in conformations that are not optimal for MAO-B binding compared to the open-chain chalcones. To illustrate this, the calculated three-dimensional structures (MMFF94) of chalcone derivative **1** and a 2-heteroarylidene-1-tetralone derivative (**4l**) in series 3 shows that **1** significantly deviates from planarity compared to chalcone **4l**, which is frozen in a coplanar conformation. The flexibility of **1** compared to **4l** may, in part, explain its ability to better bind to the MAO-B active site.

In conclusion, although not as potent as open-chain chalcones, some ring-closed derivatives of the present study possess promising MAO inhibition properties, and may find application in the treatment of PD. However, reversibility of MAO inhibition and toxicological studies are required to confirm these compounds’ viability as potential PD therapeutic drugs. Determination of reversibility of MAO inhibition is especially essential to establish safety, particularly with respect to the hypertensive response elicited by irreversible MAO-A inhibitors when taken with dietary tyramine (Da Prada, 1988). Adding to that more SARs could unfold cyclic structures with better biological and chemical interactions with the MAO enzymes which can result in more potent compounds for application as antiparkinsonian drugs.

REFERENCES

Bach, A.W.J., Lan, N.C., Johnson, D.L., Abell, C.W., Bembenek, M.E., Kwa, S., Seeburg, P.H. & Shih, J.C. 1988. cDNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymatic properties. *Proceedings of the national academy of sciences of the United States of America*, 85(13):4934-4938.

Da Prada, M., Zürcher, G., Wüthrich, I. & Haefely, W.E. 1988. On tyramine, food, beverages and the reversible MAO inhibitor moclobemide. *Journal of neural transmission supplementa*, 26:26.

Dauer, W. & Przedborski, S. 2003. Parkinson's disease: mechanisms and models. *Neuron*, 39(6):889-909.

Fernandez, H.H. & Chen, J.J. 2007. Monoamine oxidase-B inhibition in the treatment of Parkinson's disease. *Pharmacotherapy*, 27(12 pt 2):174-185.

Finberg, J.P.M. 2014. Update on the pharmacology of selective inhibitors of MAO-A and MAO-B: focus on modulation of CNS monoamine neurotransmitter release. *Pharmacology & therapeutics*, 143(2):133-152.

Gareth, T. 2007. Medicinal chemistry: an introduction. Chichester: Wiley. http://www.wiley.com/WileyCDA/WileyTitle/productCd-0470025972.html. Date of access: 31 October 2016.

Hubálek, F., Binda, C., Khalil, A., Li, M., Andrea Mattevi, A., Castagnoli, N. & Edmondson, D.E. 2005. Demonstration of isoleucine 199 as a structural determinant for the selective inhibition of human monoamine oxidase B by specific reversible inhibitors. *Journal of biological chemistry*, 280(16):15761-15766.

Lees, A.J., Hardy, J. & Revesz, T. 2009. Parkinson's disease. *The Lancet*, 373(9680):2055-2066.

Patil, C.B., Mahajan, S.K. & Katti, S.A. 2009. Chalcone: a versatile Molecule. *Journal of pharmaceutical sciences and research*, 1(3):11-22.

Prashar, H., Chawla, A., Sharma, A.K. & Kharb, R. 2012. Chalcone as a versatile moiety for diverse pharmacological activities. *International journal of pharmaceutical sciences and research*, 3(7):1000-1014.

Thanvi, B. & Lo, T. 2004. Long term motor complications of levodopa: clinical features, mechanisms, and management strategies. *Postgraduate medical journal*, 80(946):452-458.

Youdim, M.B.H. & Bakhle, Y.S. 2006. Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness. *British journal of pharmacology*, 147(Suppl. 1):S287-S296.

ANNEXURE A : GUIDES TO AUTHORS

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

The Tetrahedron Journal for Research at the Interface of Chemistry and Biology

AUTHOR INFORMATION PACK

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AUTHOR INFORMATION PACK

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Books without editor:

3. Doe, J. S.; Smith, J. J. Bioorganic Chemistry; Pergamon: Oxford, 1990, Chapter 6.

Theses:

4. Doe, J. S. Ph.D. Thesis, University of California at San Diego, January 2000.

Patent/Chem. Abstract:

5. Lyle, F. R. U.S. Patent 6,973,257, 1995; Chem. Abstr. 1995, 123, 2870.

Abstract of meeting papers:

6. Doe, J. S. Abstract of Papers, 195th National Meeting of the American Chemical Society, Anaheim, CA; American Chemical Society: Washington, DC, 1995; Abstract 3028.

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