

Monoamine oxidase inhibition by indanone and benzoquinone analogues

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Preface

This doctoral thesis is submitted in article format. All four articles are research articles and have been prepared for submission to either one of the following journals, ChemMedChem and Chemical Biology and Drug Design. The article guidelines for the respective journals are included in the addendum. The first article, chapter 4, has already been published and the third article, chapter 6, was submitted during compilation of this thesis. The remaining two chapters 5 and 7 are presented as “ready for submission”. All scientific research for this thesis was conducted by Ms S. Mostert as stated at the start of these chapters. The relevant contribution of co-authors are also stated as well as permission granted by the respective journals for the inclusion of these articles in this thesis.

I would like to express my gratitude to the North-West University, especially towards the School of Pharmacy for granting me the opportunity to pursue doctoral studies. During the period of this study, support and assistance was of great value, and I would like to thank all the people who helped me to complete this thesis. Members of the Sasol Centre for Chemistry for the numerous NMR and MS spectra. Prof JL du Preez at the Analytical Technology Laboratory for his assistance in recording HPLC spectra. The NRF for their highly appreciated financial support. My promoter, JP Petzer, and co-promoter, A Petzer for their valuable assistance during the whole study period. Lastly, friends and family for their patience, motivation and support, especially during hardships.

***The road was not always paved but through the
trudges we come and we conquered what we set
out to achieve in the first place, determination is
the key.***

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Abstract

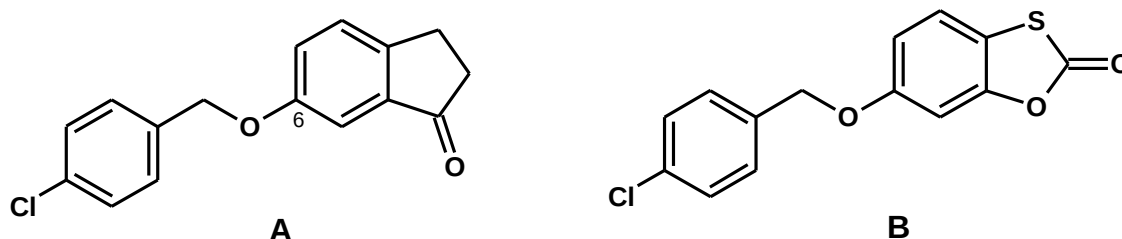
Keywords: Monoamine oxidase; Parkinson's disease; Inhibition; Indanones; Benzoxathiolones; Naphthoquinones; Benzoquinones.

Parkinson's disease (PD) is a neurological disorder of which aging is the greatest risk factor. Over the next fifteen years, the number of persons affected by PD worldwide is expected to increase from 4.6 million to 9.3 million. Although PD is not fatal, it seriously inhibits a patient's quality of life. PD is a debilitating, incurable disease of which only symptomatic treatment is available. In PD the nigrostriatal neuronal pathway degenerates leading to central dopamine deficiency, primarily in the striatal area. Symptoms of PD only present when 70-80% of dopamine in the brain has deteriorated. Levodopa, the metabolic precursor of dopamine, is the first-line treatment of PD. Unlike dopamine, which is primarily metabolised in the periphery, levodopa is able to cross the blood-brain barrier where it is metabolised to yield dopamine in the brain. This replenishes dopamine levels and results in relief of PD symptoms. Unfortunately levodopa is extensively metabolised in the periphery, which reduces its efficacy and for this reason, levodopa is combined with medications that inhibit its metabolism in the periphery. An alternative approach is to combine levodopa with medications that block the metabolism of dopamine, thereby increasing the central dopamine levels after levodopa treatment. The monoamine oxidase (MAO) enzymes are key dopamine metabolising enzymes in the brain and MAO inhibitors are thus used as adjuncts to levodopa in PD therapy. These enzymes consist of two isoforms, namely MAO A and MAO B, which are 70% identical on the amino acid sequence level. In spite of their similarity the MAOs have unique substrate specificities and are thus targets for different disease states. For example, inhibitors of MAO A have been employed as antidepressant agents since MAO A is a major metabolic enzyme of serotonin in the brain. MAO B inhibitors are used in PD therapy since MAO-B is the major metabolic enzyme of dopamine in the brain. It has also been found that many PD patients present with undiagnosed depression and that the dual inhibition of both MAO A and MAO B may be of enhanced value in the treatment of PD.

Based on the limited availability of drugs approved for the symptomatic treatment of PD, there exists a need for new therapies for PD. This thesis therefore aims to contribute in this regard by investigating the potential MAO inhibitory potencies of four chemical classes. New effective MAO inhibitors may represent candidates for the treatment of PD. For the purpose of this thesis, selected compounds of each class were synthesised or in some instances obtained from commercial sources, and their respective IC_{50} values for the inhibition of the human MAOs were determined *in vitro*. For selected compounds the modes of inhibition (e.g. competitive) and the reversibility of inhibition were examined. The reversibility of inhibition is an important consideration in the design of MAO inhibitors, especially MAO A inhibitors. It has been found that the irreversible

inhibition of MAO A in the periphery may lead to a severe and potentially fatal side effect, termed the “cheese reaction”. Irreversible MAO A inhibition blocks the metabolism of dietary tyramine (found in foods such as cheese) leading to increased systemic concentrations of tyramine. Since tyramine is a sympathomimetic amine, the result is a dangerous increase in blood-pressure. Reversible inhibitors, on the other hand, do not cause tyramine-induced changes in blood-pressure, since the inhibitors can be displaced from enzyme binding sites as substrate concentration increases.

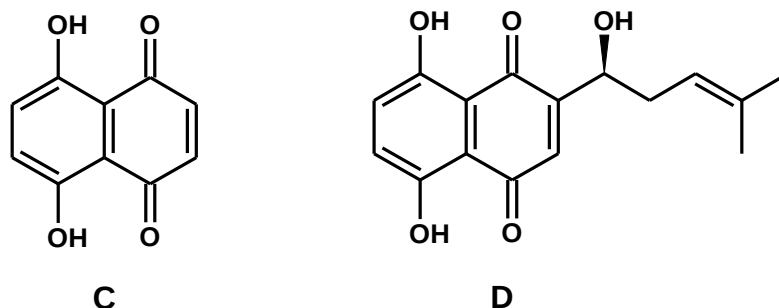
The first article focused on the synthesis of 1-indanones substituted on the C5 and C6 positions. Also included is a related series of indane derivatives. This study found that these compounds are high potency MAO inhibitors with a selectivity preference towards MAO B. The most potent inhibitors were the series of 1-indanones substituted on the C6 position. These compounds exhibited IC_{50} values ranging from 0.001-0.030 μ M for the inhibition of MAO B and 0.032-1.348 μ M for the inhibition of MAO A. Although the 1-indanones and indanes were selective inhibitors of MAO B, a number of compounds, such as 6-(4-chlorobenzoyloxy)-2,3-dihydro-1*H*-inden-1-one (**A**) may be classified as dual MAO inhibitors. This compound inhibits MAO A and MAO B with IC_{50} values of 0.032 μ M and 0.002 μ M, respectively. Further investigation showed that selected 1-indanones are reversible and competitive inhibitors of the MAOs, however, 1-indanones may possibly display tight-binding towards MAO B.



The second article investigated the human MAO inhibitory properties of a series of benzoxathiolones derivatives, which are structurally related to the 1-indanones. It was found that the benzoxathiolones are also high potency inhibitors of MAO B with IC_{50} values ranging from 0.003 to 0.051 μ M. 6-(4-Chlorobenzoyloxy)-1,3-benzoxathiol-2-one (**B**) is an example of a dual inhibitor with IC_{50} values of 0.189 μ M for the inhibition of MAO A and 0.003 μ M for the inhibition of MAO B. As with the 1-indanones, selected benzoxathiolones were found to be reversible and competitive inhibitors of the MAOs.

The third article investigated the MAO inhibition properties of 1,4-naphthoquinone derivatives. This study is based on a literature report that 2,3,6-trimethyl-1,4-naphthoquinone, isolated from flue cured tobacco leaves, is a non-specific MAO inhibitor. The most potent inhibitor of the present study was 5,8-dihydroxy-1,4-naphthoquinone (**C**) with an IC_{50} value of 0.860 μ M for the inhibition of MAO B. Another compound, shikonin (**D**), which is a component of chinese herbal medicine, was found to be a dual inhibitor with IC_{50} values of 1.5 μ M and 1.01 μ M for the inhibition of MAO A and

MAO B, respectively. Interestingly this compound was also previously investigated for cancer therapy. Literature suggests that MAO inhibitors are not only useful in PD and depression, but may find application in cancer and congestive heart failure. By mechanisms of DNA intercalation and MAO inhibition, 1,4-naphthoquinones may be of particular relevance in PD.



In the fourth article of this thesis, a series of 1,4-benzoquinone derivatives were synthesised and evaluated as MAO inhibitors. This thesis found the 1,4-benzoquinone compounds are moderate inhibitors of both MAOs. These derivatives inhibit MAO A and MAO B with IC_{50} values of 5.03-13.2 μ M and 3.69-23.2 μ M for MAO A and MAO B, respectively. Although these compounds are not considered to be highly potent MAO inhibitors, these inhibition potencies are still similar to clinically used inhibitors such as toloxatone, a MAO A inhibitor. An interesting finding was that in contrast to the 1,4-naphthoquinone compounds, 1,4-benzoquinones bind irreversibly to MAO A. This is the first report of irreversible inhibition for MAO by a quinone compound. This thesis proposes that 1,4-benzoquinones react with a nucleophile within the MAO A active site, thereby modifying the enzyme covalently. The reduced flavin cofactor may act as such a nucleophile.

This thesis therefore discovered a number of new MAO inhibitors from four chemical classes. Using molecular modelling, in certain instances, important insights were gained into the binding modes of these inhibitors to the active sites of the MAOs. In addition, useful structure-activity relationships of MAO inhibition by the selected classes of inhibitors were derived. Of particular note is that, among 1-indanone and benzoxathiolone analogues are compounds that inhibit both MAOs. Such compounds may thus find application in the treatment of PD patients also presenting with depression. This thesis thus contributes to the discovery of new MAO inhibitors, compounds that are relevant in the treatment of PD.

Uittreksel

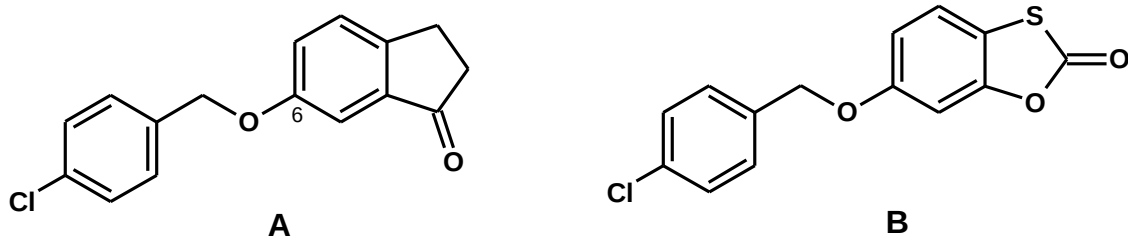
Sleutelwoorde: Monoamienoksidase; Parkinson se siekte; Inhibisie; Indanone; Bensoksatiolone; Naftakinone; Bensokinone.

Parkinson se siekte (PS) is 'n neurologiese siekte waarvan ouderdom die grootste risiko faktor is. Daar word voorspel dat oor die volgende vyftien jaar, die aantal persone in die wêreld met PS vanaf 4.6 miljoen tot 9.3 miljoen sal toeneem. Alhoewel die siekte nie tot die dood lei nie, kan dit 'n persoon se lewenskwaliteit inkort. PS is ongeneesbaar en slegs simptomatiese behandeling is tans beskikbaar. In PS degenerereer die nigrostriatale neurone wat tot 'n dopamientekort in die sentrale gedeelte van die brein lei, spesifiek in die striatum. PS simptome verskyn eers nadat dopamienvlakke in die brein tot 70-80% verlaag het. Levodopa, die metaboliese voorloper van dopamien, is die eerstelinie behandeling vir die simptome van PS. Anders as dopamien, wat hoofsaaklik in die periferie gemetaboliseer word, kan levodopa die bloedsreinskan oorsteek waar dit na dopamien gemetaboliseer word. Hierdie behandeling verhoog dopamienvlakke in die brein en bring sodoende verligting van die simptome van PS. Ongelukkig word levodopa ook tot 'n groot mate in die periferie gemetaboliseer wat tot gevolg het dat levodopa se effektiwiteit verlaag. Om hierdie rede word levodopa gekombineer met medikasie wat levodopa se metabolisme in die periferie onderdruk. 'n Ander benadering is om levodopa te kombineer met medikasie wat die metabolisme van dopamien in die brein onderdruk en sodoende tot verhoogde dopamienkonsentrasies in die brein lei na die toediening van levodopa. Monoamienoksidase (MAO) is die belangrikste ensieme wat dopamien in die brein metaboliseer, en inhibeerders van dié ensieme kan as bykomende behandeling tot levodopa gebruik word. Hierdie ensieme bestaan uit twee isovorme naamlik, MAO A en MAO B, wat 70% identies is wanneer aminosuurvolgordes vergelyk word. Ten spyte van hul ooreenkomste, het die MAO-ensieme unieke substraatspesifisiteite en die ensieme kan dus afsonderlik geteiken word vir verskillende sieketoestande. Byvoorbeeld, inhibeerders van MAO A word aangewend as antidepressante aangesien MAO A serotonien in die brein metaboliseer. Inhibeerders van MAO B word gebruik vir die behandeling van PS omdat MAO B dopamien in die brein metaboliseer. In PS pasiënte met ongediagnoseerde depressie sal nonselektiewe MAO-inhibeerders van waarde wees vir die behandeling van beide motor- en depressiesimptome.

Daar is egter slegs 'n beperkte aantal geneesmiddels beskikbaar vir die simptomatiese behandeling van PS, en gevolglik bestaan daar 'n behoefte vir die ontdekking van nuwe behandelingstrategieë. Hierdie proefskrif poog om 'n bydra te lewer deur vier chemiese klasse as potensiële MAO-inhibeerders te ondersoek. Nuwe en meer effektiewe inhibeerders van MAO mag kandidate wees vir die toekomstige behandeling van PS. Verbindings is uit elke klas gesintetiseer of in sekere gevalle aangekoop. Elke verbinding se IC_{50} -waarde vir die inhibering van die menslike

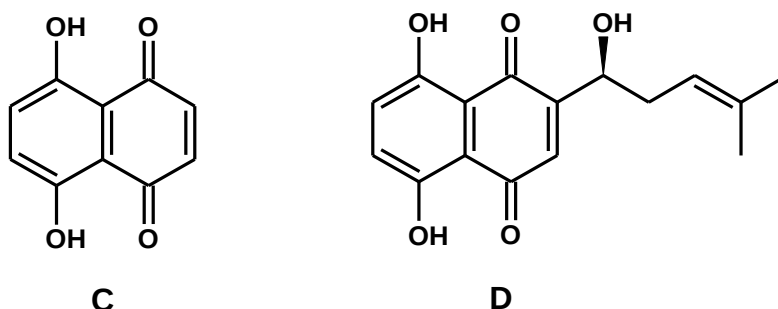
MAO-ensieme is *in vitro* bepaal. Vir sommige verbindings is die omkeerbaarheid sowel as meganisme van inhibisie bepaal. Omkeerbaarheid van MAO-inhibisie is 'n baie belangrike faktor wat in ag geneem moet word wanneer MAO-inhibeerders ontwerp word, veral MAO A-inhibeerders. Onomkeerbare inhibisie van MAO A in die periferie mag lei tot 'n ernstige newe-effek, die sogenoemde “kaasreaksie”. Onomkeerbare inhibisie van MAO A verhoed die metabolisme van tiramien wat in kos soos kaas gevind word. Die gevolg is verhoogde konsentrasies tiramien in die sistemiese sirkulasie, en omdat tiramien 'n simpatomimetiese amien is, kan dit lei tot 'n gevaarlike styging in bloeddruk. Omkeerbare inhibeerders daarenteen lei nie tot tiramien-geïnduseerde verhoging in bloeddruk nie omdat die inhibeerder uit die ensiem se bindingsetel verplaas kan word sodra die konsentrasie van die substraat verhoog.

Die eerste artikel van hierdie proefskrif fokus op die sintese van 1-indanoonderivate wat op die C5- en C6-posisies gesubstitueer is. Ook ingesluit was 'n verwante reeks van indaanderivate. Hierdie studie bevind dat die verbindings hoogs potente inhibeerders van MAO is en dat die MAO B-isoform by voorkeur geïnhibeer word. Die mees potente inhibeerders is die reeks 1-indanoonderivate wat gesubstitueer is op die C6-posisie. Hierdie verbindings toon IC_{50} -waardes van 0.001-0.030 μM vir die inhibisie van MAO B en 0.032-1.348 μM vir die inhibisie van MAO A. Alhoewel die 1-indanoon- en indaanderivate selektiewe MAO B-inhibeerders is, kan sekere verbindings, soos byvoorbeeld 6-(4-chlorobensieloksie)-2,3-dihidro-1*H*-inden-1-oon (**A**), geklassifiseer word as dubbelwerkende MAO-inhibeerders. Hierdie verbinding inhibeer die MAO-ensieme met IC_{50} -waardes van 0.032 μM vir MAO A en 0.002 μM vir MAO B. Verdere ondersoek het bewys dat geselekteerde 1-indanoonderivate omkeerbare en kompeterende inhibeerders van MAO is.



Die tweede artikel het 'n reeks bensoksatioloonderivate ondersoek as moontlike inhibeerders van menslike MAO. Hierdie verbindings is struktureel verwant aan die bogenoemde 1-indanoonderivate. Die studie het gevind dat die bensoksatiolone ook hoogs potente inhibeerders van MAO B is met IC_{50} -waardes van 0.003-0.051 μM . Die verbinding, 6-(4-chlorobensieloksie)-1,3-bensoksatiol-2-oon (**B**), is 'n voorbeeld van 'n dubbelwerkende inhibeerder met IC_{50} -waardes van 0.189 μM vir die inhibisie van MAO A en 0.003 μM vir die inhibisie van MAO B. Soos vir die 1-indanoonderivate is daar ook gevind dat die bensoksatiolone omkeerbare en kompeterende inhibeerders van MAO is.

Die derde artikel bestudeer die MAO-inhibisie eienskappe van 1,4-naftakinoonderivate. Dié studie is afgelei uit 'n vorige studie wat bevind het dat 2,3,6-trimetielfnaftakinoon, 'n verbinding wat uit tabakblare geïsoleer is, 'n nie-spesifieke inhibeerder van MAO is. In die huidige studie is gevind dat 5,8-dihidroksie-1,4-naftakinoon (**C**) die mees potente inhibeerder van MAO B is met 'n IC_{50} -waarde van $0.860\ \mu\text{M}$. Shikonien (**D**) inhibeer beide MAO A en MAO B met IC_{50} -waardes van $1.5\ \mu\text{M}$ en $1.01\ \mu\text{M}$, onderskeidelik. 1,4-Naftakinoonderivate soos shikonien is voorheen reeds ondersoek vir die behandeling van kanker. Die literatuur stel voor dat inhibeerders van MAO nie net bruikbaar is vir die behandeling van PS en depressie nie, maar kan ook aangewend word vir die behandeling van kanker en kongestiewe hartversaking. 1,4-Naftakinoonderivate mag van besondere waarde wees in kankerbehandeling deur MAO te inhibeer sowel as deur DNS-interkalering.



Vir die vierde artikel is 'n reeks 1,4-bensokinoonderivate gesintetiseer en geëvalueer as inhibeerders van MAO. Daar is gevind dat hierdie verbindings inhibeerders van MAO is met IC_{50} -waardes van $5.03\text{--}13.2\ \mu\text{M}$ vir die inhibisie van MAO A en $3.69\text{--}23.2\ \mu\text{M}$ vir die inhibisie van MAO B. Hierdie potensies is vergelykbaar met dié van toloksatoon, 'n MAO A-inhibeerder in kliniese gebruik. Hierdie studie vind ook dat, in teenstelling met die 1,4-naftakinoonderivate, 1,4-bensokinone onomkeerbare inhibeerders van MAO A is. Hierdie is die eerste studie wat vind dat die MAO-ensieme onomkeerbaar geïnhipeer kan word deur 'n kinoonderivaat. Die proefskrif stel voor dat die 1,4-bensokinone met 'n nukleofiel in die aktiewe setel van MAO A reageer en sodoende kovalent aan die ensiem bind. Die gereduseerde flavien kofaktor mag optree as die voorgestelde nukleofiel.

In hierdie proefskrif is nuwe MAO-inhibeerders uit vier chemiese klasse ontdek. Deur gebruik te maak van molekulêre modellering kon insigryke gevolgtrekkings gemaak word aangaande die bindingswyse van die inhibeerders in die aktiewe setels van MAO. Belangrike struktuuraktiwiteitsverwantskappe is gemaak rakende die inhibisie van MAO deur die verskillende chemiese klasse. Van belang is dat, onder die 1-indanoon- en bensoksatioloonderivate, verbinding is wat beide MAO-isovorme inhibeer. Hierdie verbinding mag van belang wees vir die behandeling van PS-pasiënte wat ook depressiesimptome toon. Hierdie proefskrif het dus bygedra tot die ontdekking van nuwe MAO-inhibeerders wat veral van belang is vir die behandeling van PS.

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Abbreviations

A

AADC	-	Amino acid decarboxylase
ADH	-	Aldehyde dehydrogenase
Arg	-	Arginine
ATP	-	Adenosine triphosphate

B

BBB	-	Blood-brain barrier
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C

COMT	-	Catechol-O-methyltransferase
Cys	-	Cysteine

D

DA	-	Dopamine
DBH	-	Dopamine-β-hydroxylase
DOPAC	-	3,4-Dihydroxyphenylacetic acid

E

EGCG	-	Epigallocatechin
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F

FAD	-	Flavin adenine dinucleotide
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G

Glu	-	Glutamic acid
GPO	-	Glutathione peroxidase
GSH	-	Glutathione
GSSG	-	Glutathione disulfide
Gly	-	Glycine

H

HVA	-	Homovanillic acid
H₂O₂	-	Hydrogen peroxide
5-HTP	-	5-Hydroxytryptophan

I

Ile - **Isoleucine**

M

MAO - **Monoamine oxidase**

MPP⁺ - **1-Methyl-4-phenylpyridinium**

MPTP - **1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine**

3-O-MD - **3-O-Methyldopamine**

mp - **Melting point**

P

PD - **Parkinson's disease**

Phe - **Phenylalanine**

PNMT - **Phenylethanol-amine-N-methyltransferase**

R

ROS - **Reactive oxygen species**

S

SI - **Selectivity index**

SSAO - **Semicarbazide sensitive amine oxidase**

Ser - **Serine**

5-HT - **Serotonin**

SET - **Single electron transfer**

SNpc - **Substantia nigra pars compacta**

SOD - **Superoxide dismutase**

T

Tyr - **Tyrosine**

U

UCHL-1 - **Ubiquitin C-terminal hydrolase-L1**

UPS - **Ubiquitin-proteasome pathway**

Chapter 1

Introduction, research rationale and aims

1.1 Introduction

Parkinson's disease (PD) is a well-defined chronic neurological disease first described by James Parkinson in 1817 (Parkinson, 2002). Today it is known to be the second most dominant, progressive neurological disorder affecting people over the age of 65, after Alzheimer's disease. The main characteristic of PD is the progressive deterioration of the dopaminergic neurons in the substantia nigra of the brain. This results in the key symptoms of PD such as postural instability, rigidity and tremors (Dauer & Przedborski, 2003). PD is challenging to treat because symptoms only clinically present when dopamine levels in the brain has been reduced to 70-80% (Mounsey & Teismann, 2012). Moreover, with aging, the activity and density of monoamine oxidase (MAO) B increases in most brain regions, contributing towards reduced amounts of dopamine (Fowler *et al.*, 1997). MAO B is the main enzyme responsible for the metabolism of dopamine in the brain and inhibition thereof has proven to be valuable in the treatment of PD. MAO inhibitors, especially MAO B inhibitors may prolong the activity of endogenous and exogenous derived dopamine and consequently improve PD motor symptoms (Fernandez & Chen, 2007).

The MAOs exist as two isoforms, MAO A and MAO B. These isoforms are 70% identical on the amino acid sequence level (De Colibus *et al.*, 2005). MAO A catalyses the metabolism of substrates such as serotonin and noradrenalin. Both iso-enzymes catalyse the breakdown of dopamine, tyramine and tryptamine (Glover *et al.*, 1977). Since dopamine is metabolised by both MAO isoforms, it has been suggested that, when one isoform of MAO is inhibited, the other would take over its function and metabolise dopamine. Thus the steady-state level of dopamine remains unchanged with the inhibition of a single MAO isoform (Riederer & Youdim, 1986). Thus MAO A as well as MAO B should be inhibited to affect dopamine levels in the brain. Dual MAO inhibitors are also important in PD since 40-60% of PD patients exhibit signs of depression. While MAO B inhibitors are mainly employed in the treatment of PD, MAO A inhibitors are used for the treatment of depression. Research has shown that moclobemide, a MAO A inhibitor, exhibits additional anti-symptomatic effects in PD patients in addition to treating depression comorbid with PD (Youdim & Weinstock, 2004). This suggests that dual inhibition of both MAO enzymes may be more advantageous in the treatment of PD, rather than standalone therapy with MAO B inhibitors (Youdim & Weinstock, 2004; Finberg *et al.*, 1998). It is, however, important to note that irreversible MAO A inhibitors are restricted with the simultaneous use of tyramine-containing foods, such as cheese, because of the severe hypertension response which can be provoked (Da Prada *et al.*, 1988). To overcome this obstacle, inhibitors which bind reversibly to MAO A, such as moclobemide are preferred since these drugs do not lead to tyramine-induced hypertension (Provost *et al.*, 1992).

In the design and development of novel MAO inhibitors, it is important that inhibitors bind in a reversible manner not only to MAO A, but also to MAO B since it has been found that, when used in high doses and for a long duration of time, irreversible inhibitors of MAO B may also irreversibly block MAO-A activity centrally and peripherally (Bartl *et al.*, 2014). Also irreversible inhibition possesses, in general, more side-effects compared to reversible inhibition (Balon *et al.*, 1999). Thus, this study will attempt to discover new reversible MAO B inhibitors and/or dual MAO A and MAO B inhibitors for use as potential symptomatic treatment of PD.

1.2 Rationale

This thesis will be divided into four sections, which will be presented as separate articles. In each section a different class of compound will be investigated as potential MAO inhibitors. The four chemical classes that will be considered are:

- ❖ 1-Indanones (Chapter 4)
- ❖ Benzoxathiol-2-ones (Chapter 5)
- ❖ 1,4-Naphthoquinones (Chapter 6)
- ❖ 1,4-Benzoquinones (Chapter 7)

1. The possibility that the 1-indanone class may act as MAO inhibitors is derived from a recent report that α -tetralones act as highly potent inhibitors of MAO, with inhibition potencies in the nanomolar range (Legoabe *et al.*, 2014). α -Tetralones are the six-membered ring analogues of 1-indanones. Based on the structural similarity between α -tetralone and 1-indanone, it is hypothesised that 1-indanones may very well be potent MAO inhibitors. It is also noteworthy that, rasagiline, a well-known MAO-B inhibitor that is used in the clinic, is an indane derivative with close structural similarity to 1-indanone.

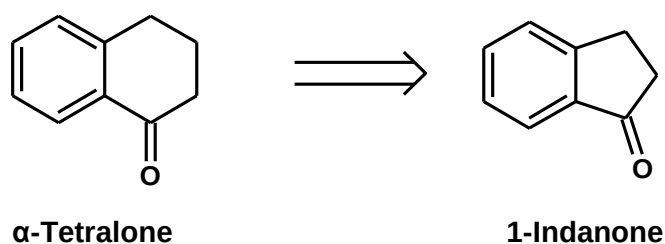


Figure 1.1: Structures of α -tetralone and 1-indanone.

2. The benzoxathiol-2-one class has not previously been considered as potential MAO inhibitors. Benzoxathiol-2-ones are, however, structurally related to a number of known MAO inhibitors including isatin and phthalimide analogues (Manley-King *et al.*, 2011a; Manley-King *et al.*, 2011b). Isatin and phthalimide are structurally similar to benzoxathiol-2-one as these are all bicyclic heterocycles incorporating the carbonyl group in a five-membered ring. It is thus plausible that benzoxathiol-2-one analogues may act as MAO inhibitors.

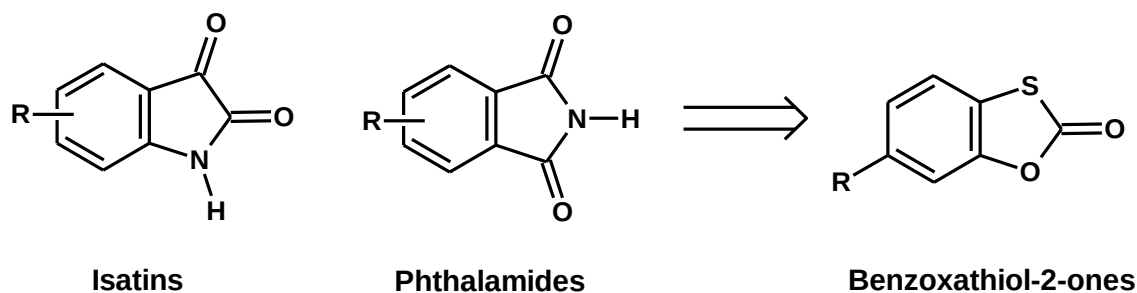


Figure 1.2: Structures of isatin, phthalimide and benzoxathiol-2-one derivatives.

3. 1,4-Naphthoquinones are known MAO inhibitors. The first report of the MAO inhibitory properties of this class of compounds showed that 2,3,6-trimethyl-1,4-naphthoquinone (TMN), present in extracts of cured tobacco leaves, is a non-selective MAO inhibitor (Khalil *et al.*, 2000). In a subsequent study, related menadione and 1,4-naphthoquinone was shown to also inhibit the MAO enzymes (Coelho-Cerqueira *et al.*, 2014). This thesis aims to further contribute by examining the MAO inhibition properties of additional 1,4-naphthoquinones. In this way, 1,4-naphthoquinones with improved MAO inhibition potencies may be discovered and the structure-activity relationships (SARs) of this class as MAO inhibitors will be expanded.

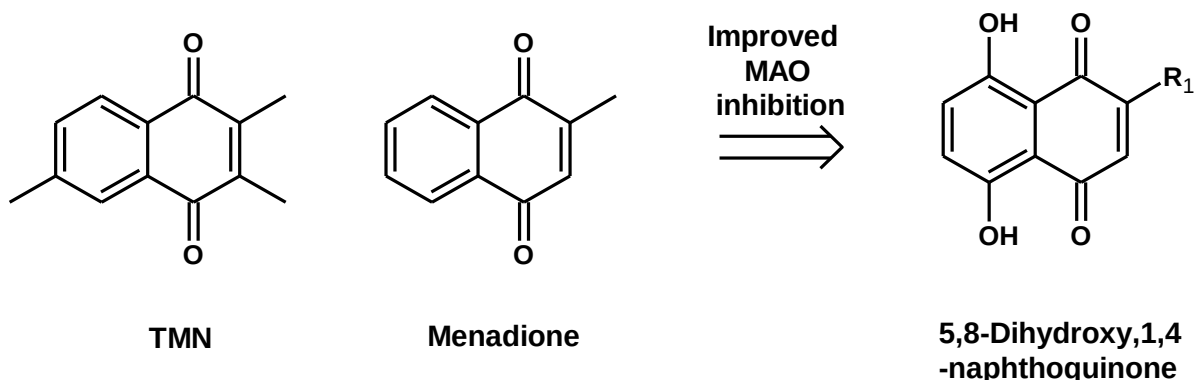


Figure 1.3: Structures of TMN, menadione and 1,4-naphthoquinone derivatives.

4. Based on reports that 1,4-naphthoquinones are MAO inhibitors, this thesis will examine the MAO inhibitory properties of a small series of 1,4-benzoquinones. These compounds are structurally very similar to 1,4-naphthoquinones and are thus expected to inhibit the MAOs. In fact, one literature report has shown that 1,4-benzoquinone indeed inhibits MAO-A and MAO-B (Naoi *et al.*, 1987). As will be shown by the results of this thesis, unexpected and exciting findings related to the mode of MAO inhibition by 1,4-benzoquinones were obtained.

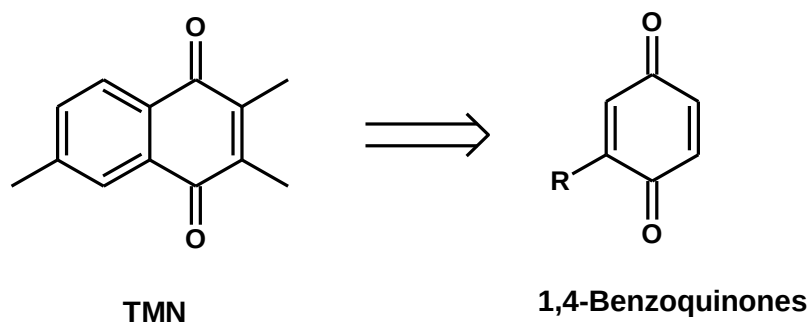


Figure 1.4: Structures of TMN and 1,4-benzoquinones.

1.3 Aims

This thesis will investigate the MAO inhibition properties of the following:

- ❖ A synthetic series of 1-indanones (Chapter 4).
- ❖ A synthetic series of benzoxathiol-2-one derivatives (Chapter 5).
- ❖ A series of natural and synthetic 1,4-naphthoquinones (Chapter 6).
- ❖ A small synthetic series of 1,4-benzoquinones (Chapter 7).

These studies will be presented as four articles destined for publication in academic journals. The objective of this thesis is thus to contribute to the discovery and characterisation of new MAO inhibitors. Such compounds may find application in various disease states such as PD and depression.

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Chapter 2

Parkinson's disease

2.1 General background

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease. The prevalence of PD is indefinite since several methodological considerations need to be taken into account, however, a prospective population-based study reported an incidence rate of 8-18 per 100 000 person-years (De Lau & Breteler, 2006). PD slowly progresses over a period of 10-20 years and the typical onset is over the age of 50, with a drastic increase over 60. A prevalence of 0.3% in the general population rising to 1% in people above 60 years of age has been reported (Nussbaum & Ellis, 2003). It has also been shown that about 25-40% of PD patients eventually develop dementia (Emre, 2003) and epidemiological studies reported that PD patients are sixfold more at risk than healthy people in this regard (Aarsland *et al.*, 2003). PD patients also present with depressive illness which can affect the patient's quality of life and cognitive function. It's been estimated that 17% of PD patients suffer from major depressive disorder and that 35% suffer from clinically significant depressive symptoms (Reijnders *et al.*, 2008).

The pathological hallmark of PD is the deterioration of pigmented melanin neurons along the nigrostriatal pathway in the substantia nigra pars compacta (SNpc) (Figure 2.1). These neurons use dopamine as neurotransmitter and send projections from the midbrain to two forebrain nuclei, the caudate and putamen (striatum). This degenerative process is characterised by the presence of intracytoplasmic inclusions in the SNpc, known as Lewy bodies. They are responsive to immunostaining with antibodies of α -synuclein and ubiquitin (Ehringer & Hornykiewicz, 1960). This is used as the standard histochemical method for routine diagnostic purposes of neurodegenerative diseases (Wakabayashi *et al.*, 2007). Also, the degenerative process is characterised by SNpc depigmentation of melanin (Marsden, 1983). Degeneration of these neurons leads to the depletion of dopamine resulting in the clinical symptoms of PD, such as resting tremor, rigidity, slow movement and postural instability. These symptoms only present when 80% of dopamine in the putamen and 60% of SNpc dopaminergic neurons have deteriorated (Uhl *et al.*, 1985). Dopamine depletion in the putamen is more severe than the SNpc, for example when the SNpc contain 15% residual dopamine, the putamen will have only 2%. Thus, it has been postulated that PD pathogenesis may involve 'the dying back' of the dopaminergic nigrostriatal pathway (Wooten, 1997).

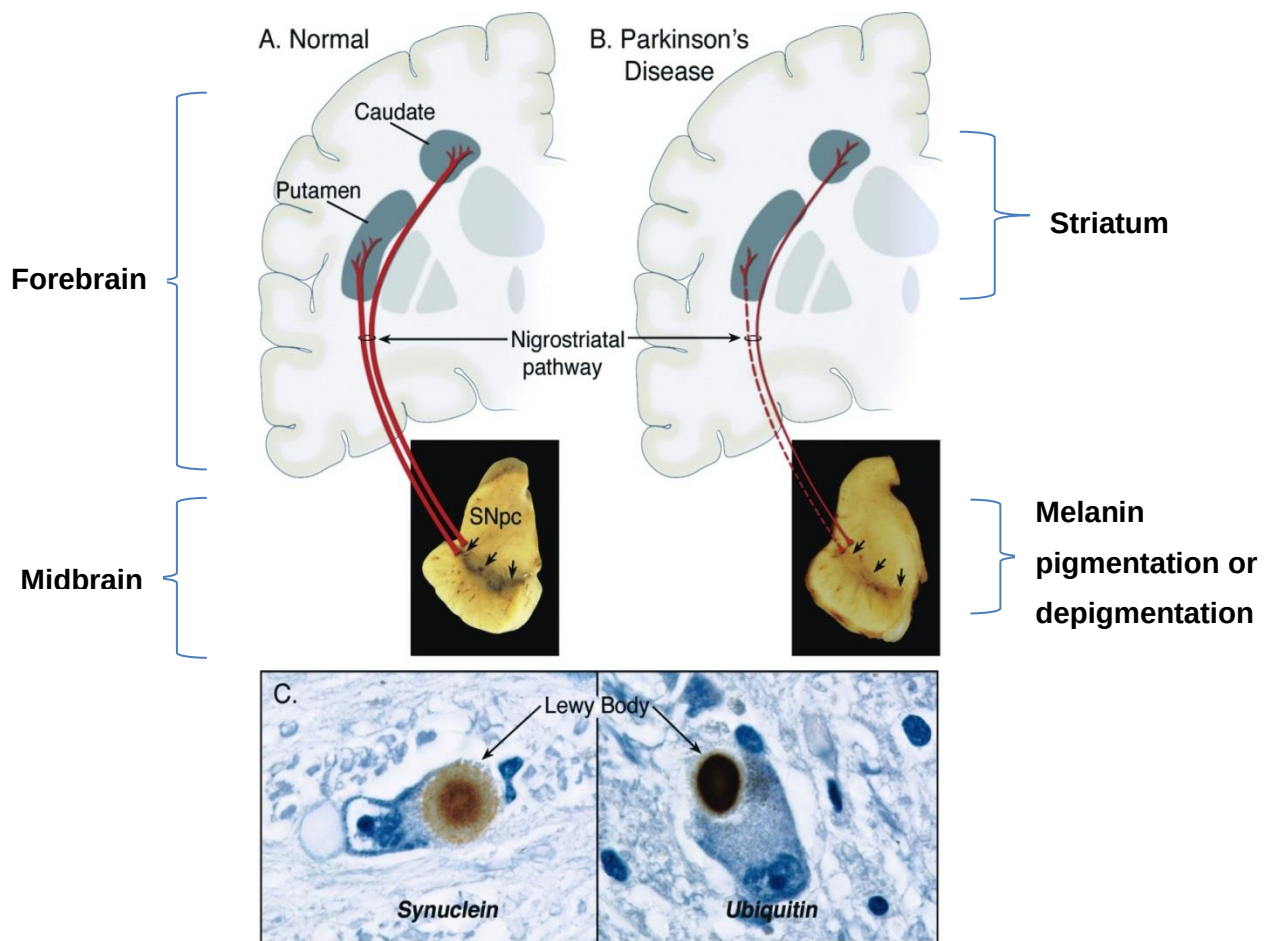


Figure 2.1: Neuropathological representation of a normal patient (A) and of a PD patient (B). The intracytoplasmic inclusions, Lewy bodies, are represented in (C). (Dauer & Przedborski, 2003)

The exact cause of PD is unknown but genetic and environmental factors have been identified. Epidemiological studies suggest that environmental risk factors include, rural living and the exposure to herbicides (paraquat), pesticides (rotenone) and heavy metals. Neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) are also known to cause parkinsonian symptoms (Langston *et al.*, 1983). Paraquat, rotenone and MPTP are selective complex-1 inhibitors and induce dopamine depletion through nigrostriatal degeneration. Also long-term exposure to heavy metals such as iron, lead and copper results in excess accumulation in the substantia nigra and contributes towards oxidative stress and eventually neurodegeneration (Lai *et al.*, 2002). Genetic factors include gene mutations in the following genes: α -synuclein, parkin, and ubiquitin C-terminal hydrolase-L1 (UCHL-1) (Dauer & Przedborski, 2003). Evidence suggests that these genes exert an effect on the ubiquitin-proteasome pathway (UPS) which is responsible for protein degradation. Mutations interfere with the normal clearance of misfolded proteins through the UPS, leading to the accumulation of aggregated and misfolded proteins, which may contribute toward familial and sporadic onset of PD (McKnaught *et al.*, 2001). This is based, in part, on the

observation that α -synuclein is one of the components of Lewy bodies. Thus two hypotheses, which may be interlinked, are suggested to explain the death of nigrostriatal neurons, first through the aggregation and misfolding of proteins and secondly mitochondrial dysfunction and oxidative stress (Dauer & Przedborski, 2003).

The diagnosis of PD mainly focusses on the clinical observation of the motor symptoms typically associated with the disease. However, when the diagnosis is made, 60% of the dopaminergic neurons have already deteriorated. PD is incurable and treatment strategies mainly focus on the replacement of dopamine to improve the key motor symptoms. Neuroprotection is an additional treatment strategy, however, effective neuroprotective treatments are not available. The first-line treatment of PD is the administration of levodopa in combination with a peripheral dopa decarboxylase inhibitor, typically benserazide or carbidopa. This combination reduces the amount of levodopa degraded in the periphery and increases the amount that crosses the blood-brain barrier, up to fourfold (Cedarbaum *et al.*, 1986). Within one week noticeable improvement in symptoms can be observed. Nevertheless most patients develop motor complications and fluctuations after 4-6 years on levodopa treatment. These complications include levodopa dyskinesia and the end of dose “wearing off” phenomenon. During the “on” period, dyskinesia occurs, and during the “off” period, voluntary movement is severely diminished (Fahn *et al.*, 2004). To reduce these complications and to prolong levodopa’s efficacy, alternative drugs are co-administered, for example dopamine agonists and catechol-O-methyltransferase (COMT) inhibitors. MAO B inhibitors are also beneficial in the treatment of motor symptoms. They increase the levels of dopamine in the striatum by inhibiting dopamine metabolism. It was also found that MAO B inhibitors may exhibit their benefit after a week and also may delay the need for levodopa. Thus MAO B inhibitors can be used as monotherapy or as adjunct therapy with levodopa. It has also been suggested that MAO B inhibitors may be neuroprotective (Fernandez & Chen, 2007).

Since only a selected few MAO inhibitors are approved for the treatment of PD, the design and development of MAO inhibitors may be of great importance in the future symptomatic treatment of PD.

2.2 Pathogenic mechanisms in PD

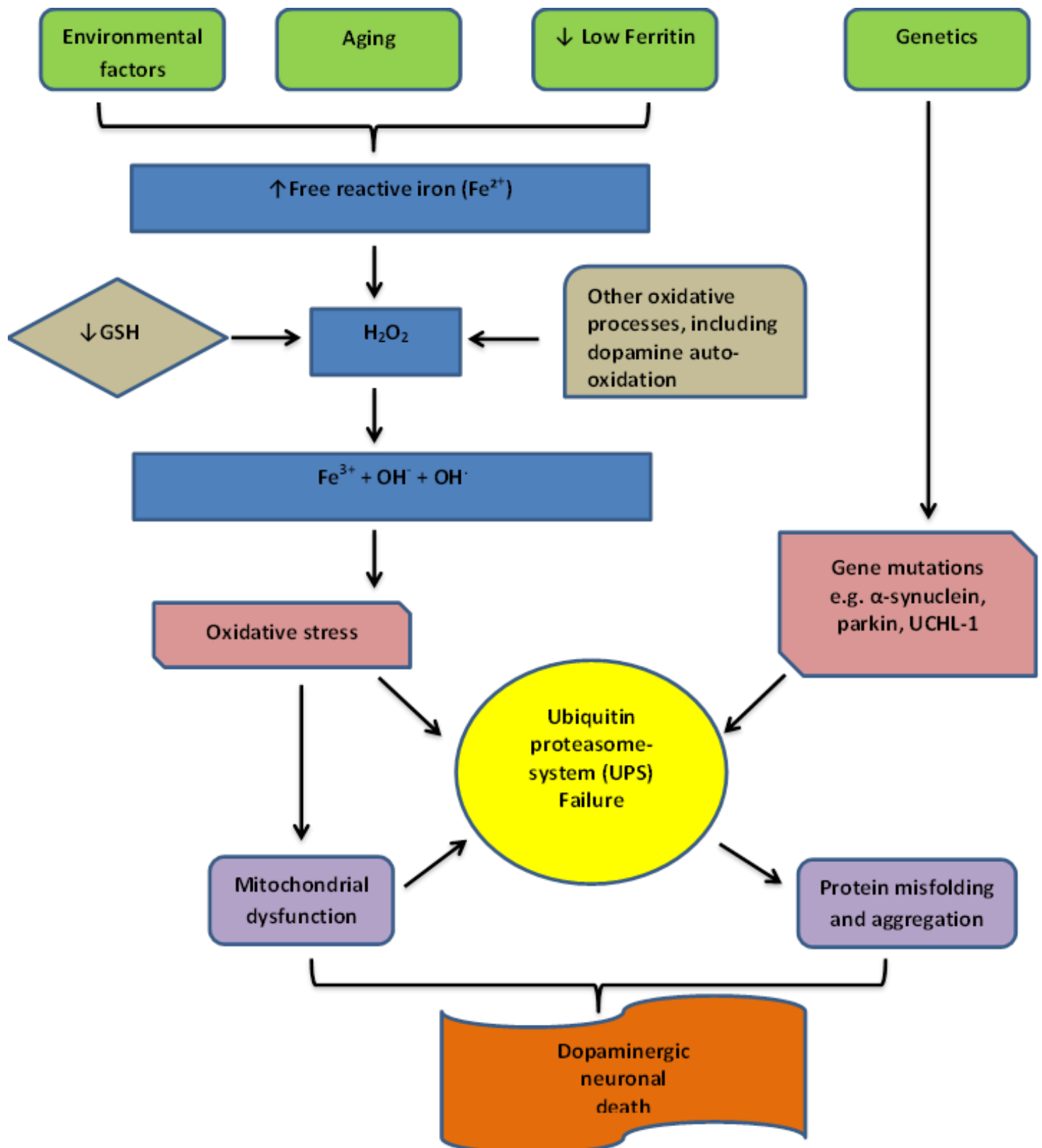


Figure 2.2: Schematic summary of the mechanisms leading to neurodegeneration.

Oxidative stress in PD: The mechanism of dopaminergic neuronal death has not yet been fully elucidated but several theories have been postulated. The main hypothesis is mitochondrial dysfunction through oxidative stress (Figure 2.2). This hypothesis of oxidative stress is founded on the evidence of free radical involvement. Dopamine oxidation yields toxic by-products; dopaldehyde and reactive oxygen species (ROS) e.g. hydrogen peroxide (H₂O₂), superoxide anions and hydroxyl radicals (Fowler *et al.*, 1980). Also, the discovery that MPTP induces PD strengthens the hypothesis of mitochondrial dysfunction. MPTP is transported to the brain where it is metabolised to its metabolite 1-methyl-4-phenylpyridinium (MPP⁺) by MAO B. MPP⁺ is the active toxin, which then accumulates in the dopaminergic neurons where it exerts its effect by inhibiting complex 1 of the electron-transport chain inside mitochondria. This results in ATP depletion which generates ROS and ultimately leads to cell death (Javitch *et al.*, 1985).

Aging is the most important risk factor for neurodegenerative diseases. It has been found in post-mortem studies that the brains of PD patients possess 35% higher amounts of iron age-matched compared to normal brains (Dexter *et al.*, 1987). Normal levels of iron are vital for physiological functions such as DNA synthesis, mitochondrial respiration and oxygen transport. Iron also acts as a co-factor for tyrosine hydroxylase, an enzyme involved in the synthesis of dopamine (Rausch *et al.*, 1988). Thus iron is the most abundant metal in the brain, though excess levels can be toxic through the mechanism of oxidative stress (Dexter *et al.*, 1987).

It has also been observed that MAO B levels increase four- to fivefold in the brain with ageing, thus increasing the metabolism of dopamine and the formation of ROS (Fowler *et al.*, 1980). Dopamine autooxidation (see Figure 3.10) leads to the formation of dopamine quinone which is then converted into neuromelanin (Graham, 1978). Since MAO B activity increases in PD, more dopamine is metabolised by MAO instead of undergoing autooxidation and hence the characteristic presentation of SNpc depigmentation of melanin.

The ferritin protein is responsible for transport of iron. When iron is bound to ferritin, it is nontoxic but as soon as it is unbound, iron returns to its free radical state and may lead to toxicity. Thus, the levels of ferritin must be regulated (Thomson *et al.*, 1999). Also, neuromelanin has the ability to bind free heavy metals such as iron. Since, neuromelanin is reduced in the brain of PD patients, an increase in nonheme iron (Fe³⁺) may occur with an increase in redox activity and oxidative stress (Faucheux *et al.*, 2003).



Figure 2.3: The Fenton reaction.

The Fenton reaction, displayed in figure 2.3, is a normal metabolic reaction which is responsible for the redox balance between the two forms of iron, reduced iron (Fe^{2+}) and oxidised iron (Fe^{3+}). In normal brains the ratio is 1:1; however, in PD the ratio is 1:3. This suggests that in PD, unbound iron levels are increased which, in turn, leads to the formation of toxic hydroxyl radicals (Youdim *et al.*, 2004).

Under normal physiological conditions the body has its own antioxidant systems equipped to inactivate toxic radicals (Perry *et al.*, 1982). The first-line antioxidant mechanism is glutathione (GSH). GSH is a free radical scavenger and predominantly effective against hydrogen peroxide, a molecule that may yield radicals via the Fenton reaction. GSH is converted to an oxidised dimer (GSSG) by utilizing glutathione peroxidase (GPO). GPO uses GSH as cofactor to inactivate H_2O_2 (Figure 2.4). Upon ageing and in PD, GSH levels decrease in the brain. This may result in the accumulation of H_2O_2 and thus increased amounts of Fe^{3+} radicals may be formed in the Fenton reaction, further contributing towards oxidative stress (Riederer *et al.*, 1989).

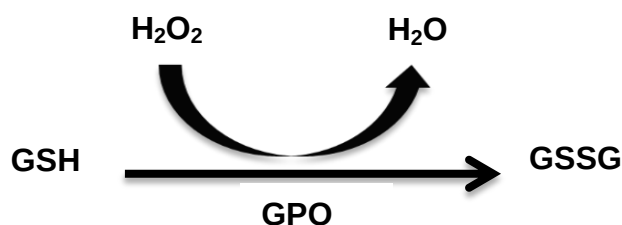


Figure 2.4: The glutathione peroxidase (GPO) reaction pathway.

Other cellular antioxidant defence mechanisms include superoxide dismutase (SOD), catalase and vitamin E. SOD exerts its effect by catalysing the conversion of superoxide anions to oxygen and H_2O_2 . Catalase, in turn, is responsible for the degradation of high concentrations of H_2O_2 (Figure 2.5). However, in PD brains, SOD activity is increased, which contributes to H_2O_2 formation. Catalase activity, in contrast, is reduced leading to reduced clearance of H_2O_2 . Both these factors greatly contribute to oxidative stress in PD (Ambani *et al.*, 1975). Vitamin E is an oxygen radical scavenger that protects against oxidation and autooxidation of lipids, nucleic acids and proteins (Tappel, 1962), and has been suggested as a potential protectant against neurodegeneration in PD.

In PD there is thus an increase in iron levels and a decreased capacity of antioxidant mechanisms leading to the enhanced generation of toxic hydroxyl radicals. Hydroxyl radicals are the most toxic of the free radicals formed, and together with unbound iron, exert deleterious effects on cells. Hydroxyl radicals thus induce oxidative stress through DNA fragmentation, lipid peroxidation and damage to proteins leading in cell death (Warren *et al.*, 1987). The mitochondrion has been identified as the main site for the production of toxic radicals, especially, superoxide. In this

respect, the electron transport system is implicated in superoxide production. The MAO enzymes, which are bound to the outer membrane of the mitochondria, also contribute to the production of ROS. For each mole of dopamine oxidised by the MAOs, one mole of H_2O_2 is produced. As mentioned above, H_2O_2 is a substrate for the Fenton reaction, yielding hydroxyl radicals. In PD oxidative stress and mitochondrial dysfunction appear to function in synergy.

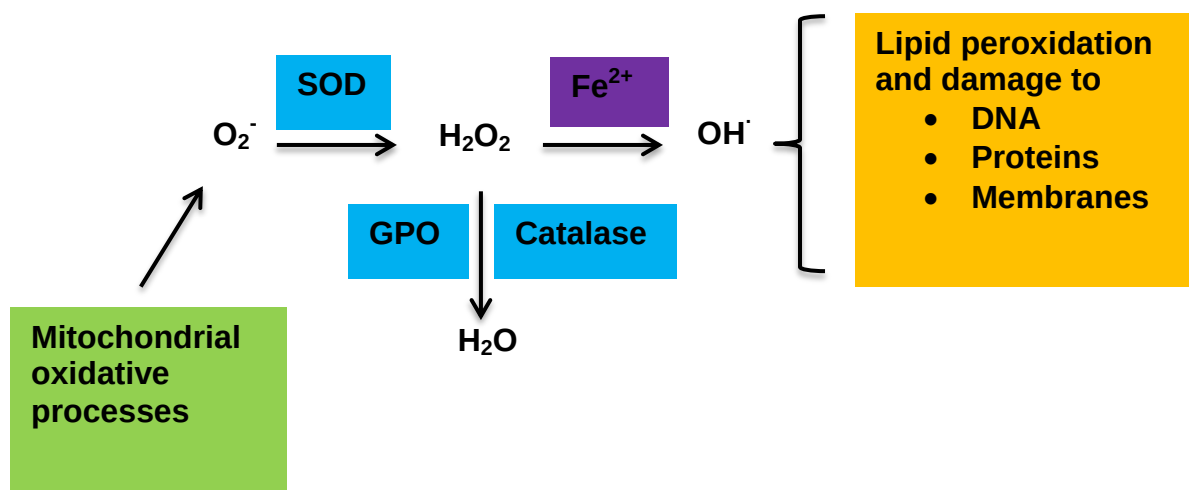


Figure 2.5: Summary of antioxidant defence mechanisms.

Protein aggregation and misfolding in PD: Protein aggregation and misfolding causes abnormal functioning or conformation of proteins, which may possibly be neurotoxic. The primary mechanism responsible for the clearance of misfolded proteins is the UPS (Figure 2.6). The UPS utilises ubiquitin that marks misfolded proteins for inactivation and clearance. The first step is to activate the ubiquitin through ubiquitin-activating enzymes (E1). The activated ubiquitin is then transported to an ubiquitin conjugating enzyme (E2) and is then covalently linked to the misfolded protein. This bondage is catalysed by ubiquitin protein ligase (E3), which utilises ATP. ATP is thus crucial for UPS function. Thus a chain of ubiquitin molecules are formed. This chain then enters into the proteasome core of the UPS. Just before entry, the ubiquitin/misfolded protein complex is detached from the chain and separated, inactivating the mutant protein and releasing the free ubiquitin molecules back for activation in E1. This detachment is catalysed by UCHL-1 (Pickart, 2001). When the integrity of the UPS is compromised, these misfolded proteins may accumulate and form insoluble inclusion bodies which can compromise cellular homeostasis and lead to cell death (Sherman & Goldberg, 2001).

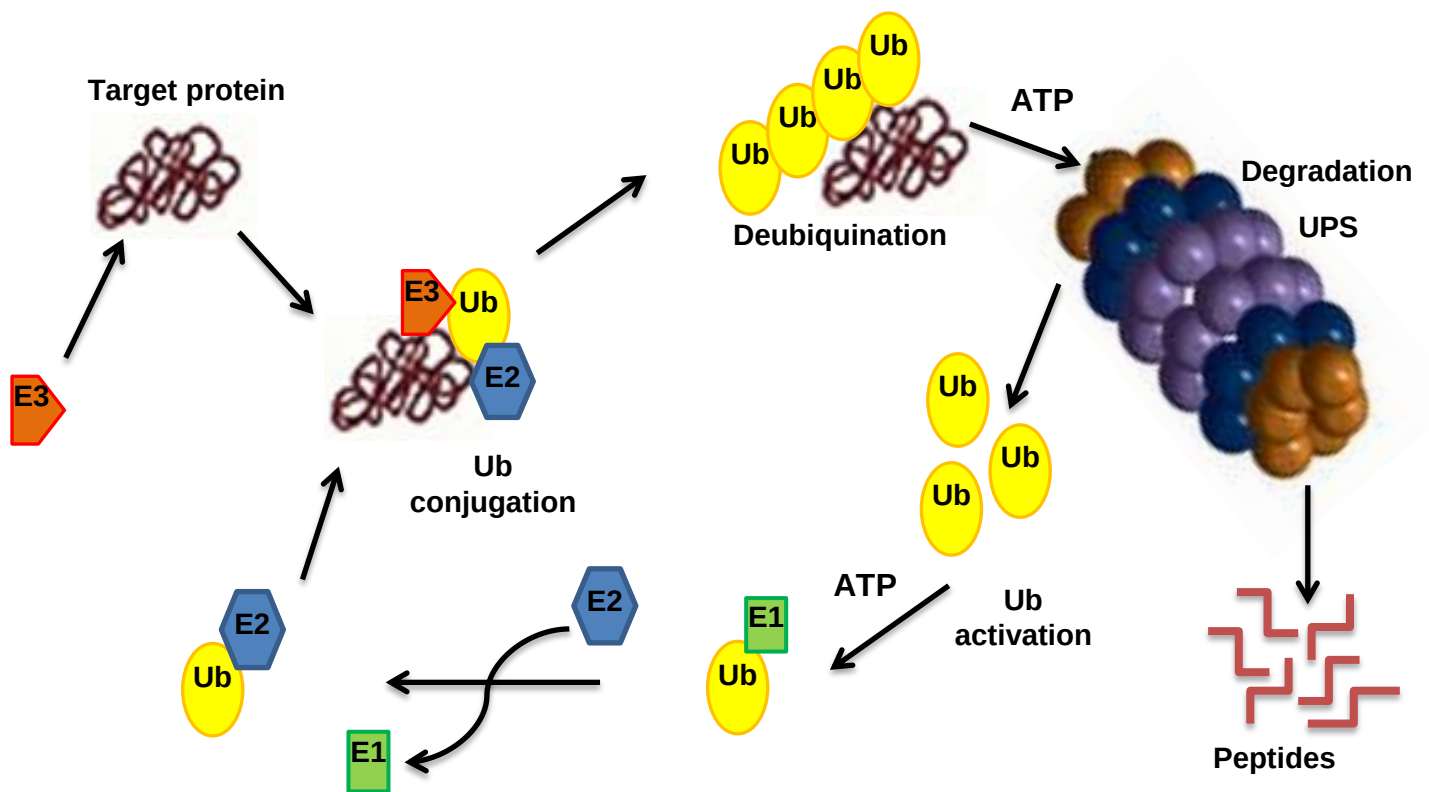


Figure 2.6: Schematic representation of the function of the ubiquitin proteasome system (UPS).

Gene mutations found in certain forms of PD can obstruct the efficacy of the UPS. Parkin is an E3 ubiquitin protein ligase and mutations in this gene can result in decreased UPS activity. Mutant α -synuclein is suggested to inhibit the UPS mechanism (Masliah *et al.*, 2000) and mutations in UCHL-1 may lead to reduced amounts of free ubiquitin used for marking abnormal proteins for inactivation (McKnaught & Olanow, 2003). Mitochondrial dysfunction and oxidative stress can also obstruct the UPS. Oxidative damage to α -synuclein contributes to the misfolding and aggregation of this protein and mitochondrial complex 1 dysfunction may reduce ATP production, which may inhibit the function of UPS at E3 (Giasson *et al.*, 2000). Dysfunctioning or suboptimal functioning of the UPS therefore appears to be a central mechanism in neurodegeneration as seen in PD.

From above discussion it is clear that numerous factors contribute towards dopaminergic neuronal death in PD. Two hypotheses for the pathogenesis of PD were suggested, mitochondrial dysfunction and aggregation/ misfolding of proteins. It can be reasoned that these two hypotheses may be interlinked and that the cause of PD is multifactorial.

2.3 Treatment

Currently there are two treatment strategies in PD. The first focuses on symptomatic treatment and the second on neuroprotection, slowing disease progression for example through reduction of oxidative stress and altered apoptotic pathways. Most of the drugs approved for clinical use are intended for the treatment of the psychiatric and motor symptoms of PD. Research has shown that, if PD patients remain untreated, rapid deterioration of motor functions occur and quality of life is severely diminished within 10 years or less (Löhle & Reichmann, 2010). There is thus a great need to develop therapies which can delay neurodegeneration or even restore neuronal functions.

2.3.1 Symptomatic treatment of motor symptoms

- **Levodopa and amino acid decarboxylase inhibitors**

Levodopa was the first drug used in the treatment of PD. Dopamine itself cannot be administered since it is unable to cross the blood-brain barrier (BBB), whereas levodopa is the metabolic precursor of dopamine and able to cross the BBB. In 1961, Birkmayer and Hornykiewicz first initiated a clinical trial of levodopa, but it was not until 1967 when Cotzias *et al.* (1967) discovered that a chronic high levodopa dosage leads to the improvement of PD symptoms. These high dosages, however, lead to side effects because of the rapid metabolism of levodopa to dopamine in the periphery (Cotzias *et al.*, 1967).

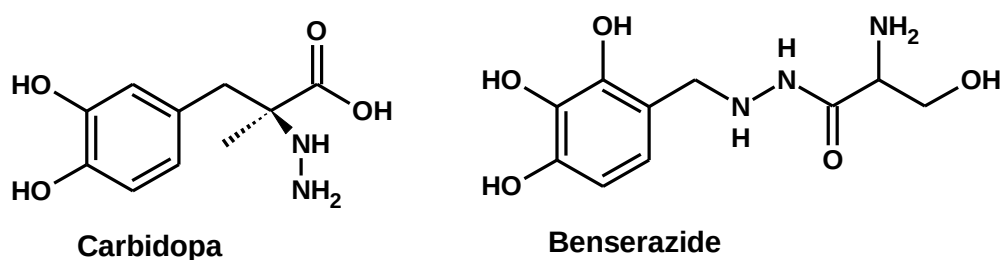


Figure 2.7: Structures of amino acid decarboxylase inhibitors.

Levodopa is metabolised by COMT and amino acid decarboxylase (AADC) to inactive metabolites and dopamine, respectively. Overcoming this obstacle, conversion of levodopa in the periphery must be inhibited. AADC is present in the periphery and brain, however inhibitors thereof such as carbidopa and benserazide (Figure 2.7) cannot cross the BBB and thus inhibition only takes place in the periphery, increasing the amount of dopamine that reaches the brain (Bartholini & Pletscher, 1975).

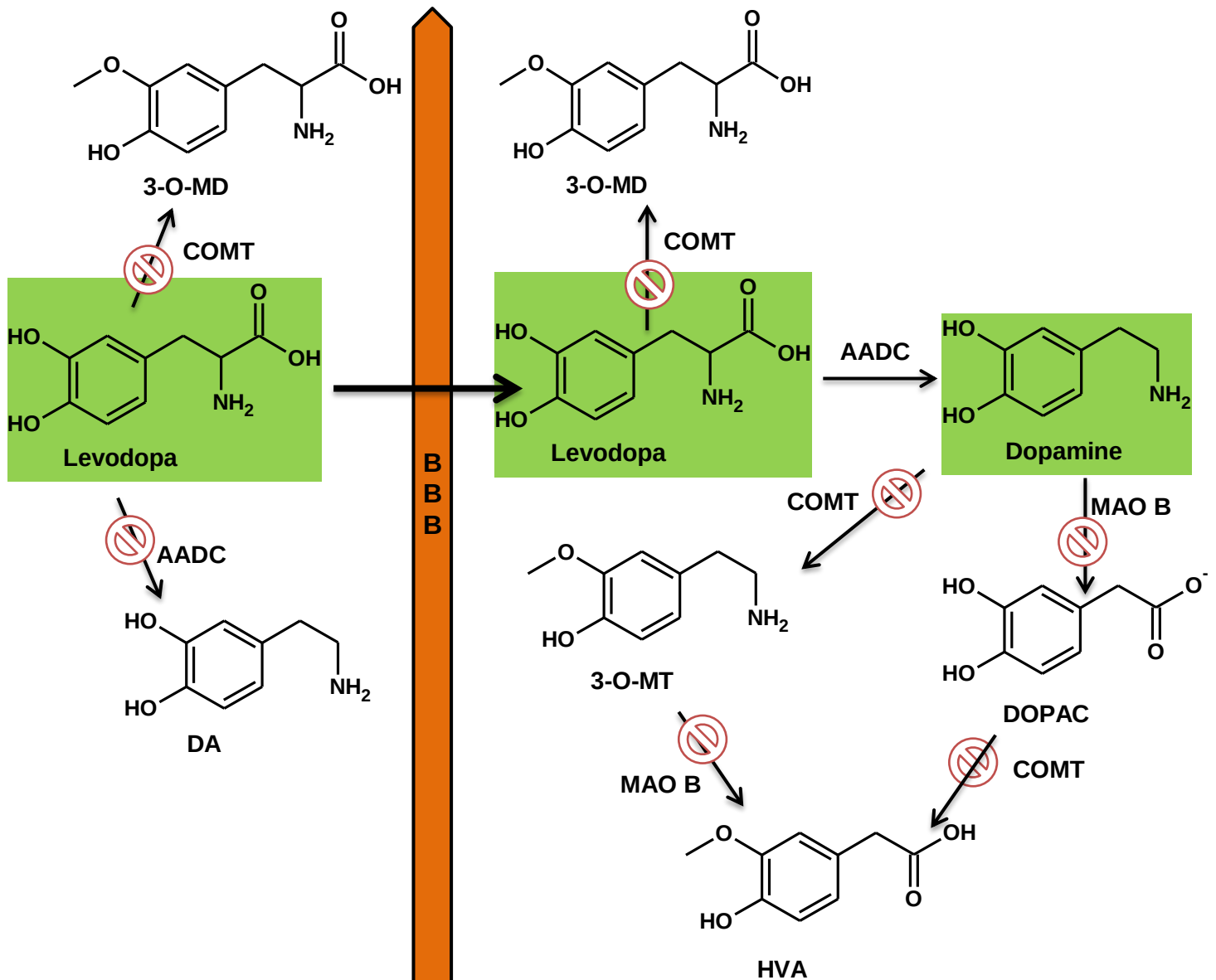


Figure 2.8: Representation of dopamine enhancement through inhibition of selected enzymes for PD treatment.

Figure 2.8 gives an overview of levodopa's metabolism and what drug actions will increase the levels of dopamine in the PD brain. Levodopa is metabolised in the periphery by COMT and AADC, yielding the metabolites 3-O-methyldopamine (3-O-MD) and dopamine, respectively. When levodopa crosses the BBB it is once again metabolised by COMT and AADC to its corresponding metabolites. Dopamine, however, can also be further metabolised to 3-O-methoxytyramine (3-O-MT) and 3,4-dihydroxyphenylacetic acid (DOPAC), which in turn are both metabolised to homovanillic acid (HVA) (Wade & Katzman, 1975).

- **COMT inhibitors**

Inhibition of COMT in the periphery and brain will lead to an increase of available dopamine. COMT inhibitors such as tolcapone and entacapone (Figure 2.9) can increase the half-life of levodopa. This will result in a higher percentage of levodopa crossing the BBB, leading to more stable levels of levodopa in the brain and subsequently decreases in “off” time and improving motor fluctuations (Pellicano *et al.*, 2009). COMT inhibitors are, however, associated with liver toxicity, especially tolcapone (Canesi *et al.*, 2008).

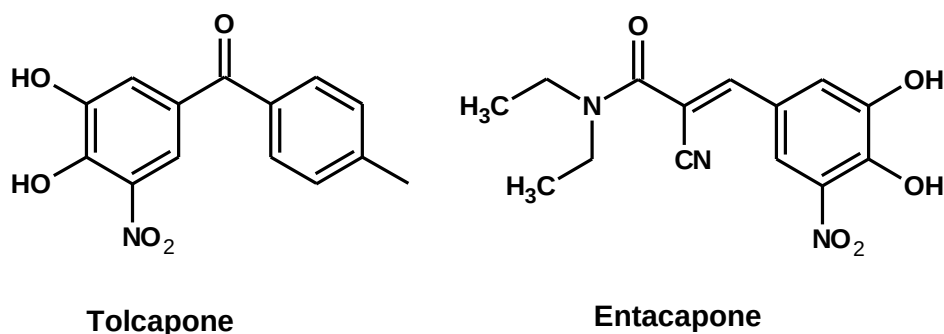


Figure 2.9: Structures of COMT inhibitors.

- **Monoamine oxidase B inhibitors**

Inhibition of MAO B in the striatum prevents the metabolism of dopamine to its metabolites. When administered in combination therapy with levodopa, motor fluctuations are reduced and a reduced dosage of levodopa is allowed. MAO B inhibitors (Figure 2.10) may even postpone the need of levodopa therapy (Lew *et al.*, 2007). The percentage ratio of MAO A and MAO B present in the brain is 20:80. In the intestines this ratio is 80:20. Thus MAO B inhibitors are considered for PD treatment since MAO B is the predominant isoform in the human brain. In addition, inhibitors of MAO A in the periphery may cause the severe hypertensive crisis, the “cheese” reaction. This will be further discussed in chapter 3 (Youdim & Bahkle, 2006).

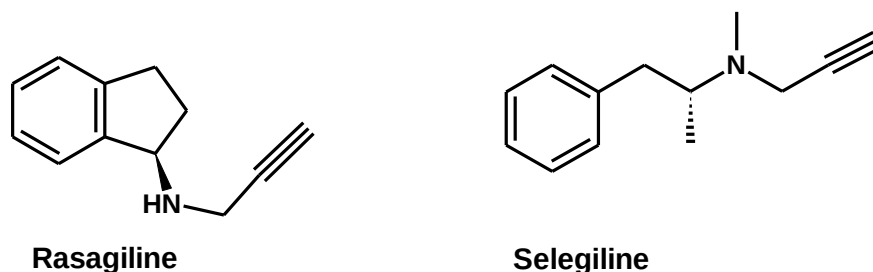


Figure 2.10: Structures of MAO B inhibitors approved for PD therapy.

- **Dopamine agonists**

Dopamine agonists (Figure 2.11) exert their effect by directly stimulating postsynaptic dopamine receptors present in the striatum, thus reducing and delaying the onset of motor fluctuations. They are less efficacious than levodopa and cause side-effects such as hallucinations and sleep disturbances (Antonini & Barone, 2008). Nevertheless, a previous study done has shown that PD patients that received dopamine agonist therapy in combination with levodopa were 50% less probable to develop motor fluctuations compared to levodopa monotherapy (Binder *et al.*, 2009).

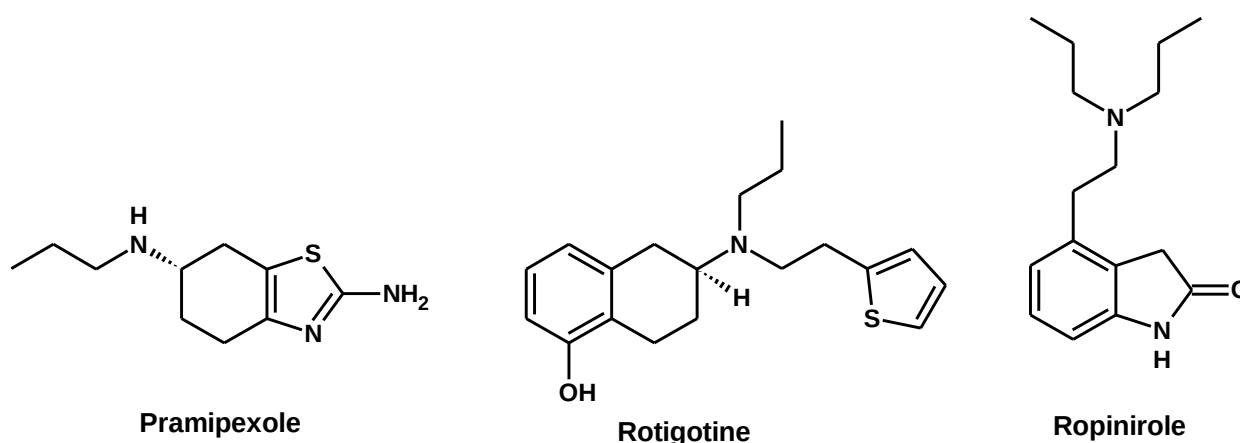


Figure 2.11: Structures of dopamine agonists.

- **Amantadine**

Amantadine (Figure 2.12) is an antiviral agent, which also possesses anticholinergic effects. Amantadine also stimulates the release of dopamine and block dopamine reuptake into presynaptic nerve endings (Schwab *et al.*, 1969). A study has shown that amantadine improves akinesia, rigidity and tremor, especially decreasing levodopa-induced dyskinesia (Luginger *et al.*, 2000).

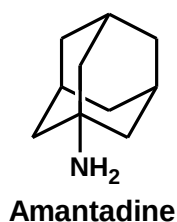


Figure 2.12: Structure of amantadine.

- **Anticholinergic drugs**

Anticholinergic drugs are mainly used to treat dyskinesia in PD by inhibiting postsynaptic muscarinic receptors and thus restoring the equilibrium between dopamine and acetylcholine in the striatum. They are, however, associated with many side effects such as dry mouth, urine incontinence and dementia (Lang, 1984). Examples of anticholinergic drugs used in PD are trihexyphenidyl and bztropine (Figure 2.13).

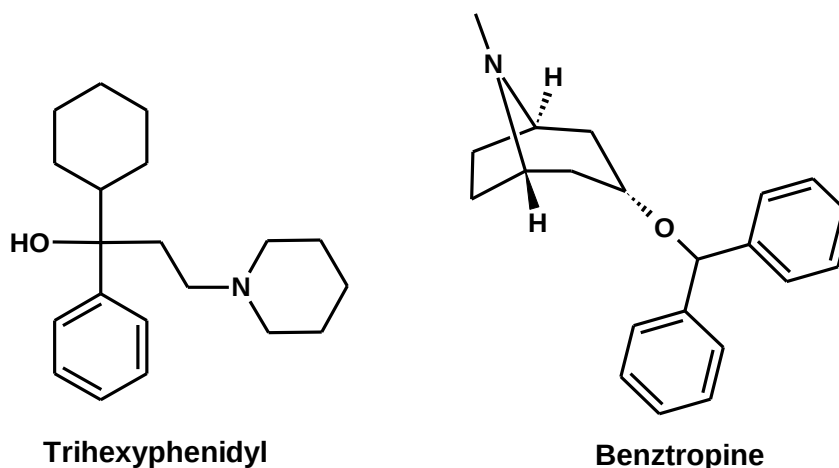


Figure 2.13: Structures of anticholinergic drugs used in PD.

2.3.2 Neuroprotection

- **Glutathione**

Glutathione (Figure 2.14) has been suggested as an antioxidant in the treatment of PD. It has been found that, in the midbrain of PD patients, the activities of glutathione peroxidase and catalase are reduced. This suggests that antioxidant mechanisms are disrupted in PD (Ambani *et al.*, 1975).

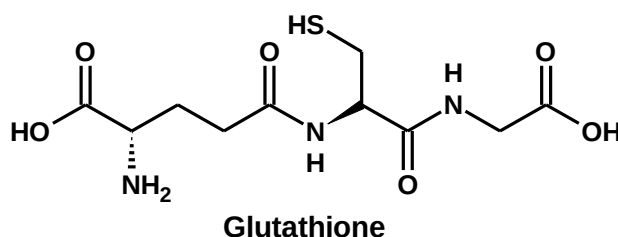


Figure 2.14: Structure of the antioxidant, glutathione.

Glutathione does not cross the BBB, thus oral administration alone is ineffective. Current clinical trials are evaluating the administration of glutathione with N-acetylcysteine, which is a rate limiting amino acid in the production of glutathione. Intranasal administration is also investigated as improved route of administration (Goetz & Pal, 2014).

- **Antioxidants**

Antioxidants are abundantly found in the natural world in for example fruits, vegetables and tea. They are of interest since it has been shown that antioxidants inhibit lipoprotein oxidation *in vitro* (Dufresne & Farnworth, 2001). When considering the hypothesis of mitochondrial dysfunction and the role of the metabolic antioxidant mechanism in PD pathogenesis, it is expected that administered antioxidants may reduce oxidative stress. Examples of antioxidant agents investigated for PD treatment are coenzyme Q10, ginkgo biloba, green tea extracts and vitamin E (Figure 2.15).

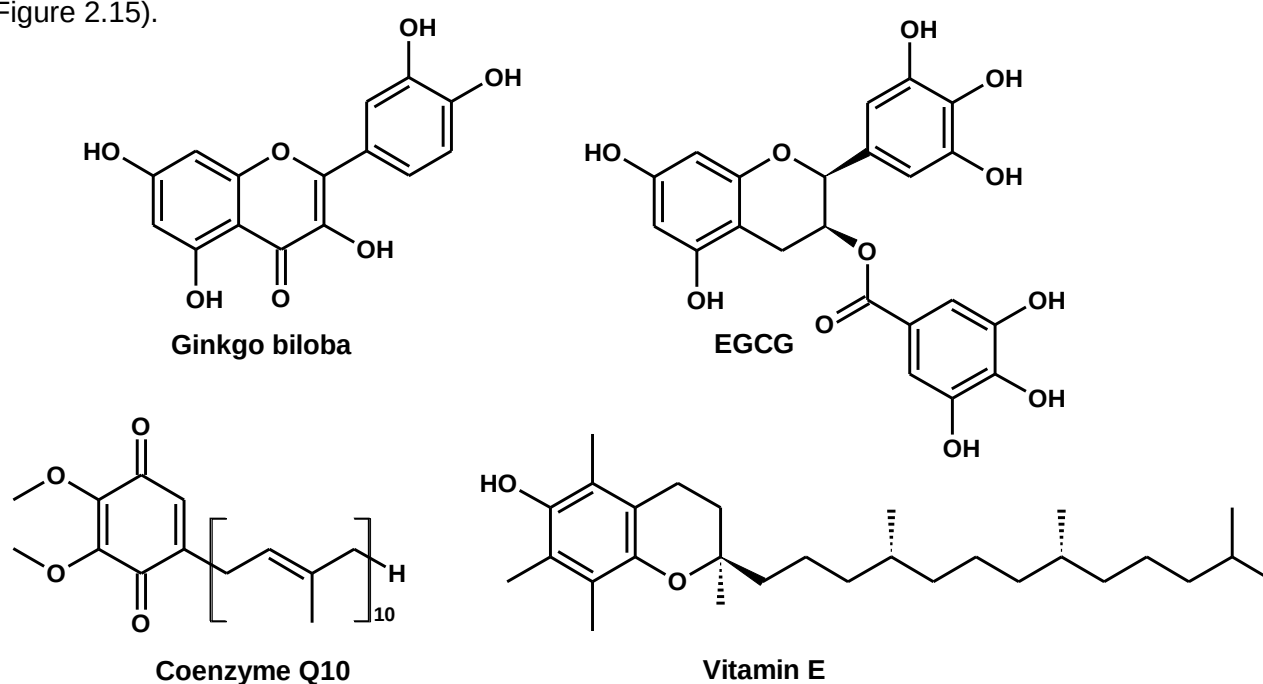


Figure 2.15: Structures of antioxidant agents for possible treatment of PD.

Coenzyme Q10, also known as ubiquinone, acts as an electron acceptor for complex 1 of the mitochondrial electron transport chain, and is actively involved in the synthesis of ATP (Abdin & Hamouda, 2008). Ginkgo biloba is enriched with flavonoids and possesses free radical scavenger activity. Ginkgo biloba may protect cells against GSH depletion thus acting as a neuroprotective agent (Hibatallah *et al.*, 1999). Green tea extracts contains flavonoids and polyphenols such as epigallocatechin (EGCG). A study has shown that EGCG prevents dopamine and tyrosine hydroxylase depletion in the substantia nigra of MPTP-treated mice (Levites *et al.*, 2001). Green tea is nontoxic and components thereof can cross the BBB where they may reduce ferric iron (Fe^{3+}). Epidemiological studies have shown that drinking two cups of green tea per day may reduce the risk of PD (Chan *et al.*, 1998). Vitamin E (α -tocopherol) is an endogenous free radical scavenger that protects lipids against ROS by forming stable radicals that can be degraded by vitamin C and glutathione (McCay, 1985). Regrettably clinical trials did not provide evidence of the beneficial effect of vitamin E therapy in PD, but larger dosages and longer treatment periods may prove otherwise (Fahn, 1992).

- **Iron chelators**

Iron chelators are readily available, however, their use in neurodegenerative processes are mainly hindered because of their inability to cross the BBB.

- Desferal or otherwise known as deferoxamine is a high affinity iron-chelating compound excreted by bacteria (Miller, 1989). It is reported to reduce oxidative stress and mitochondrial dysfunction due to excess iron (Sangchot *et al.*, 2002). Desferal does not cross the BBB and is effective only when injected into the brain, which drastically limits its practical clinical use.
- 8-Hydroxyquinoline (8-HQ) is highly lipophilic and thus able to cross the BBB. 8-HQ forms stable 5-membered rings with Fe^{3+} and thus is a very effective iron chelator for oral use (Pierre *et al.*, 2003). The structure of 8-HQ was used as lead in the design of other effective oral administered chelators such as VK-28, M30 and clioquinol (Figure 2.16). These compounds are, however, still in animal trials (Mounsey & Teismann, 2012).

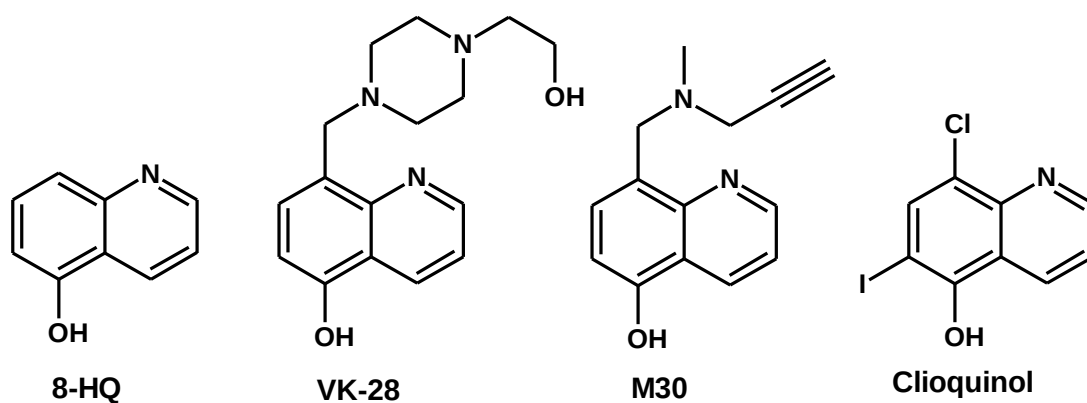


Figure 2.16: Structures of iron chelators.

VK-28 has been reported to possess similar potency to desferal, but can cross the BBB (Sachar *et al.*, 2004). M30 is a potent inhibitor of both MAO isoforms in the brain and may thus reduce MAO-mediated H_2O_2 formation as well as scavenge excess iron. Interestingly this compound inhibits MAO in the brain with little to no inhibition in the gut (Zheng *et al.*, 2005). MAO inhibition by M30 is dependent upon its propargylamine group, also present in the well-known MAO inhibitors, rasagiline and selegiline (Youdim, 2003). Clioquinol is a well-known highly effective antibiotic against fungal and protozoal infections (Rohde *et al.*, 1976). It was later discovered that high dosages lead to neurotoxicity due to clioquinol's contribution towards increased vitamin B12 excretion (Yassin *et al.*, 2000). Clioquinol is known to chelate iron, but was only recently investigated as a potential therapeutic agent in neurodegenerative diseases (Kidani *et al.*, 1974). Even though high concentrations lead to neurotoxicity, clioquinol is highly lipophilic and can thus cross the BBB at very low concentrations. In MPTP-treated mice clioquinol treatment resulted in decreased motor symptoms (Kaur *et al.*, 2003).

- **Pioglitazone**

Pioglitazone (Figure 2.17) is a drug approved to treat type 2 diabetes and is currently in clinical trials being evaluated as a possible disease modifying agent in PD. It is suggested that pioglitazone may reduce neuroinflammation thereby reducing oxidative stress and contributing towards the restoration of mitochondrial function. Pioglitazone is also an inhibitor of MAO B (Aviles-Olmos *et al.*, 2013).

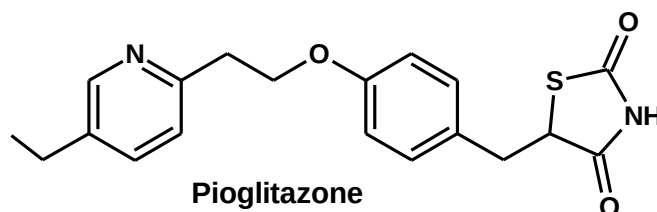


Figure 2.17: Structure of pioglitazone.

2.4 Conclusion

Although a number of hypotheses have been formulated, the aetiology of PD remains unknown. Key mechanisms that may contribute to neurodegeneration in PD include mitochondrial dysfunction, oxidative stress, misfolding and aggregation of proteins and the dysfunction of the UPS. In this respect, both environmental and genetic factors may induce or accelerate these mechanisms. The treatment of PD is focused on symptomatic relief of the motor and psychiatric symptoms. Levodopa is the most important and widely used drug in PD therapy, but has severe limitations. Other drugs, often co-administered with levodopa, include AADC inhibitors, COMT inhibitors and MAO inhibitors. These drugs block the metabolism of levodopa, thus increasing its therapeutic efficiency. This thesis will focus on the discovery of new MAO inhibitors. Such drugs have the additional benefit of also treating depression comorbid with PD. MAO inhibitors also may slow the degenerative processes in PD by reducing MAO-generated H_2O_2 .

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Chapter 3

Monoamine oxidase

3.1 General background

The catabolism of amines is an essential pharmacological process ranging from dietary digestion to prevent their functioning as false neurotransmitters to regulation of cell functioning. The MAOs are enzymes which can catalyse the oxidation of either endogenous or dietary amines. MAO is not the only enzyme responsible for the oxidation of amines, and semicarbazide sensitive amine oxidase (SSAO) which is copper containing, can also oxidise primary amines, diamines and histamine. For the purpose of neurodegenerative diseases, only MAO is a major contributor to neurodegeneration since SSAO is absent in the nerves and glial cells of the brain (Lyles, 1996).

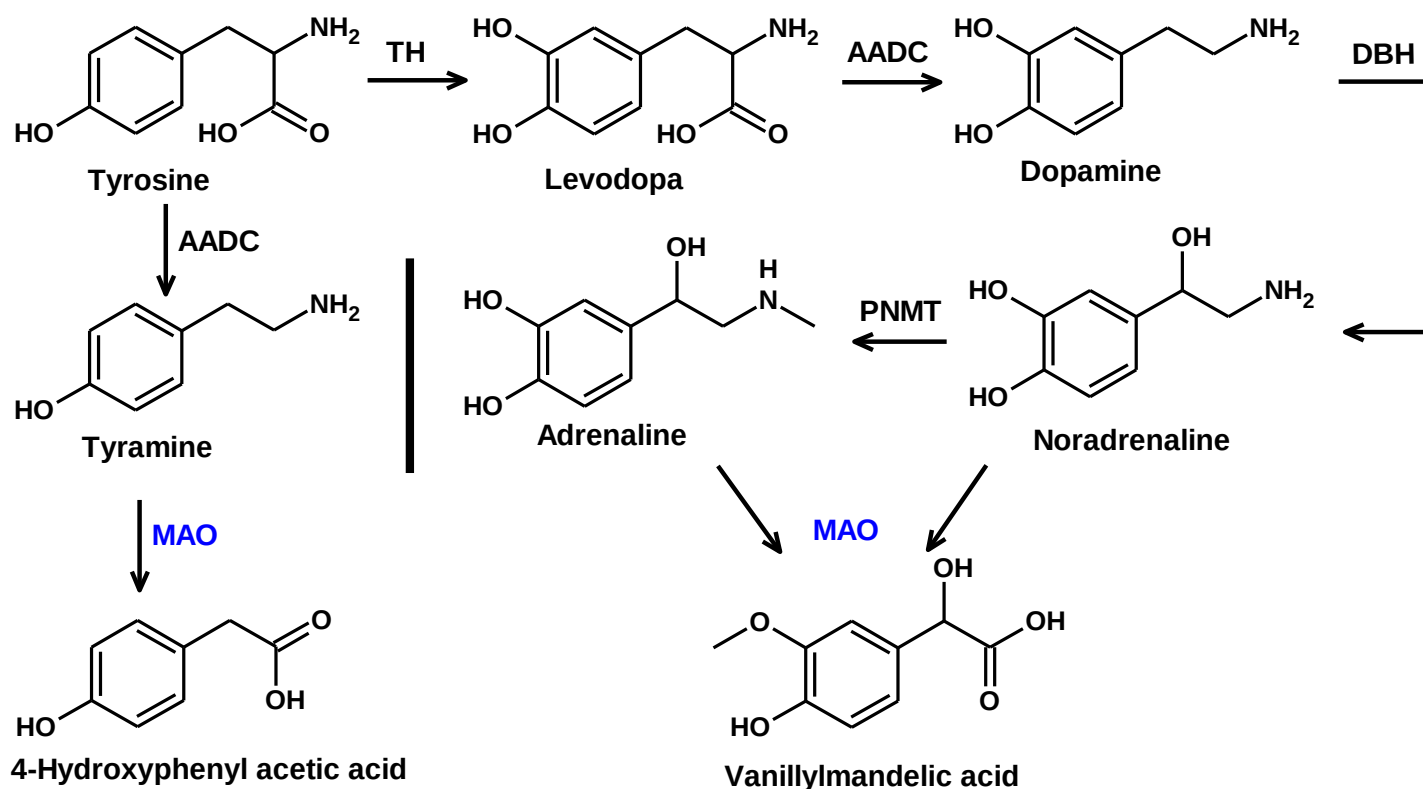


Figure 3.1: Synthesis of neurotransmitters from tyrosine and condensed metabolisms by MAO. TH: Tyrosine hydroxylase; AADC: Aromatic amino acid decarboxylase; DBH: Dopamine-β-hydroxylase; PNMT: Phenylethanol-amine-N-methyltransferase.

MAO was first discovered in 1928 and named tyramine oxidase since it was found to catalyse the oxidative deamination of tyramine (Hare, 1928). Hugh Blaschko made the discovery that tyramine oxidase is responsible for the metabolism of primary, secondary and tertiary amines but not of diamines (Figure 3.1). In 1938, tyramine oxidase was renamed to MAO (Zeller, 1938). In 1967 it was found that the MAO enzymes are expressed on the outer membranes of mitochondria

(Schnaitman *et al.*, 1967). This finding resulted in the use of bovine liver and later bovine brain as the foremost and convenient sources of MAO (Gomes *et al.*, 1969; Harada *et al.*, 1971). MAO purification led to the finding that MAO contains a flavin adenine dinucleotide (FAD) cofactor (Harada & Nagatsu, 1969) which is covalently bound to a cysteine amino acid (Walker *et al.*, 1971). In recent years purified recombinant MAO is sourced from a *Pichia pastoris* yeast system (Edmondson *et al.*, 2009).

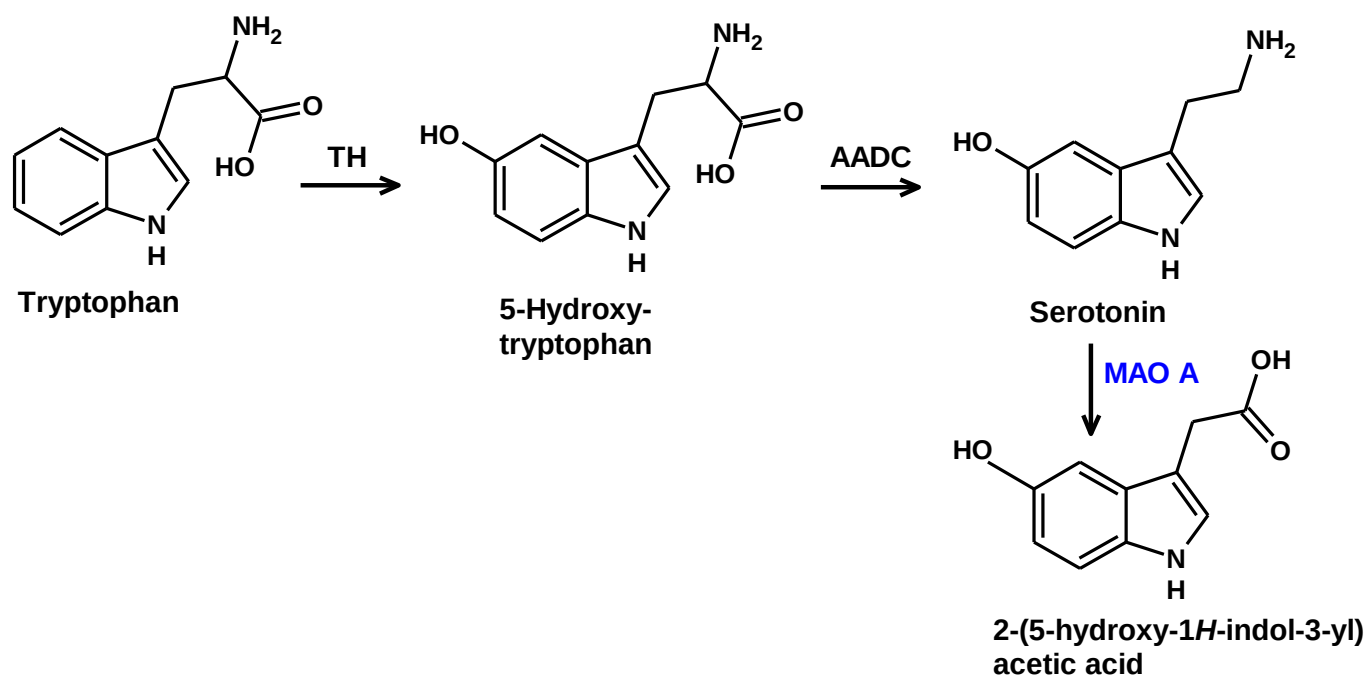


Figure 3.2: Synthesis of the neurotransmitter, serotonin, and its condensed metabolism by MAO A.

Additional studies suggested that there may be multiple forms of MAO (Collins *et al.*, 1970) and was confirmed by the discovery of Johnston in 1968 that clorgyline can be used to distinguish between these forms, MAO A and MAO B (Johnston, 1968). Clorgyline is a selective inhibitor of MAO A and thus MAO B remains unaffected. Later it was discovered that (R)-deprenyl is a selective inhibitor of MAO B. Favoured substrates of MAO A are serotonin (Figure 3.2), whereas preferential substrates for MAO B (Figure 3.3) are benzylamine and 2-phenylethylamine (Knoll, 1978). Both MAO isoenzymes oxidise tyramine, dopamine, noradrenaline and adrenaline (Glover *et al.*, 1977).

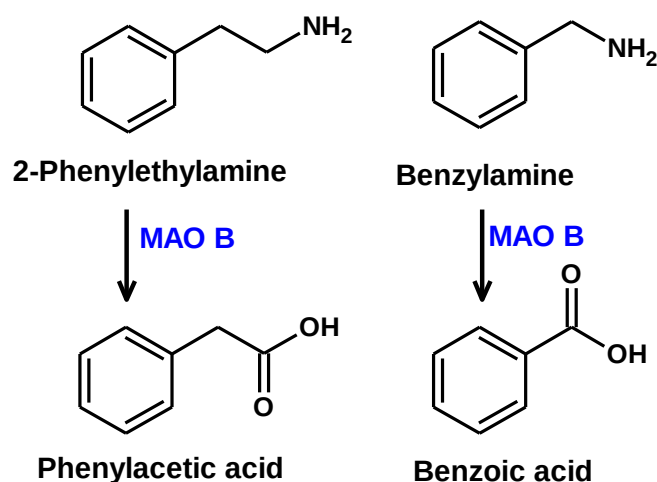


Figure 3.3: Structures of β -phenylethylamine and benzylamine and their condensed metabolism by MAO B.

MAO is ubiquitous in the human body, however, the two isoforms are distributed at different percentages in body tissues. For example MAO A is predominantly expressed (80%) in the gastrointestinal tract, but constitutes only 20% of the total MAO activity in the brain. MAO A is also the predominant isoform in the placenta and heart tissues. MAO B is mainly expressed in the dopaminergic neurons of the brain, platelets and in glial cells, but is only 20% of the total MAO activity in the gastrointestinal tract. The liver has equal amounts of the MAOs because, in the liver, biogenic amines should be quickly metabolised to lesser bioactive forms for excretion (Youdim & Bahkle, 2006).

When the concentrations of the neurotransmitters present in the brain, such as adrenaline, noradrenaline and dopamine fluctuate, an effect can be perceived in cognition, emotion and movement. These neurotransmitters are closely regulated by the MAOs. Low levels of neurotransmitters are evidently linked to hyperactivity and depression (Gillman, 2006). Higher levels are linked to increased risk-taking (Bartolatto *et al.*, 2009). Thus it was theorised that MAO A and MAO B are linked to neurological and psychiatric disorders such as Parkinson's disease and depression (Finberg *et al.*, 1998).

3.2 The therapeutic potential of MAO A

MAO A is the main MAO isoenzyme that influences mood and behaviour. Studies have shown that a genetic defect in MAO A, causing the enzyme to be inactive, leads to aggressiveness in males functioning under stress (Alia-Klein *et al.*, 2008). Research has also discovered that the metabolism of neurotransmitters by MAO A in the heart may contribute to heart failure (Kaludercic *et al.*, 2010) and age-related cardiomyopathies leading to enlarged heart muscles (Bianchi *et al.*, 2005).

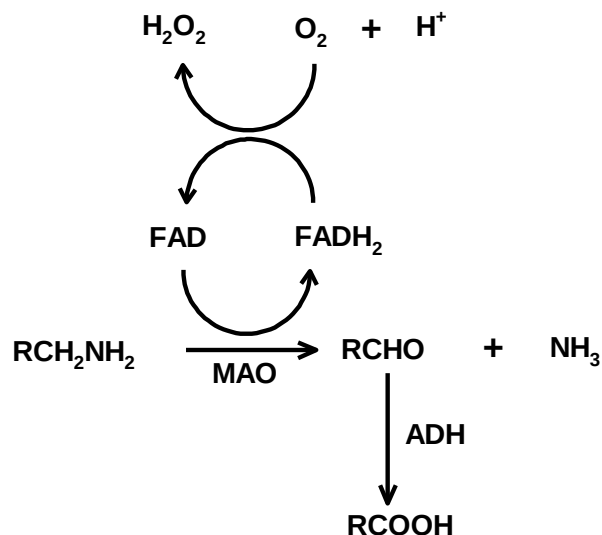


Figure 3.4: Oxidative deamination of amines by MAO.

Oxidative deamination of amines by MAO follows two catalytic pathways but ultimately both yields the amine-derived aldehyde as principal product and ammonia and H_2O_2 as by-products (discussed in more detail later). These products may all contribute towards oxidative stress. The aldehyde is rapidly metabolised by aldehyde dehydrogenase (ADH) to the corresponding acid (Figure 3.4). ADH is inhibited when operating under conditions of oxidative stress and this may result in the accumulation of aldehyde intermediates, which in turn may lead to toxicity towards biological systems (Chen *et al.*, 2008).

In the heart, oxidative stress impairs the normal functioning of cardiomyocytes causing contractile failure. MAO A is the main contributor of ROS in the heart and increased MAO A activity results in increased ROS facilitated damage. Thus MAO A overexpression and activity may cause cardiomyocyte necrosis (Villeneuve *et al.*, 2013) and heart failure (Kaludercic *et al.*, 2010). However, research has shown that inhibition of MAO A and even irreversible inhibition of MAO B reduces the formation of ROS and subsequently protects heart function (Poon *et al.*, 2010).

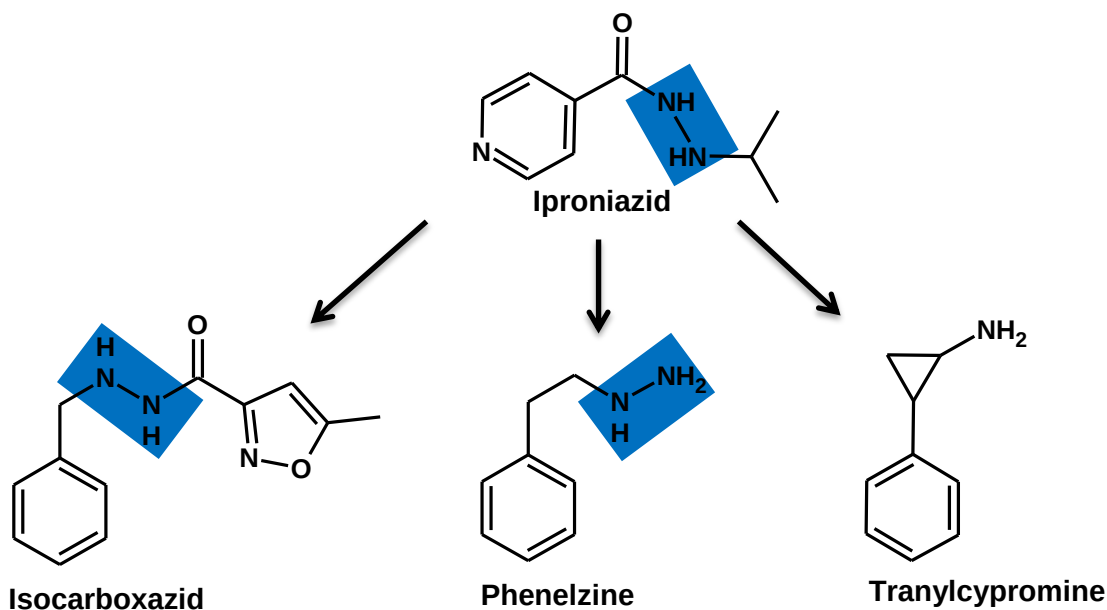


Figure 3.5: Structures of non-selective irreversible MAO inhibitors. The hydrazine functional group is highlighted in blue.

However, after it was revealed that there are two functional forms of MAO and their substrates specificities were confirmed, it was discovered that these compounds are nonselective irreversible inhibitors of the MAOs. This explained their side-effect profile with respect to the “cheese reaction” (Figure 3.6). It was thusly named after it was observed that hypertensive crisis develops when cheese and these inhibitors are taken together. Later all types of food containing amines (such as red wine and chocolate), especially tyramine, were found to provoke this reaction (Asatoor *et al.*, 1963).

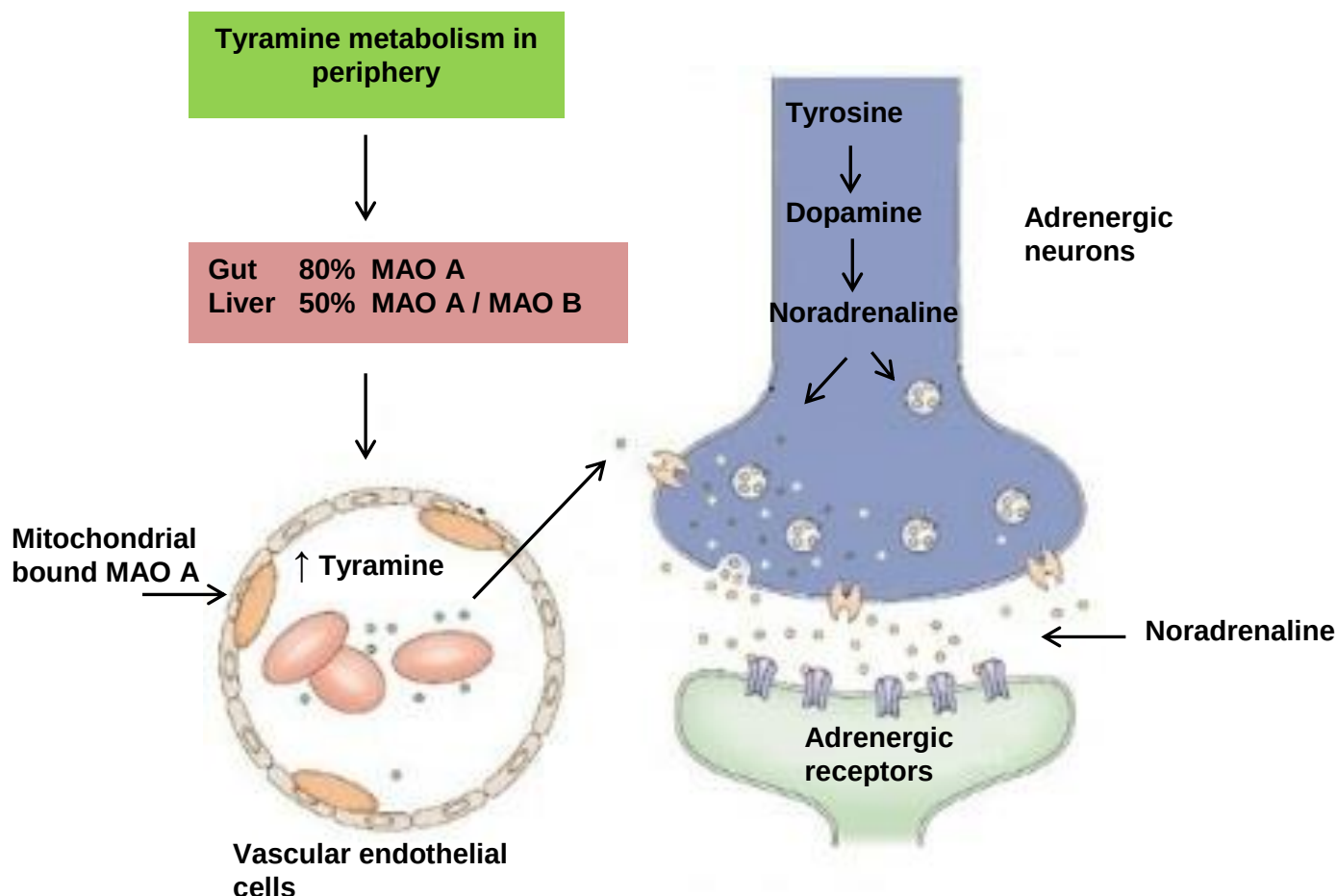


Figure 3.6: A schematic representation of the “cheese reaction”.

Under normal physiological conditions dietary amines undergo extensive inactivation firstly in the gut then the liver. The remaining tyramine is absorbed into vascular endothelial cells in which it is further deaminated by MAO A. The amount that reaches the adrenergic neurons is carefully regulated. Irreversible inhibition of MAO A prevents the first pass inactivation of tyramine in the gut, resulting in a high influx into the systemic circulation. In the adrenergic neurons, the high concentration of tyramine stimulates the synthesis and release of noradrenaline. The resulting increase in noradrenaline-mediated neurotransmission results in a hypertensive crisis (Youdim *et al.*, 1988).

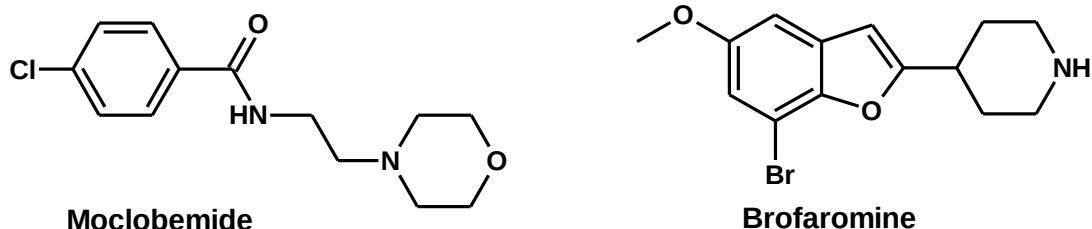


Figure 3.7: Structures of moclobemide and brofaromine.

Since it was already established that MAO A plays an important role in depression, the concept of developing compounds with selectivity and reversibility was initiated to overcome this obstacle. Selectivity towards one isoenzyme will cause the other isoenzyme to remain active to perform the metabolic actions of MAO e.g. deaminating ingested tyramine to prevent the “cheese reaction”. Reversible and competitive inhibition will allow the substrates such as tyramine to displace the inhibitor from the enzyme, so that tyramine in the periphery is normally metabolised by MAO (Da Prada *et al.*, 1988). The first compound to be registered under these conditions was moclobemide (Youdim, 1972) then later brofaromine (Figure 3.7), both selective reversible inhibitors of MAO A (Möller *et al.*, 1991).

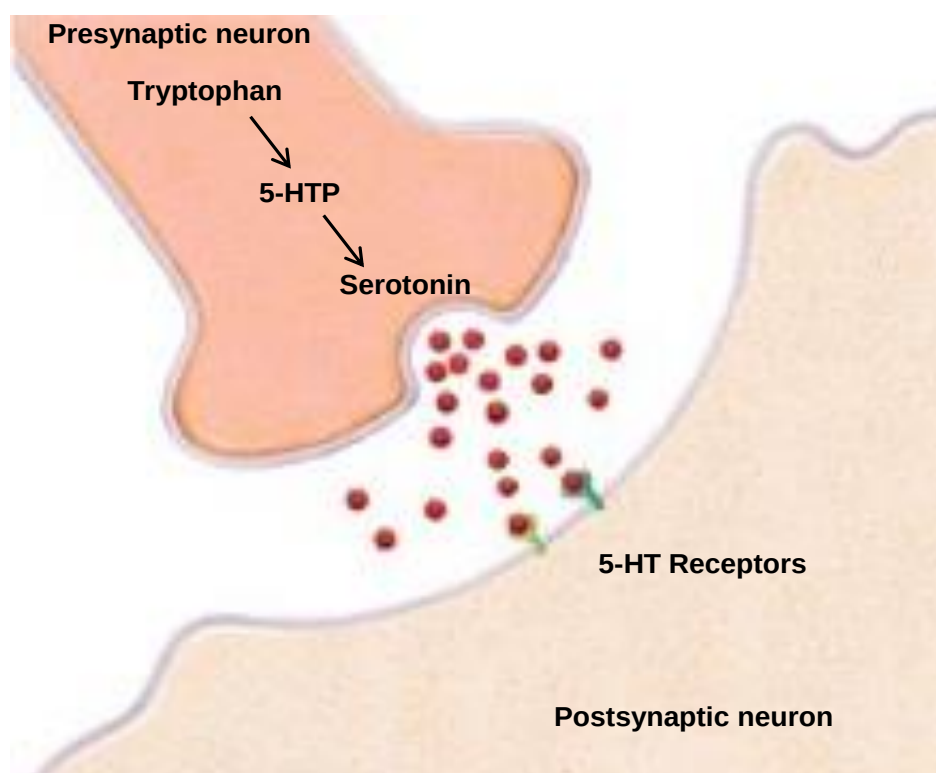


Figure 3.8: A schematic representation of serotonin syndrome. Serotonin, 5-HT; 5-Hydroxytryptophan, 5-HTP.

Nevertheless reversible MAO inhibitors also presented with another serious side effect, “serotonin syndrome”. It was first described in 1960 when a study was done on the effect of co-administering tryptophan and a hydrazine MAO inhibitor. They noted symptoms which were comparable to alcohol intoxication (Oates & Sjoerdsma, 1960). Serotonin syndrome presents with symptoms such as altered mental status, tremors, uncontrollable muscle spasms, motor restlessness, tachycardia

and hyperthermia (Sampson & Warner, 1999). Later it became more apparent that the simultaneous use of MAO A inhibitors and drugs that stimulate serotonin release, cause this side-effect. This is because MAO A is the main isoenzyme responsible for the degradation of serotonin in the brain (Figure 3.8). Together with serotonin enhancing drugs, serotonin receptors are overstimulated resulting in the serotonin syndrome. This syndrome can present with potentially life-threatening toxicity (Isbister & Whyte, 2002).

Recently, it was discovered that it may be possible to inhibit MAO activity in the brain, but not in the periphery. Two drugs have been identified, ladostigil and M30 (Figure 3.9). Research has shown that these drugs exert antiparkinsonian and antidepressant effects without inducing the “cheese reaction” or the serotonin syndrome. Also of interest is that ladostigil is classified as an acetylcholine esterase inhibitor, which can be used in the treatment of Alzheimer’s disease (Weinstock *et al.*, 2000). M30 also functions as an iron chelator (Zheng *et al.*, 2005). Thus these drugs are examples of multitarget drugs and possibly possess disease modifying properties.

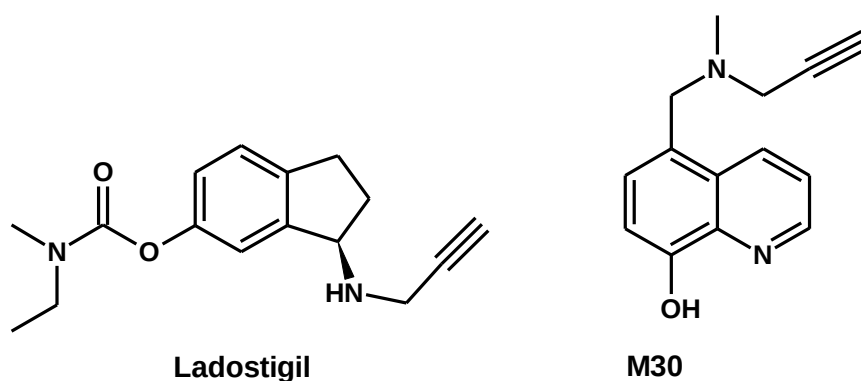


Figure 3.9: Structures of ladostigil and M30.

MAO A inhibitors may be of much value in the treatment of PD. It has been established that depression in PD is often undiagnosed and untreated, thus affecting the quality of life of PD patients negatively. Only 35% of PD patients are treated adequately for depression (Weintraub *et al.*, 2003). Effective and well-tolerated antidepressants are thus urgently needed as part of the therapy of PD. MAO A inhibitors may thus represent a potential treatment strategy of depression in PD patients. As shown below, non-selective MAO inhibitors may be used to address both the motor and depression symptoms in PD.

3.3 Therapeutic potential of MAO B

Metabolism of amines in the brain is important for normal physiological functioning. Low levels or absence of MAO B can alter a person's behaviour. For example, low platelet MAO B is associated with addictive behaviour such as Type 2 alcoholism (Lenders *et al.*, 1996). Elevated levels and activity of MAO B have been proposed as possible contributing factors in neurodegenerative mechanisms. This is because MAO B is responsible for the degradation of dopamine.

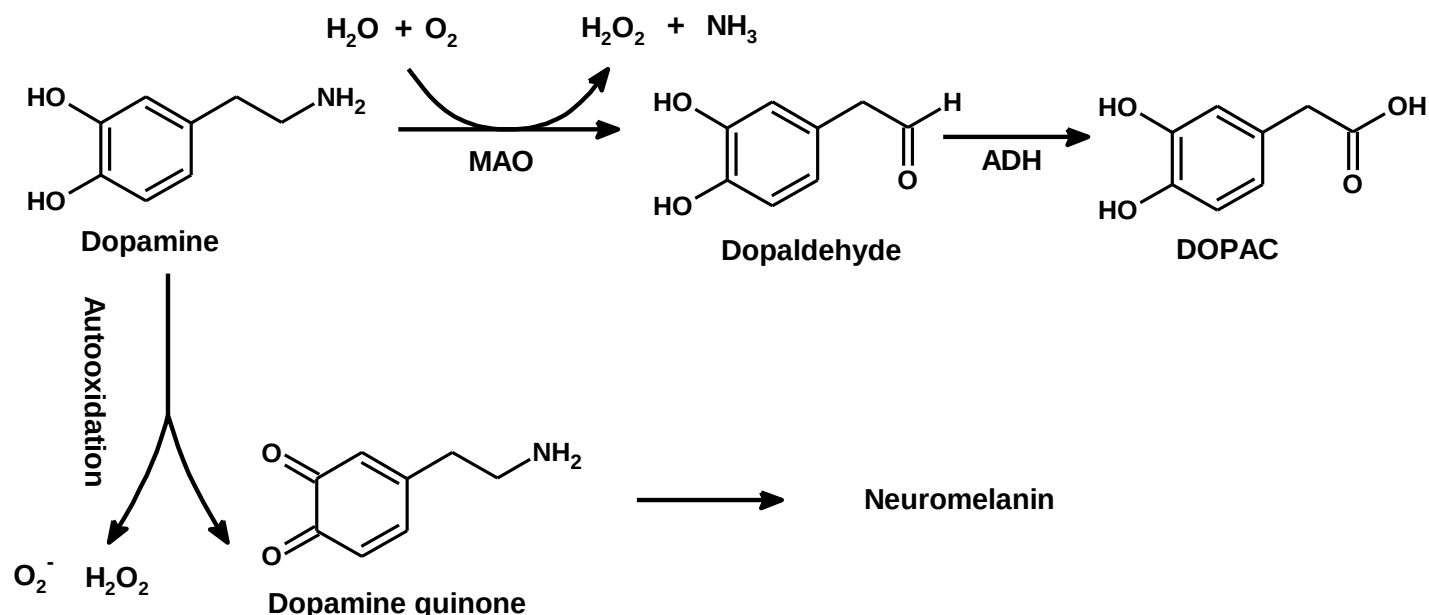


Figure 3.10: Oxidation of dopamine.

MAO B levels in the human brain increase as a person ages, thus the activity of MAO B in the brain also increases. This has the consequence that dopamine in the aged brain is oxidised more rapidly resulting in low dopamine levels. Furthermore, dopaldehyde produced by the oxidation of dopamine by MAO B is toxic to dopaminergic neurons. Aldehydes induce inactivation of macromolecules including the UPS and mitochondrial electron transport chain. It also inactivates ADH, which is responsible for inactivating toxic aldehydes (Burke *et al.*, 2003). The increased dopamine oxidation by MAO also contributes towards oxidative stress, further contributing to neurodegeneration.

Early 1970, after it was discovered that there are two isoforms of MAO, and that MAO A inhibition leads to the “cheese reaction”, another inhibitor was discovered, (R)-deprenyl. (R) Deprenyl is an irreversible inhibitor of MAO B, and a derivative of propargylamine. This compound inhibits the MAO B-mediated oxidative deamination of dopamine, phenylethylamine and benzylamine, when administered at low dosages. However, when higher dosages are administered, its selectivity towards MAO B is reduced and (R)-deprenyl may also inhibit MAO A (Knoll & Magyar, 1972). Nevertheless, (R)-deprenyl was introduced as a PD drug, although early trials as monotherapy yielded disappointing results. However, as adjunct therapy with levodopa, a decrease in

levodopa's wearing off effect was noted (Birkmayer *et al.*, 1975). After long-term research and clinical trials, (R)-deprenyl was suggested to act as a neuroprotective agent, since a reduced rate of neurodegeneration in dopaminergic neurons was witnessed compared to levodopa monotherapy (Birkmayer *et al.*, 1985). (R)-Deprenyl was approved for PD treatment and renamed to selegiline. Apart from the "cheese reaction" at high dosages, selegiline has additional side-effects. Selegiline is an amphetamine derivative, which undergoes first pass metabolism in the liver to form potentially neurotoxic amphetamine metabolites (Figure 3.11). These metabolites can lead to cardiovascular (Churchyard *et al.*, 1997) and psychiatric adverse effects (Monastruc *et al.*, 2000). The major metabolites of (R)-deprenyl are desmethylselegiline, l-methamphetamine and l-amphetamine (Riederer *et al.*, 1978).

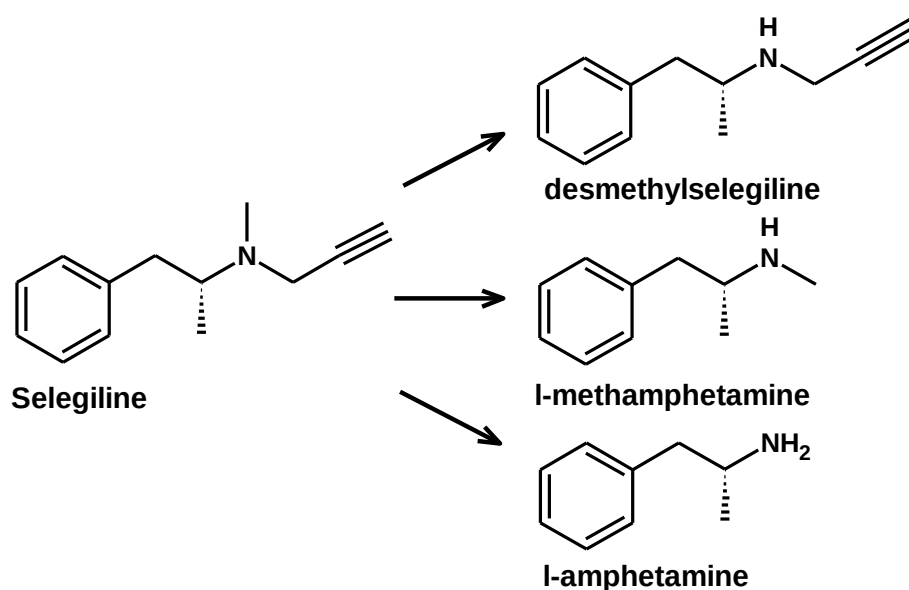


Figure 3.11: Structures of selegiline and three of its principal metabolites.

The mechanism underlying the disease modifying potential of selegiline is still unknown. The MPTP model of PD, however, supports the proposal that selegiline may act as neuroprotectant. MPTP is metabolised by MAO B to MPP⁺, a potent parkinsonian-inducing neurotoxin. The discovery that selegiline protects nigrostriatal neurons against MPTP-induced toxicity is in accordance to its inhibitory effect on MAO B (Heikkila *et al.*, 1984). This strengthens the hypothesis that MAO B is involved in neurodegeneration and possibly activates PD-inducing neurotoxins present in the environment. These observations, in part, led to the development of additional MAO B inhibitors for the treatment of PD.

In 1978 a drug originally developed for hypertension was showed to possess MAO inhibition properties. AGN 1133 is a non-selective irreversible MAO inhibitor. However, the N-demethylated derivative, AGN 1135, is a potent irreversible selective MAO B inhibitor (Finberg *et al.*, 1981). AGN 1135 is a mixture of enantiomers and the R(+)-enantiomer, rasagiline, is the more potent inhibitor. Rasagiline is a nonamphetamine aminoindan propargylamine that undergoes first pass metabolism to yield the inactive non-toxic aminoindan metabolite. Thus it has the safety benefit that, unlike

selegiline, rasagiline does not form toxic methamphetamine metabolites (Finberg & Youdim, 1985). Studies have shown that rasagiline is more potent than selegiline and is effective as both mono- and adjunct therapy with levodopa to reduce wearing off time (Pahwa *et al.*, 2006).

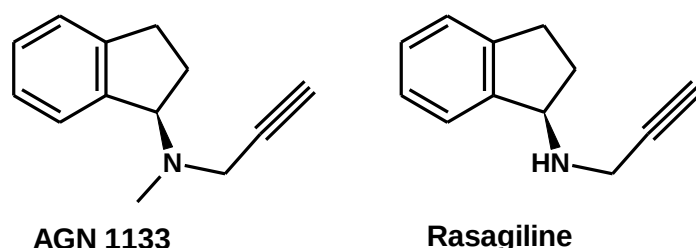


Figure 3.12: Aminoindan propargylamine derivatives.

Other drugs identified as MAO B inhibitors are safinamide and zonisamide, both of which are reversible inhibitors. Safinamide was originally developed as an anti-convulsant, but was found to be 5000-fold more potent towards MAO B than MAO A making it an exclusive inhibitor for MAO B. It is an α -aminoamide derivative with the added benefit of inhibiting dopamine reuptake (Fariello, 2007). Safinamide has entered phase III clinical trials and have recently been approved in Europe, as an antiparkinsonian drug (Borgohain *et al.*, 2014). Zonisamide is an anti-epileptic drug in use since 1980 and was later discovered to have beneficial effects in PD. In addition to its selective inhibition of MAO B, zonisamide is suggested to increase dopamine synthesis by decreasing the loss of tyrosine hydroxylase. Zonisamide is registered in Japan as an antiparkinsonian drug for adjunct therapy with levodopa (Murata, 2010).

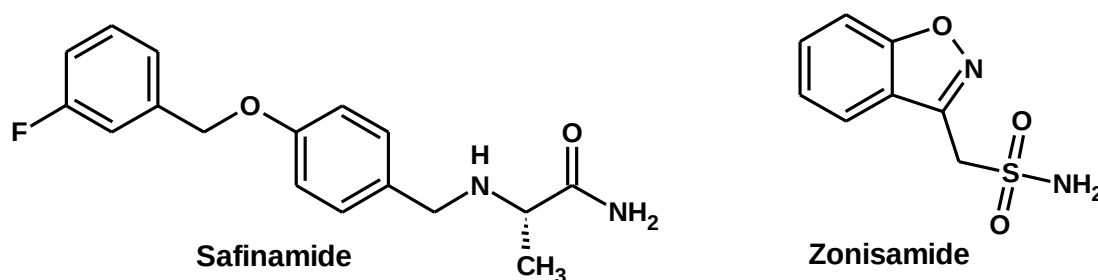


Figure 3.13: Structures of safinamide and zonisamide.

Selegiline, rasagiline and recently, safinamide, are the only MAO B inhibitors approved for the treatment of PD in the clinic. There is thus a need for the design and development of more MAO B inhibitors with acceptable efficacy and safety profiles. Examples of experimental inhibitors include resveratrol; trans, trans-farnesol and PF-9601 N.

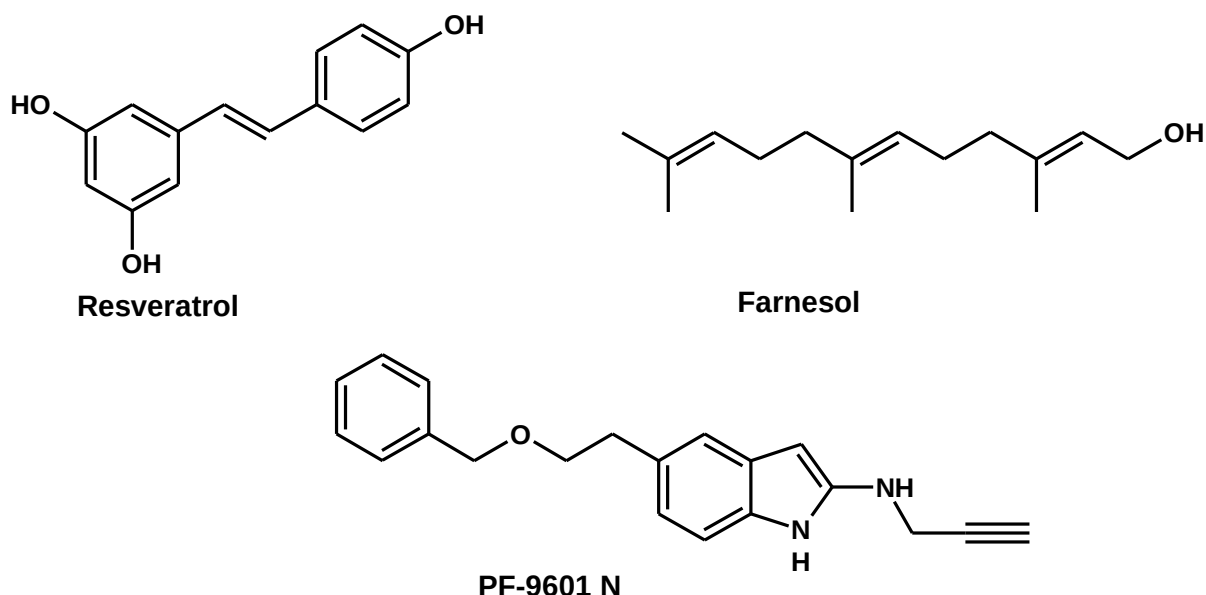
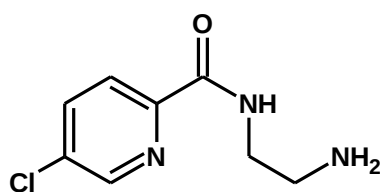


Figure 3.14: Structures of experimental MAO B inhibitors.

Resveratrol is a reversible MAO B inhibitor with antioxidant properties. This compound may thus possess neuroprotective activity since it can reduce oxidative stress (Feretta *et al.*, 2014). Trans, trans-farnesol is a compound found in tobacco smoke and is a potent reversible inhibitor of MAO B (Hubálek *et al.*, 2005). This may be of interest since some studies have found that smokers have a lower probability of developing PD (Hernán *et al.*, 2001). PF-9601 N is an irreversible inhibitor of MAO B and also displays neuroprotective properties. This compound inhibits the autooxidation of dopamine thereby increasing available dopamine in the brain (Perez *et al.*, 2003).

Alzheimer's disease is a neurodegenerative disorder responsible for 60-70% of dementia with advanced aging (Brookmeyer *et al.*, 1998). Dementia also occurs in PD. Oxidative damage by ROS appears to be central to the development of Alzheimer's disease. This is based on the fact that neurons are particularly sensitive to free radical damage because of their low GSH levels, increased oxygen requirement for metabolic processes and increased MAO B activity, resulting in oxidative stress (Christen, 2000). The only approved treatment for Alzheimer's disease is acetylcholine esterase inhibitors, however, these drugs only improve cognition with no effect on disease progression (Bliss & Collingridge, 1993). Since selegiline may be neuroprotective in PD, this compound was evaluated in Alzheimer's disease. It was found that selegiline improves behaviour, memory and attention in Alzheimer's disease patients (Piccinin *et al.*, 1990). Most importantly, in moderately severe Alzheimer's disease selegiline was shown to reduce disease progression (Sano *et al.*, 1996). Based on these findings, ladostigil, an analogue of selegiline, was developed as treatment for Alzheimer's disease (Figure 3.9). Ladostigil is currently in phase II clinical trials for the treatment of mild cognitive impairment and to prevent progression of Alzheimer's disease (Weinreb *et al.*, 2011). Another noteworthy MAO B inhibitor is lazabemide. Lazabemide is a highly selective, irreversible inhibitor of MAO B. It was also found to act as an antioxidant and decreases oxidative stress by inhibiting lipid peroxidation (Mason *et al.*, 2000). Unfortunately clinical trials have been discontinued because of toxicity issues with lazabemide.



Lazabemide

Figure 3.15: Structure of lazabemide.

Considering the central role of MAO in neurodegenerative disorders, it is unfortunate that only a few inhibitors are approved for this purpose. In PD, MAO inhibitors may be particularly advantageous since these compounds improve a patient's quality of life, delay the need for levodopa, allow for the reduction of the dosage of levodopa and possibly protect against further neurodegeneration. In addition, MAO inhibitors may be used to treat depression which is frequently associated with PD. There is thus a need for the development of new MAO inhibitors. It is however important that these inhibitors possess acceptable safety profiles.

3.4 The structure of MAO

The crystal structure of MAO is of great importance and revolutionised the design of novel MAO inhibitors. Analysing the crystal structure in complex with inhibitors provides a better understanding of the interactions between the enzyme and the inhibitors. This also provides insight into the selectivity profiles of inhibitors. The crystal structure of MAO B was first published in 2002 (Binda *et al.*, 2002) and that of MAO A in 2005 (De Colibus *et al.*, 2005). In order to solve the structures, the enzymes had to be purified and crystallised for analysis. This proved difficult since the MAOs are mitochondrial bound enzymes, and any attempt to remove the C-terminal helix, which functions as an anchor, destabilises the enzyme. The breakthrough was the discovery that a *Pichia pastoris* yeast expression system could be used to produce both enzymes, which after purification remains stable and active. For successful crystal growth the active site cavities requires a bound ligand. The first successful crystal structure of MAO B with a reversible inhibitor bound, was with 1,4-diphenyl-2-butene, which is a contaminant of polystyrene labware used in the experiment. As mentioned, this compound binds reversibly to MAO B. MAO B crystallises as a dimer and MAO A as a monomer, however, when bound to the mitochondrial membrane both enzymes are expressed as dimers (Binda *et al.*, 2003). It has been suggested that during purification of MAO A, the enzyme dissociates resulting in a mixture of monomers and dimers in which the monomer structure is more favourable for crystallisation (Binda *et al.*, 2011).

Elucidation of the crystal structures of MAO A and MAO B shows that the two isoenzymes share an approximate 70% amino acid sequence identity and are structurally similar. The major differences between the two enzymes are in their active site cavities, where inhibitors bind. MAO A has an active site cavity of 550 Å³, termed the substrate cavity, whereas MAO B has an active site cavity of ~700 Å³. The active site cavity of MAO B is bipartite and consists of a substrate cavity of

~400 Å³ and an entrance cavity of 290 Å³. Both enzymes have a C-terminal helix that protrudes into the outer mitochondrial membrane. MAO A and MAO B also possess cavity shaping loops which control ligand entry into the active site cavities (De Colibus *et al.*, 2005).

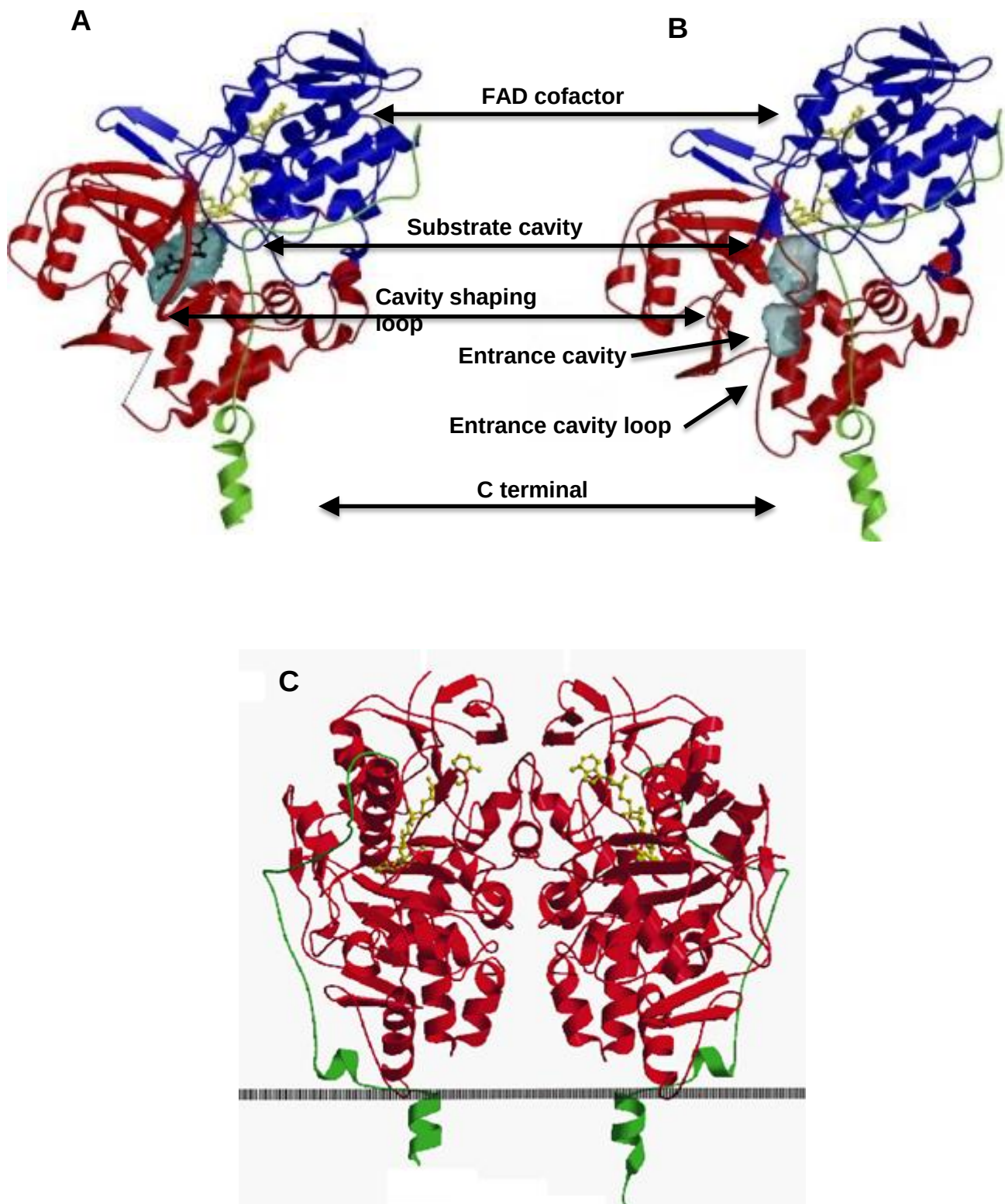


Figure 3.16: Crystal structures of human MAO A (A) and B (B) (Edmondson *et al.*, 2007). Also shown is the dimeric structure of MAO B (C) (Binda *et al.*, 2002).

MAO A consists of 527 amino acids with a subunit molecular weight of 59,700, while MAO B consists of 520 amino acids with a weight of 58,000. The FAD cofactor, illustrated in Figure 3.16, is covalently bound to both MAO enzymes through a cysteine (Cys) residue in the pentapeptide amino acid sequence chain Ser-Gly-Gly-Cys-Tyr. This cysteine is residue 397 in MAO A and residue 406 in MAO B (Bach *et al.*, 1988). In both enzymes the isoalloxazine ring of the FAD cofactor is in a bent conformation around the N(5)-N(10) axis, and can vary between 5-32° from planarity (Ramsay, 2012).

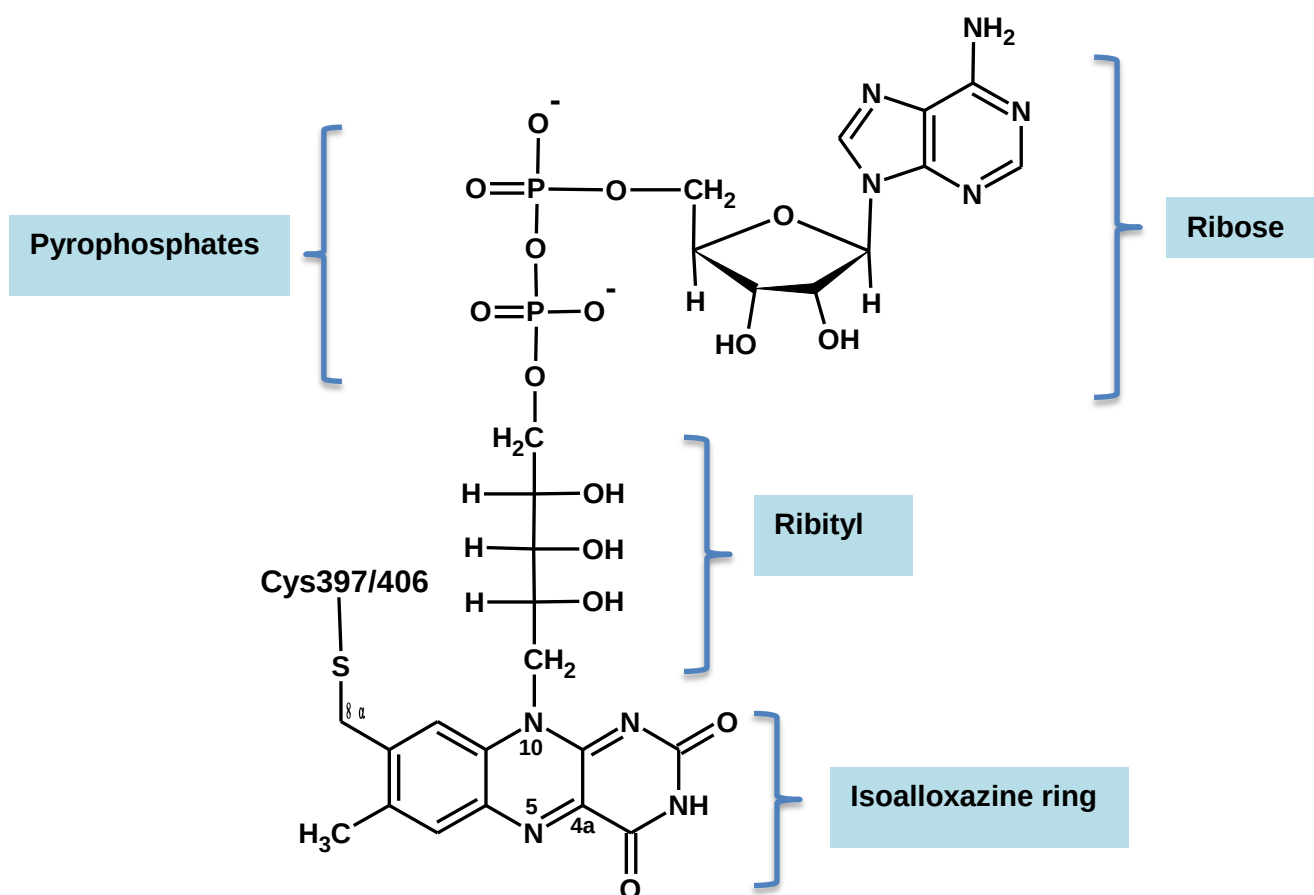


Figure 3.17: Structure of the covalent FAD cofactor of MAO.

The FAD cofactor lies at the end of a hydrophobic tunnel within the enzyme, which ends within an aromatic cage. The FAD is stabilised within this tunnel through various interactions such as ionic interactions between an arginine residue 42 (Arg42) and the pyrophosphates. Extensive hydrogen bonding occurs between the ribose moiety and glutamic acid 34 (Glu34), Arg36 and a water molecule. Also the ribityl side chains stabilise the FAD by hydrogen bonding between glycine 434 (Gly434) and the oxygen of the pyrophosphate linkage (Edmondson *et al.*, 2004). The aromatic cage is formed between two tyrosine (Tyr) residues situated opposite each other, Tyr406 and Tyr444 in MAO A, and Tyr398 and Tyr435 in MAO B. The purpose of this aromatic cage is to align the substrate towards the C4-N5 positions of the flavin (Moureau *et al.*, 1994). Research has shown that these two residues do not play a role in catalysis, but is essential to align the substrate correctly. Mutations of the aromatic cage residues are expected to negatively affect the affinity and

turnover of substrates. The tyrosine residues also exert a dipole effect on the substrate which makes it more susceptible to oxidation, thus polarising the lone pair of electrons on the amine (Li *et al.*, 2006).

Another similarity between the two MAO isoenzymes is a six residue amino acid sequence, termed the cavity shaping loop, which controls the entry of inhibitors into the substrate cavities. It has previously been suggested that these loops determine substrate specificities, but this possibility has now been ruled out. The loop, however, possesses a certain degree of flexibility enabling the binding of substrates and determining substrate affinities. In MAO A the six residues are 210-216 and in MAO B, residues 201-206.

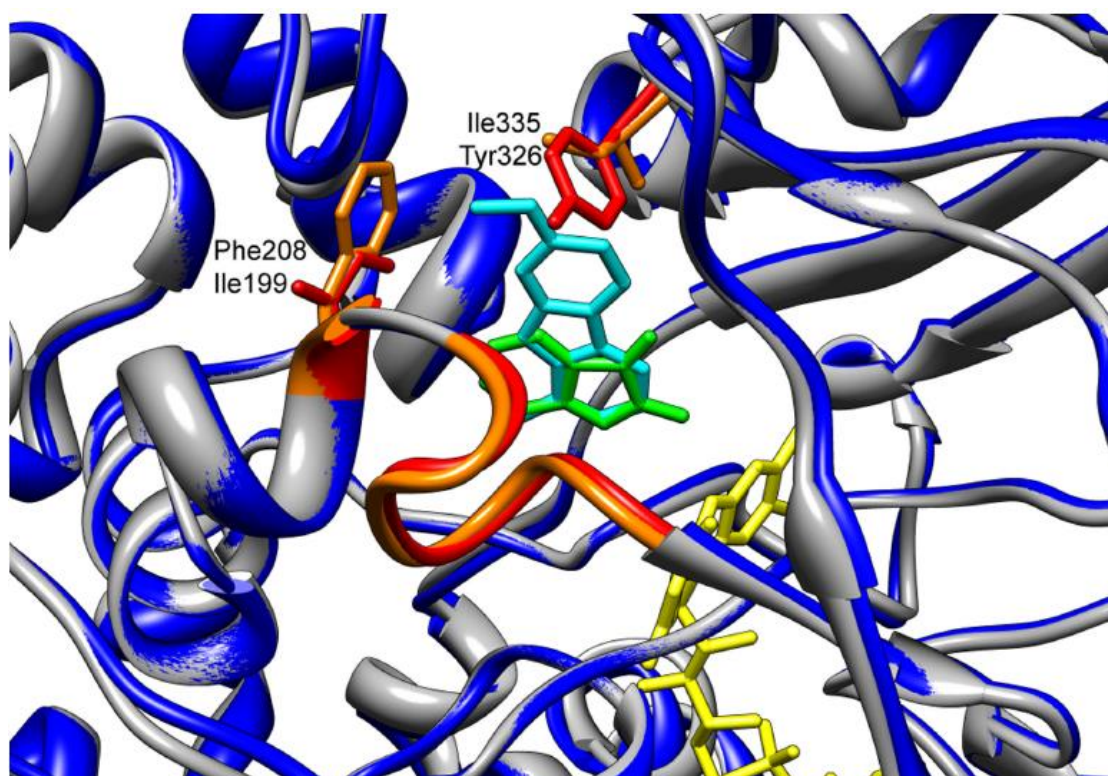


Figure 3.18: Overlay of the cavity shaping loops in MAO A and MAO B (Gaweska & Fitzpatrick, 2011).

Illustrated in figure 3.18 is the overlay of the substrate binding cavities of MAO A (grey) and MAO B (blue). The cavity shaping loop and amino acid residues of MAO A and B is displayed in orange and red, respectively. The FAD cofactor is displayed in yellow and the inhibitors, harmine (cyan) and isatin (green), are displayed for MAO A and MAO B, respectively. There are, however, differences in the substrate binding sites. The four residues displayed, exert effects on inhibitor inhibition and selectivity. Isoleucine 335 (Ile335) in MAO A and Tyr 326 in MAO B are crucial for substrate/inhibitor selectivity. Harmine a selective inhibitor of MAO A could not be fitted within the substrate cavity of MAO B because of structural overlap with Tyr326. Thus Tyr326 restricts the orientation of inhibitors within the substrate cavity of MAO B (Figure 3.18). In MAO A this restriction

is less prominent allowing inhibitors more flexibility of orientation due to the smaller size of Ile335, which is capable of induced fit. The orientation of Ile335 in MAO A is shown in figure 3.19 (Son *et al.*, 2008).

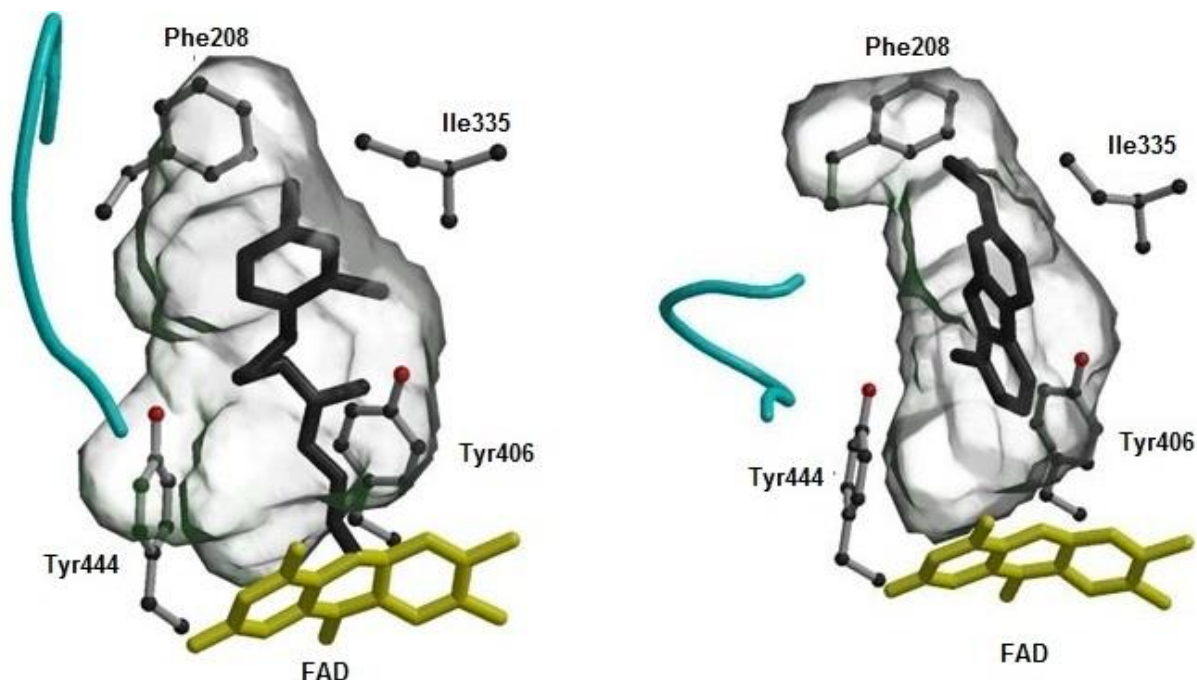


Figure 3.19: Illustration of the MAO A active site cavity in complex with clorgyline (left) and harmine (right) (Edmondson *et al.*, 2009).

The two residues, phenylalanine 208 (Phe208) in MAO A and Ile199 in MAO B, also play crucial roles in inhibitor binding and selectivity. A MAO B-selective inhibitor such as 1,4-diphenyl-2-butene will not fit within the active site cavity of MAO A because of steric hindrance caused by the Phe208 residue in MAO A (Figure 3.19). The Ile199 residue, however, acts as a gating residue between the substrate cavity and entrance cavity within MAO B. This residue can exist in an open or closed conformation depending on the bound substrate. Thus MAO B is capable of binding inhibitors in either cavity or both cavities (Figure 3.20). Isatin is bound in the substrate cavity where the Ile199 is in the closed conformation, whereas 1,4-diphenyl-2-butene traverses both active site cavities of MAO B and the Ile199 gate is in the open conformation (Edmondson *et al.*, 2009). This has the implication that larger inhibitors can bind to MAO-B when the Ile199 gate is in the open position. In MAO A, the corresponding residue, Phe208 cannot exist in an open orientation similar to that seen for Ile199, and this residue thus restricts the binding of larger compounds to MAO A.

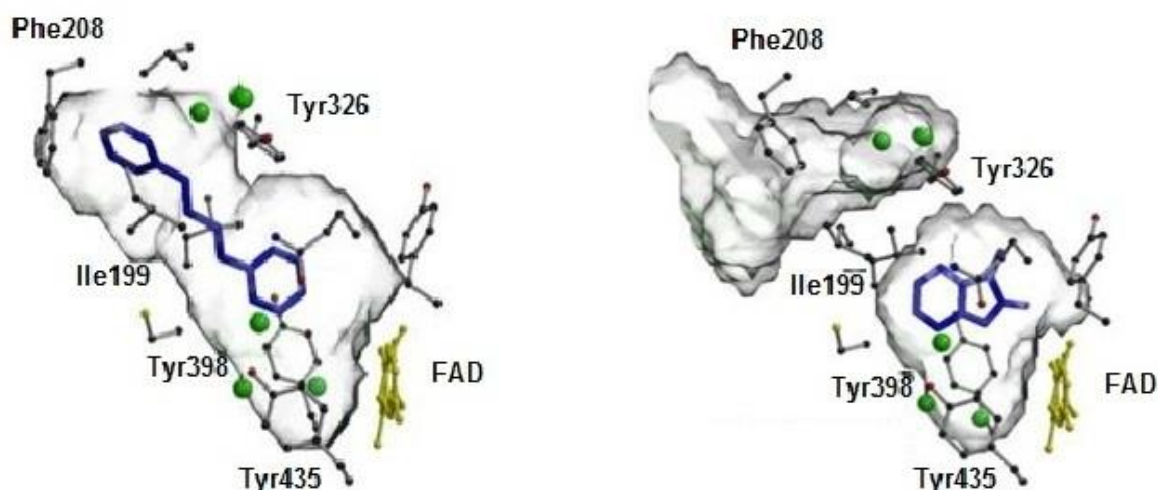


Figure 3.20: Illustration of the open and closed conformations of residue Ile199 in the active site cavity of MAO B, with 1,4-diphenyl-2-butene (left) and isatin (right) bound (Edmondson *et al.*, 2009).

Also shown in figure 3.20 are green spheres which represent conserved water molecules in the MAO B active site. Even though the MAOs have hydrophobic active sites, these fixed water molecules are present in all reported MAO structures. They participate in direct hydrogen bonding with the ligands and are thought important in MAO inhibitor specificities and affinities (Binda *et al.*, 2011).

Equipped with the knowledge of the structures of the MAOs it is possible to follow a structure-based design approach for the discovery of new MAO inhibitors. This involves docking of potential inhibitors into models of the MAO enzymes and analysing the resulting binding interactions and calculated affinities.

3.5 Proposed mechanisms of MAO catalysis

The MAOs are flavoproteins, which catalyse the oxidation of amine neurotransmitters and utilise molecular oxygen (O_2) as electron acceptor. Firstly, the amine substrate binds in the active site cavity of the enzyme to form $E.FAD_{ox}\text{-S}$. The substrate is oxidised by two electrons with the concomitant reduction of the oxidised FAD cofactor, forming an enzyme bound complex ($E.FAD_{red}\text{-P}$). This complex then reacts with atmospheric oxygen, which reoxidises the FAD (to yield $E.FAD_{ox}\text{-P}$) and releases H_2O_2 . Lastly the complex separates, releasing the imine product to form aldehyde and ammonia through non-enzymatic hydrolysis. Alternatively, the reduced complex ($E.FAD_{red}\text{-P}$) may dissociate first before it is reoxidised (Edmondson *et al.*, 2009).

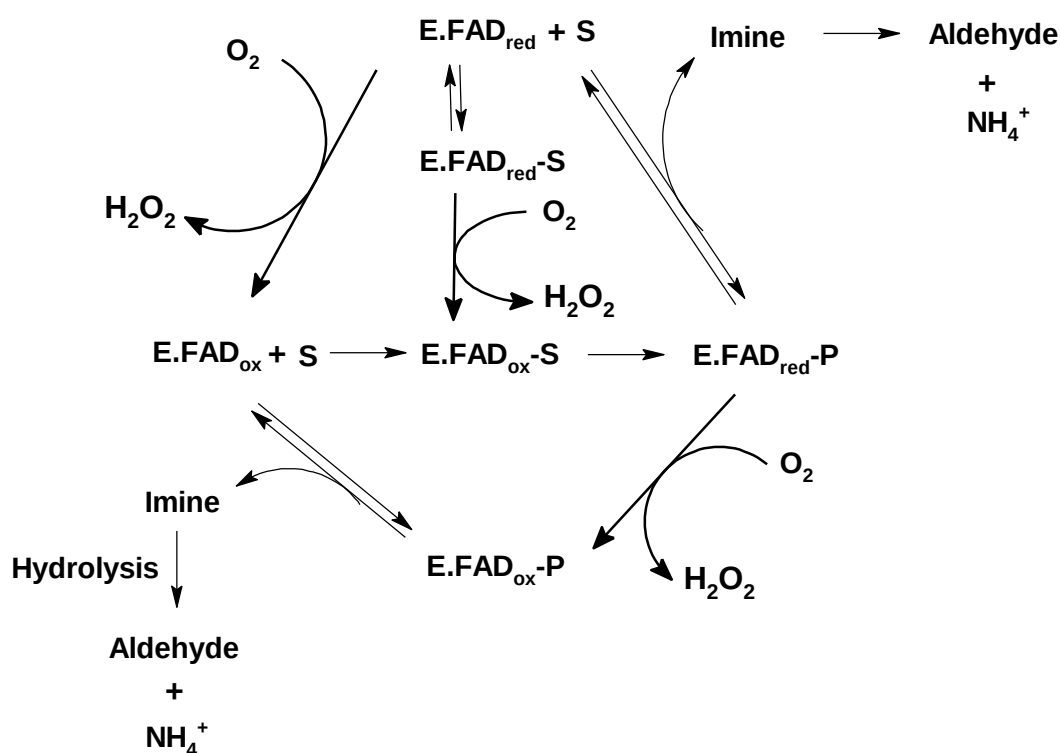


Figure 3.21: General schematic of the catalytic pathways of the MAOs.

Thus for MAO there are two possible catalytic pathways, the oxidative and reductive half reaction pathways. The reductive pathway is more favourable for most substrates and is represented by the lower loop in figure 3.21. The enzyme-product complex reacts with oxygen before the imine is separated. It is believed that the deprotonated amine moiety bind to the enzyme and is oxidised to the protonated imine (Edmondson *et al.*, 1993). This half reaction is instigated by a cleavage of the C α -H of the amine with the two electron reduction of the FAD, forming the imine and flavin hydroquinone, respectively. Stereochemistry experiments showed that the pro-R hydrogen is removed during catalysis and abstraction may take place through three possibilities; heterolytic hydride transfer, heterolytic H⁺ abstraction and homolytic H[•] abstraction (Yu *et al.*, 1986). Within these possibilities three mechanisms have been proposed, the polar nucleophilic, single electron transfer (SET) and hydride transfer mechanisms.

The polar nucleophilic mechanism was first proposed by Miller and Edmondson (1999) in a kinetic study on human MAO A catalysis with a series of para-substituted benzylamine analogues (Figure 3.22). Based on the structural similarity between the two MAO enzymes, it is thought that both enzymes follow the same mechanistic pathway. In MAO A, the catalytic site is unconstrained allowing the aromatic ring of substituted benzylamine to access different conformations to enable C-H cleavage. In contrast, the catalytic site of MAO B is more constrained so that benzylamine has lesser conformations but nevertheless is still able to bind in conformations allowing transfer of electrons.

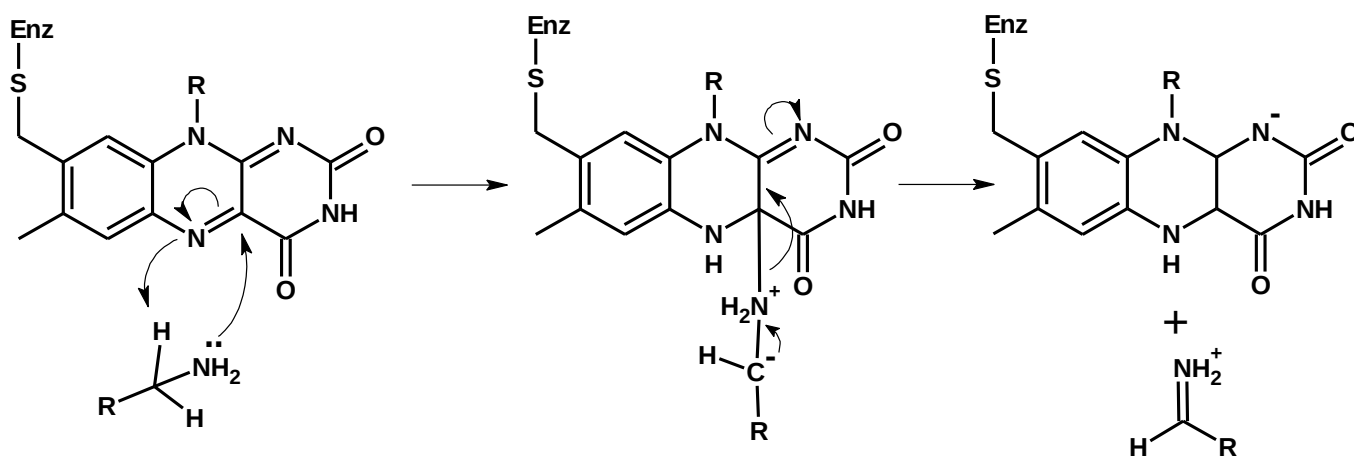


Figure 3.22: Schematic representation of the polar nucleophilic mechanism.

The isoalloxazine ring of the FAD cofactor in both MAO's is in a 30° bent conformation around the N(5), N(10) axis, when a substrate is bound (Binda *et al.*, 2003). This places the N(5) position in a sp^3 conformation opposed to a sp^2 conformation when the ring is planar. Thus the N(5) position is in a higher electron density compared to the C(4a) position. This facilitates the nucleophilic attack from the lone pair of the basic amine substrate on the C(4a) position of the enzyme resulting in a flavin-substrate adduct. Additionally for adduct formation the N(5) position should also become very basic. Thus the amine attack on C(4a) leads to the formation of a strong base at N(5) which then abstracts the α -proton from the substrate. The adduct collapses leaving the FAD in a reduced form and the substrate in an oxidised state (Miller & Edmondson, 1999). Further evidence for this mechanism is provided by structural experiments conducted with farnesol in the active site cavities of MAO B. The isoprenoid structure binds to MAO B with the hydroxyl moiety and the α -CH₂, at the C(4a) and N(5) positions of the flavin ring, respectively. The placements of these atoms are similar to those expected for an amine substrate should the polar nucleophilic mechanism be valid (Edmondson *et al.*, 2007).

The rationale behind the SET mechanism is that one-electron oxidation of the amine substrate nitrogen would cause the α -CH bond to become labile and allow hydrogen abstraction. This mechanism was first proposed by Silverman and co-workers (Figure 3.23). They suggested that the pK_a of the α -CH amine substrate required a strong base for hydrogen abstraction and that this could not be carried out by basic amino acid side-chains. In order for this hydrogen abstraction to take place the pK_a had to be lowered through acidification of the α -CH group. This may be achieved by oxidising the amine by one electron to form the cation radical, which will readily lose a proton. One-electron reduction of the FAD yields the flavin semiquinone radical, which after a second one-electron reduction, accepts a proton and forms the flavin hydroquinone (Silverman *et al.*, 1980).

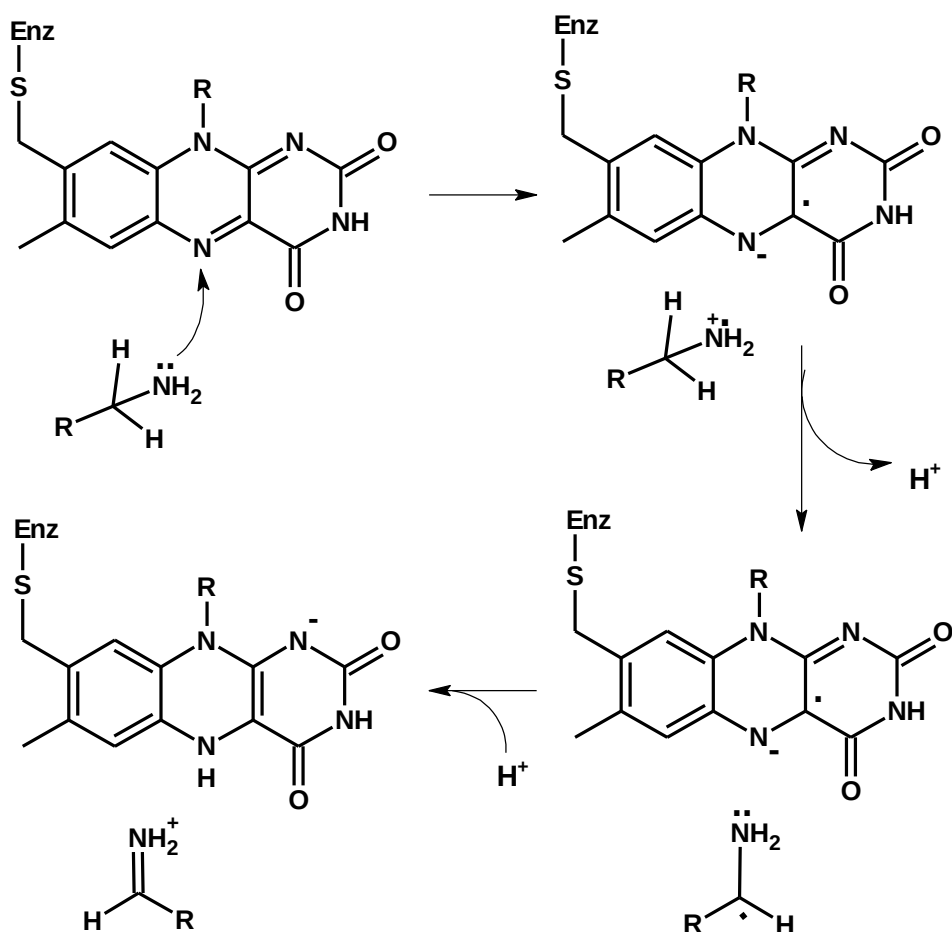


Figure 3.23: SET mechanism of MAO catalysis.

There is, however, doubt whether this mechanism is probable. Experiments failed to detect flavin semiquinone and aminium cation radicals, but it may be argued that these intermediates do not accumulate and is thus difficult to detect (Gaweska & Fitzpatrick, 2011). Furthermore, this mechanism relies on the ability of the enzyme to overcome unfavourable thermodynamics of the first electron transfer. Amines have a high redox potential and there is no oxidant in the active sites of the MAOs capable of extracting an electron from the substrate (Miller & Edmondson, 1999).

Another possible mechanism of MAO action is through direct hydride transfer from the amine substrate to the FAD cofactor with no intermediate steps (Figure 3.24). The α -CH hydrogen of the amine is transferred as a hydride to the N(5) position of the flavin. The products of this reaction are the iminium cation and reduced flavin. This hydride mechanism is a generally accepted mechanism for the oxidation of other amine oxidases such as D-amino acid oxidase. This is the most favoured mechanism since transfer of the hydride is not dependent on the involvement of basic amino acids in the active site (Fitzpatrick, 2010). Additional support provided by computational analyses indicates that the hydride mechanism also has lower transition energy than the above mentioned mechanisms (Atalay & Erdem, 2013).

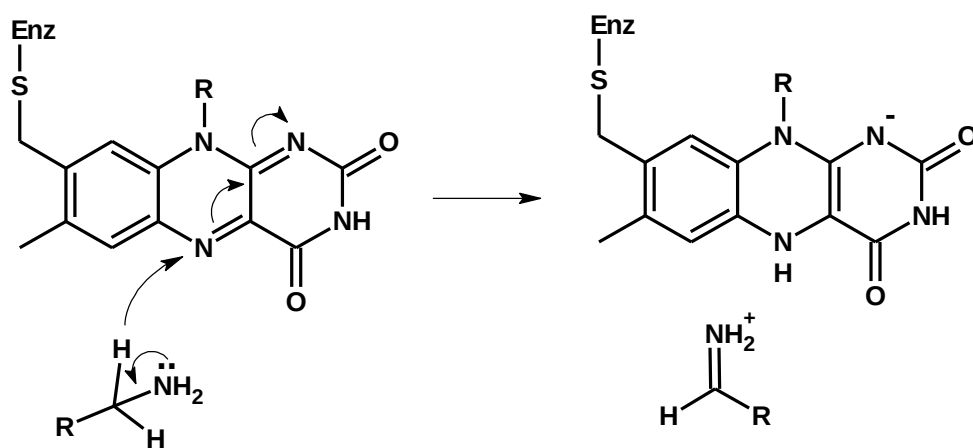


Figure 3.24: Hydride transfer mechanism of MAO catalysis.

3.6 Conclusion

The MAO enzymes are important pharmacological targets for the treatment of neuropsychiatric and neurodegenerative disorders. MAO B inhibitors have been employed in the treatment of Parkinson's disease and are under investigation for the treatment of Alzheimer's disease. MAO A inhibitors are used in the clinic for the treatment of depression, and more recently it has been found that MAO A inhibitors may protect against age-dependent damage of cardiac tissues. Inhibition of MAO A, however, leads to severe side-effects, a factor which should be taken into consideration when designing or utilising these inhibitors. Most of the severe adverse effects of MAO A inhibitors can be overcome by designing reversible inhibitors or selective inhibitors of the MAO B isoform. In the design of new MAO inhibitors, the crystal structures of these enzymes are useful, especially when following a structure-based design approach. Based on the clinical utility of MAO inhibitors, this thesis aims to contribute towards the discovery of new MAO inhibitors.

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Chapter 4

Manuscript A

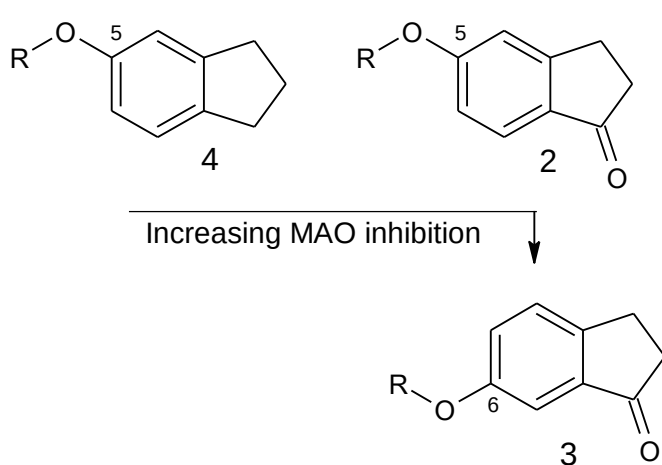
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Indanones as high potency reversible inhibitors of monoamine oxidase

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Graphical abstract



C6-Substituted indanones (**3**) are highly potent and selective MAO-B inhibitors, with IC_{50} values ranging from 0.001-0.030 μ M. C5-Substituted indanone (**2**) and indane (**4**) derivatives are comparatively weaker MAO-B inhibitors. C6-Substituted indanones are thus promising leads for the design of therapies for Parkinson's disease.

Authors' contributions

- The experimental work, interpretation of results and documentation of this study was carried out by **S. Mostert**.
- This study was conceptualised and documented with the assistance of **J.P Petzer**.
- The enzymology section of this study was conducted under the supervision of **A. Petzer**.

All co-authors provided permission to use this manuscript as part of **S Mostert's Ph.D thesis**.

Indanones as high potency reversible inhibitors of monoamine oxidase

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Keywords: indanone; inhibitors; molecular modelling; monoamine oxidase; Parkinson's disease.

Abstract

Recent reports document that α -tetralone (3,4-dihydro-2H-naphthalen-1-one) is an appropriate scaffold for the design of high potency monoamine oxidase (MAO) inhibitors. Based on the structural similarity between α -tetralone and 1-indanone, the present study synthesised 34 1-indanone and related indane derivatives as potential inhibitors of recombinant human MAO-A and MAO-B. The results show that C6-substituted indanones are particularly potent and selective MAO-B inhibitors with the IC₅₀ values ranging from 0.001–0.030 μ M. C5-Substituted indanone and indane derivatives are comparatively weaker MAO-B inhibitors. Although the 1-indanone and indane derivatives are selective inhibitors of the MAO-B isoform, a number of homologues also are potent MAO-A inhibitors with 3 homologues possessing IC₅₀ values <0.1 μ M. Dialysis of enzyme–inhibitor mixtures further establish that a selected 1-indanone is a reversible MAO inhibitor with a competitive mode of inhibition. It may be concluded that 1-indanones are promising leads for the design of therapies for neurodegenerative and neuropsychiatric disorders such as Parkinson's disease and depression.

4.1 Introduction

The monoamine oxidase (MAO) enzymes are mitochondrial-bound flavoproteins and catalyse the α -carbon 2-electron oxidation of amine substrates.^[1,2] MAO consists of two separate isoforms, MAO-A and MAO-B, which are encoded by distinct genes.^[3] Although the substrate specificities of MAO-A and MAO-B often overlap, certain substrates are specifically metabolised by one MAO isoform. In this regard, serotonin is preferentially metabolised by MAO-A, while the false neurotransmitter, β -phenylethylamine, is a MAO-B specific substrate. The dietary amine, tyramine, dopamine, epinephrine and norepinephrine are considered to be substrates for both MAO isoforms.^[4] The MAO enzymes are of pharmacological importance since they terminate the physiological actions of amine neurotransmitters in the brain and peripheral tissues.^[5,6] Inhibitors of MAO-A and MAO-B have thus been extensively used in the treatment of disease states that result from deficient levels of neurotransmitters. For example, reduced levels of serotonin in the brain are associated with depressive illness, and MAO-A inhibitors are thus used as antidepressants.^[7,8] In Parkinson's disease, MAO-B inhibitors are used to inhibit the MAO-catalysed breakdown of dopamine,^[9] which relieves dopamine depletion in the basal ganglia. MAO-B inhibitors are often combined with L-dopa, the metabolic precursor of dopamine, in Parkinson's disease in order to prolong the action dopamine derived from L-dopa.^[10,11] Interestingly, clinical evidence supports a role for MAO-B inhibitors as an aid in smoking cessation.^[12]

It is noteworthy that MAO-B inhibitors are considered to possess neuroprotective properties in Parkinson's disease. While the mechanism is unclear, this neuroprotective effect of MAO-B inhibitors may be associated with a reduction of the central levels of H_2O_2 and aldehydes.^[5,13] H_2O_2 and aldehyde intermediates are formed by the MAO catalytic cycle, and may injure neuronal cells if not adequately inactivated.^[5,13] In this regard, MAO-B inhibitors are considered more effective than MAO-A inhibitors in Parkinson's disease since MAO-B activity, and not MAO-A activity, increases as the human brain ages. This suggests that injurious species produced by MAO in the aged Parkinsonian brain is more likely to be as a result of the action of MAO-B than the MAO-A isoform.^[14] H_2O_2 and aldehyde intermediates of the MAO catalytic cycle may also have roles in cardiovascular pathophysiology. By reducing the concentrations of these species in the myocardium, MAO inhibitors may improve cardiac function in patients with congestive heart failure.^[15]

Based on the various pharmacological roles of MAO inhibitors, the discovery of novel classes and scaffolds are of value. Considering their isoform selectivities, MAO inhibitors may be categorized as MAO-A selective, MAO-B selective and MAO-A/B non-selective inhibitors. MAO inhibitors may be further categorized as irreversible and reversible inhibitors. While MAO-B selective inhibitors, including irreversible inhibitors have an excellent safety profile, MAO-A inhibitors and MAO-A/B non-selective inhibitors are used with caution in the clinical setting. This is due to the ability of MAO-A inhibitors to cause a severe hypertensive response when taken with dietary tyramine.^[16]

Tyramine is, to a large degree, metabolised in the intestine by MAO-A which limits its entry into the systemic circulation. The inhibition of MAO-A thus results in high plasma levels of tyramine. Since tyramine induces the release of norepinephrine from peripheral neurons, excessive amounts of this amine result in severe hypertension.^[16] It is important to note that this effect is associated with irreversible MAO-A inhibition, and reversible MAO-A inhibitors do not cause tyramine-induced hypertension.^[17,18] When designing MAO inhibitors, a reversible mode of inhibition is thus desirable, particularly for the MAO-A isoform.

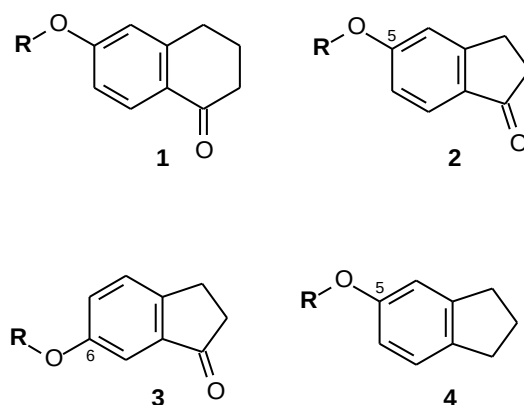


Figure 1: The structures of compounds discussed in the text.

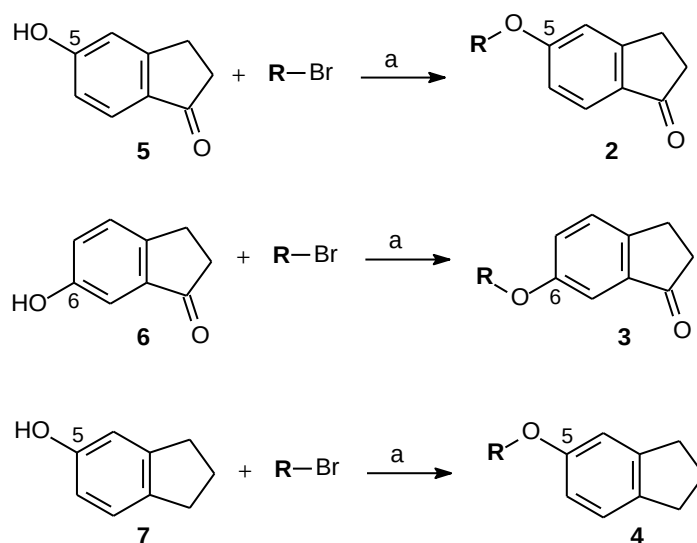
We have recently reported that a series of α -tetralone (3,4-dihydro-2*H*-naphthalen-1-one) derivatives (**1**) acts as highly potent MAO-B inhibitors with all compounds (fifteen) exhibiting IC_{50} values in the nanomolar range (Figure 1).^[19] The α -tetralones were also found to be potent MAO-A inhibitors with ten compounds exhibiting IC_{50} values in the nanomolar range. With the objective of discovering additional high potency MAO inhibitors, the present study synthesised a series of 1-indanone derivatives (**2**, **3**) as potential MAO inhibitors. 1-Indanone may be viewed as the 5-membered ring analogue of α -tetralone and may thus represent a suitable scaffold for MAO inhibitor design. For the purpose of this study, 1-indanone was substituted on the C5- or C6-positions with the benzyloxy moiety, which has been shown to be a suitable substituent for high potency MAO inhibition by α -tetralones derivatives, when placed on the C6-position. Since halogen substitution enhances both MAO-A and MAO-B inhibition potencies of such α -tetralones, the 1-indanone derivatives were substituted on the *meta* and *para* positions of the benzyloxy phenyl ring with halogens (Cl, Br). To further derive structure-activity relationships (SARs) for MAO inhibition by the 1-indanone class of compounds, homologues substituted on C5 or C6 with the cyclohexylmethoxy, cyclohexylethoxy, phenylpropoxy, 4-methylpentoxy and 2-phenoxyethoxy moieties were included. In addition, derivatives substituted on the *para* positions of the 2-phenoxyethoxy phenyl ring with halogens (Cl, Br) were also considered. This study also synthesised a homologous series of indane derivatives (**4**) for comparison with the 1-indanones. From the analysis above, it is evident that 1-indanone and indane derivatives should act as reversible MAO inhibitors, particularly when the MAO-A isoform is inhibited. Active MAO inhibitors

may represent leads for the design of new therapies for a variety of disorders where MAO is implicated in the pathophysiology. The proposal that 1-indanone and indane derivatives may act as MAO inhibitors is supported by the observation that rasagiline, a MAO-B inhibitor used in the clinic, is an indane derivative. In addition, a number of rasagiline analogues, also indane derivatives, have been found to act as MAO inhibitors.^[20]

4.2 Results and discussion

4.2.1 Chemistry

The 1-indanone (**2**, **3**) and indane (**4**) derivatives were synthesised in poor to good yields (6–85%) according to a literature procedure (Scheme 1).^[21] Commercially available 5-hydroxy-1-indanone (**5**), 6-hydroxy-1-indanone (**6**) or 5-indanol (**7**) was dissolved in N,N-dimethylformamide (DMF) and treated with an appropriately substituted alkyl bromide in the presence of K₂CO₃. After stirring the reaction for 5–24 h at room temperature, ethyl acetate was added to the reaction. The ethyl acetate phase was washed with water and brine and dried over MgSO₄. The crude obtained after evaporation of the solvent was purified by recrystallization and the structures of the target compounds were verified by ¹H NMR, ¹³C NMR and mass spectrometry.



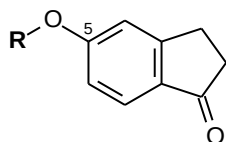
Scheme 1: Synthetic route to the 1-indanone (**2**, **3**) and indane (**4**) derivatives. Reagents and conditions: (a) DMF, K₂CO₃, rt.

4.2.2 IC₅₀ values for MAO inhibition

The 1-indanone and indane derivatives were evaluated as inhibitors of recombinant human MAO-A and MAO-B. The inhibition potencies of the derivatives were expressed as the IC₅₀ values, which were calculated from sigmoidal graphs of enzyme catalytic rate versus inhibitor concentration. The MAO-A and MAO-B catalytic activities were measured by employing the MAO-A/B mixed substrate, kynuramine, and measuring the MAO-catalysed formation of 4-hydroxyquinoline via

fluorescence spectrophotometry.^[22] By measuring the fluorescence of 4-hydroxyquinoline in the absence and presence of the test inhibitors, it was established that the 1-indanone and indane derivatives do not fluoresce under the specific assay conditions and concentrations used here, and thus do not interfere with the fluorescence measurements.

Table 1: The IC₅₀ values for the inhibition of recombinant human MAO-A and MAO-B by the 1-indanone derivatives **2**.

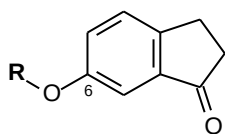


	R	IC ₅₀ (μM) ^[a]		SI ^[b]
		MAO-A	MAO-B	
2a	C ₆ H ₅ CH ₂ –	10.75 ± 0.799	0.135 ± 0.007	79.6
2b	3-ClC ₆ H ₄ CH ₂ –	2.56 ± 0.159	0.015 ± 0.002	170.6
2c	3-BrC ₆ H ₄ CH ₂ –	3.068 ± 0.193	0.019 ± 0.005	161.5
2d	4-ClC ₆ H ₄ CH ₂ –	0.504 ± 0.162	0.058 ± 0.002	8.7
2e	4-BrC ₆ H ₄ CH ₂ –	0.659 ± 0.066	0.052 ± 0.013	12.7
2f	C ₆ H ₁₁ (CH ₂) ₂ –	1.607 ± 0.081	0.053 ± 0.023	30.3
2g	C ₆ H ₅ (CH ₂) ₃ –	2.296 ± 0.346	0.04 ± 0.007	57.4
2h	(CH ₃) ₂ CH(CH ₂) ₃ –	0.537 ± 0.061	0.081 ± 0.024	6.6
2i	C ₆ H ₅ O(CH ₂) ₂ –	1.583 ± 0.136	0.113 ± 0.01	14.0
2j	4-ClC ₆ H ₄ O(CH ₂) ₂ –	0.812 ± 0.129	0.298 ± 0.083	2.7
2k	4-BrC ₆ H ₄ O(CH ₂) ₂ –	0.86 ± 0.309	0.16 ± 0.038	5.4
2l	C ₆ H ₁₁ CH ₂ –	0.971 ± 0.191	0.05 ± 0.025	19.4

^[a] All values are expressed as the mean ± SD of triplicate determinations.

^[b] The selectivity index is the selectivity for the MAO-B isoform and is given as the ratio of IC₅₀ (MAO-A)/IC₅₀ (MAO-B).

Table 2: The IC₅₀ values for the inhibition of recombinant human MAO-A and MAO-B by the 1-indanone derivatives **3**.

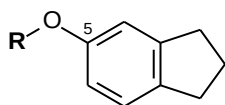


		IC ₅₀ (μM) ^[a]		SI ^[b]
	R	MAO-A	MAO-B	
3a	C ₆ H ₅ CH ₂ –	0.555 ± 0.096	0.015 ± .003	37.0
3b	3-ClC ₆ H ₄ CH ₂ –	0.084 ± 0.009	0.002 ± 0.00003	42.0
3c	3-BrC ₆ H ₄ CH ₂ –	0.116 ± 0.013	0.002 ± 0.0002	58.0
3d	4-ClC ₆ H ₄ CH ₂ –	0.032 ± 0.004	0.002 ± 0.0001	16.0
3e	4-BrC ₆ H ₄ CH ₂ –	0.039 ± 0.001	0.003 ± 0.0005	13.0
3f	C ₆ H ₁₁ (CH ₂) ₂ –	0.757 ± 0.11	0.03 ± 0.006	25.2
3g	C ₆ H ₅ (CH ₂) ₃ –	0.203 ± 0.022	0.019 ± 0.006	10.7
3h	(CH ₃) ₂ CH(CH ₂) ₃ –	0.364 ± 0.027	0.02 ± 0.005	18.2
3i	C ₆ H ₅ O(CH ₂) ₂ –	1.348 ± 0.043	0.001 ± 0.0005	1348
3j	4-ClC ₆ H ₄ O(CH ₂) ₂ –	0.161 ± 0.011	0.007 ± 0.001	23.0
3k	4-BrC ₆ H ₄ O(CH ₂) ₂ –	0.323 ± 0.03	0.016 ± 0.001	20.2

^[a] All values are expressed as the mean ± SD of triplicate determinations.

^[b] The selectivity index is the selectivity for the MAO-B isoform and is given as the ratio of IC₅₀ (MAO-A)/IC₅₀ (MAO-B).

Table 3: The IC₅₀ values for the inhibition of recombinant human MAO-A and MAO-B by the indane derivatives **4**.



		IC ₅₀ (μM) ^[a]		SI ^[b]
	R	MAO-A	MAO-B	
4a	C ₆ H ₅ CH ₂ –	35.315 ± 0.530	0.353 ± 0.056	100.0
4b	3-ClC ₆ H ₄ CH ₂ –	3.14 ± 0.247	0.041 ± 0.009	76.6
4c	3-BrC ₆ H ₄ CH ₂ –	3.966 ± 0.406	0.081± 0.016	49.0
4d	4-ClC ₆ H ₄ CH ₂ –	4.086 ± 0.561	0.512 ± 0.13	8.0
4e	4-BrC ₆ H ₄ CH ₂ –	3.329 ± 0.411	0.148 ± 0.016	22.5
4f	C ₆ H ₁₁ (CH ₂) ₂ –	3.916 ± 2.268	0.059 ± 0.012	66.4
4g	C ₆ H ₅ (CH ₂) ₃ –	9.158 ± 0.313	1.179 ± 0.055	7.7
4h	(CH ₃) ₂ CH(CH ₂) ₃ –	7.588 ± 1.564	0.413 ± 0.076	18.4
4i	C ₆ H ₅ O(CH ₂) ₂ –	9.312 ± 0.384	0.309 ± 0.1	30.1
4j	4-ClC ₆ H ₄ O(CH ₂) ₂ –	>100	>100	–
4k	4-BrC ₆ H ₄ O(CH ₂) ₂ –	25.677 ± 0.43	5.918 ± 2.041	4.3

^[a] All values are expressed as the mean ± SD of triplicate determinations.

^[b] The selectivity index is the selectivity for the MAO-B isoform and is given as the ratio of IC₅₀(MAO-A)/IC₅₀(MAO-B).

The MAO inhibition potencies of the 1-indanone (**2**, **3**) and indane (**4**) derivatives are given as the corresponding IC₅₀ values in tables 1–3. From the inhibition data it is evident that the 1-indanone (**2**, **3**) and indane (**4**) derivatives are selective inhibitors of MAO-B with SI values > 2.7. Compound **3i** is a particularly selective inhibitor, and was found to be 1348-fold more selective for MAO-B over the MAO-A isoform. The 1-indanone derivatives substituted on the C6-position (**3**) proved to be the most potent MAO-B inhibitors of the series evaluated with all homologues possessing IC₅₀ values < 0.030 μM. All of the C6-substituted 1-indanones may thus be considered as highly potent MAO-B inhibitors. Compared to the reference MAO-B inhibitor, lazabemide (IC₅₀ = 0.091 μM), the C6-

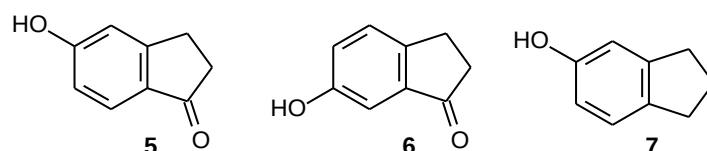
substituted 1-indanones are thus higher potency MAO-B inhibitors.^[23] Although, in general less potent, the C5-substituted 1-indanones (**2**) also are potent MAO-B inhibitors with eight (of twelve) homologues possessing IC_{50} values $< 0.10 \mu M$. Among the three series, the indane derivatives (**4**) are the least potent with three (of eleven) homologues possessing IC_{50} values $< 0.10 \mu M$. Some SARs may be derived from the inhibition data. For both series of 1-indanone derivatives **2** and **3**, substitution on the benzyloxy phenyl ring with halogens (Cl, Br) enhanced MAO-B inhibition potency compared to the unsubstituted homologues **2a** and **3a**. For the indane derivatives **4**, chlorine and bromine substitution on *meta* position, and bromine substitution on the *para* position of the benzyloxy phenyl ring lead to enhanced MAO-B inhibition potency. In contrast, halogen (Cl, Br) substitution on the 2-phenoxyethoxy phenyl ring did not enhance MAO-B inhibition for any of the three series when compared to the unsubstituted homologues **2i**, **3i** and **4i**. Also noteworthy is that, for all three series, the cyclohexyl-containing homologues may be considered as highly potent MAO-B inhibitors with IC_{50} values $< 0.059 \mu M$. This suggests that a terminal cyclohexyl moiety is beneficial for MAO-B inhibition and in certain instances (as for the C5-substituted 1-indanones) may even lead to enhanced MAO-B inhibition compared to the phenyl substituted homologues (compare **2i** and **2f** vs. **2a**). For the series of 1-indanone derivatives **2** and **3**, the 4-methylpentoxy substituent is also well tolerated with the homologues containing this substituent displaying IC_{50} values $< 0.081 \mu M$. Similarly, for the series of 1-indanone derivatives, but not the indanes, substitution with the phenylpropoxy moiety also yields compounds with potent MAO-B inhibition activity with IC_{50} values $< 0.04 \mu M$.

Similar to MAO-B, the 1-indanone (**2**, **3**) and indane (**4**) derivatives also were inhibitors of MAO-A, with the 1-indanone derivatives substituted on the C6-position (**3**) displaying more potent inhibitory activities compared to the C5-substituted 1-indanones and indanes. In fact, among the C6-substituted 1-indanones, with the exception of one homologue, all compounds possess IC_{50} values for the inhibition of MAO-A in the submicromolar range. The C5-substituted 1-indanones, in turn, are more potent MAO-A inhibitors than the indanes. The most potent MAO-A inhibitors among the compounds evaluated are **3b**, **3d** and **3e**, which display IC_{50} values $< 0.084 \mu M$. Since these compounds are also highly potent MAO-B inhibitors, they may be viewed as MAO-A/B non-selective inhibitors. These inhibitors are also significantly more potent than the reversible MAO-A inhibitor, toloxatone, which inhibits human MAO-A with an IC_{50} value of $3.92 \mu M$.^[23] Interesting SARs may also be derived from the MAO-A inhibition data. Among all three series, substitution on the benzyloxy phenyl ring with halogens (Cl, Br) enhances MAO-A inhibition potency. For the 1-indanones (**2**, **3**), halogen substitution on the *para* position is more beneficial than the *meta* position. Halogen (Cl, Br) substitution on the 2-phenoxyethoxy phenyl ring of the 1-indanones (**2**, **3**) also enhances MAO-A inhibition potency. For all three series, the 4-methylpentoxy substituent enhances MAO-A inhibition potency compared to the benzyloxy-substituted homologues, while for the C5-substituted 1-indanones and indanes, the cyclohexyl-containing homologues are more potent than the corresponding benzyloxy-substituted homologues. For all three series, substitution

with the phenylpropoxy moiety also enhances MAO-A inhibition potency compared to the benzyloxy-substituted homologues, which suggests that elongation of the C5/6 side chains of 1-indanone and indane derivatives may be a strategy to enhance MAO-A inhibition potency.

To show that the C5 and C6 substituents of the 1-indanone (**2**, **3**) and indane (**4**) derivatives are indeed structural requirements for MAO inhibition, 5-hydroxy-1-indanone (**5**), 6-hydroxy-1-indanone (**6**) or 5-indanol (**7**) were examined as potential inhibitors of human MAO. The results, shown in table 4, document these compounds are weak MAO inhibitors with IC₅₀ values ranging from 61.7 to > 100 μ M. Compared to the benzyloxy-substituted derivatives **2a**, **3a** and **4a**, these compounds are thus weak MAO-A and MAO-B inhibitors. This result demonstrates that appropriate C5 and C6 substitution is a requirement for the MAO inhibitory activities of the 1-indanone (**2**, **3**) and indane (**4**) derivatives investigated in this study.

Table 4: The IC₅₀ values for the inhibition of recombinant human MAO-A and MAO-B by compounds **5–7**.



	IC ₅₀ (μ M) ^[a]		SI ^[b]
	MAO-A	MAO-B	
5	61.7 $\pm$ 3.87	>100	–
6	64.7 $\pm$ 4.28	85.4 $\pm$ 5.82	0.8
7	>100	87.8 $\pm$ 4.71	–

^[a] All values are expressed as the mean $\pm$ SD of triplicate determinations.

^[b] The selectivity index is the selectivity for the MAO-B isoform and is given as the ratio of IC₅₀ (MAO-A)/IC₅₀ (MAO-B).

4.2.3 Reversibility of MAO inhibition

Based on the finding that 1-indanone derivatives are potent inhibitors of MAO, with the C6-substituted homologues, **3b**, **3d** and **3e**, displaying highly potent inhibition of both MAO isoforms, the reversibility of MAO-A and MAO-B inhibition was examined. For this purpose, compound **3d** was selected as representative inhibitor. To determine if **3d** is a reversible MAO inhibitor, this compound (at a concentration of $4 \times \text{IC}_{50}$) was incubated with MAO-A and MAO-B for a period of 15 min and subsequently dialyzed for 24 h. The residual MAO catalytic activities were measured and compared to the MAO activities of control samples, which consisted of dialysis experiments carried out in the absence of the test inhibitor.^[24] For reversible inhibition it is expected that, following dialysis of enzyme–inhibitor mixtures, enzyme activity will recover to levels equivalent to those of the control values. As shown in Figure 2, after dialysis of MAO-A–**3d** mixtures, MAO-A activity is completely reversed with the catalytic activity at 112% of the control value (recorded in the absence of inhibitor). In contrast, the MAO-A activity of undialyzed mixtures of MAO-A and **3d** is 43% of the control value. This result suggests that compound **3d** interacts reversibly with MAO-A. After similar treatment and dialysis of mixtures of MAO-A and pargyline, an irreversible MAO-A inhibitor, the enzyme activity is not recovered, with the residual enzyme activity at a level of only 1.6% of the control value.

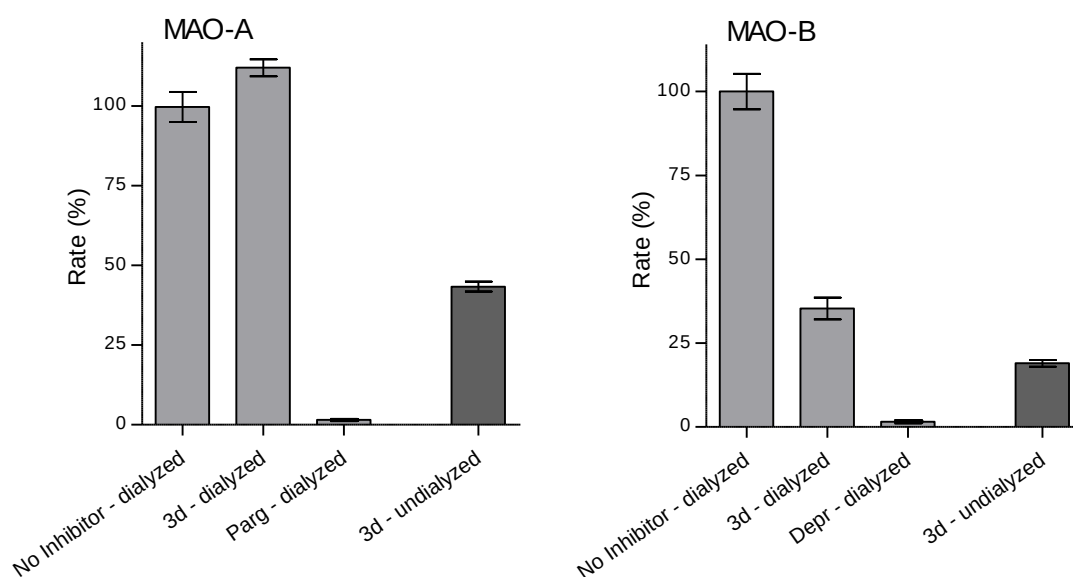


Figure 2: The reversibility of the inhibition of human MAO-A and MAO-B by **3d**. The MAO enzymes and **3d** (at $4 \times \text{IC}_{50}$) were preincubated for 15 min, dialyzed for 24 h and the residual enzyme activities were measured (**3d**–dialyzed). The enzymes were similarly preincubated in the absence (No inhibitor–dialyzed) and presence of the irreversible inhibitors, pargyline (Parg–dialyzed) or (R)-deprenyl (Depr–dialyzed), and dialyzed. For comparison, the residual MAO activities of undialyzed mixtures of the MAOs with **3d** are also shown (**3d**–undialyzed).

The results also document that, after dialysis of MAO-B–**3d** mixtures, MAO-B activity is recovered to a small degree after 24 h of dialysis, with the catalytic activity at 36% of the control value. For comparison, the enzyme activity of undialyzed mixtures of MAO-B and **3d** is 19% of the control value. This result suggests that the inhibition of MAO-B by **3d** cannot be readily reversed by dialysis. This behaviour has previously been observed for the inhibition of MAO-B by a variety of inhibitor classes, including α -tetralones.^[19] Based on these literature reports, a possible explanation may be that **3d** exhibits tight-binding to MAO-B. For example, oxadiazolones are reported to bind reversibly and time-dependently to rat brain MAO-B,^[25] while other reports describe this class of compounds to be reversible and extremely tight-binding MAO-B specific inhibitors.^[26] Further investigation is required to determine the exact mechanism of MAO-B inhibition by 1-indanone derivatives. As expected, after dialysis of mixtures of MAO-B and (R)-deprenyl, an irreversible MAO-B inhibitor, enzyme activity is not recovered, with only 1.6% activity (of the control value) remaining.

To investigate the modes of MAO-A and MAO-B inhibition by the representative inhibitor **3d**, sets of Lineweaver-Burk plots for the inhibition of each MAO isoform were constructed. Each set of Lineweaver-Burk plots was constructed by measuring MAO activity at 8 different kynuramine concentrations (15–250 μ M), in the absence of inhibitor, and presence of five different concentrations of the representative inhibitor. The Lineweaver-Burk plots for the inhibition of MAO-A and MAO-B by **3d** are shown in Figure 3. For the inhibition of both MAO isoforms, the lines of the Lineweaver-Burk plots are linear and intersect on the y-axis. This suggests that compound **3d** is a competitive inhibitor of both MAO-A and MAO-B, and may be viewed as further evidence that 1-indanones are reversible MAO inhibitors. From plots of the slopes of the Lineweaver-Burk plots versus the concentration of **3d**, K_i values of 0.047 μ M and 0.0017 μ M for the inhibition of MAO-A and MAO-B, respectively, are estimated. Global (shared) fitting of the inhibition data directly to the Michaelis-Menten equation yielded similar K_i values of 0.040 ± 0.00029 μ M ($r^2 = 0.99$) and 0.0011 ± 0.0001 μ M ($r^2 = 0.99$) for MAO-A and MAO-B, respectively. Fitting of the MAO-B inhibition data to the modification of the Michaelis-Menten equation previously reported, yielded a $K_{i(\text{red})}$ value of 0.0061 ± 0.001 μ M and a $K_{i(\text{ox})}$ value of 0.0033 ± 0.001 μ M ($r^2 = 0.99$).^[27] This approach takes into account that the inhibitor binds to both the oxidised and reduced enzyme and may better describe the observed MAO-B kinetic data.

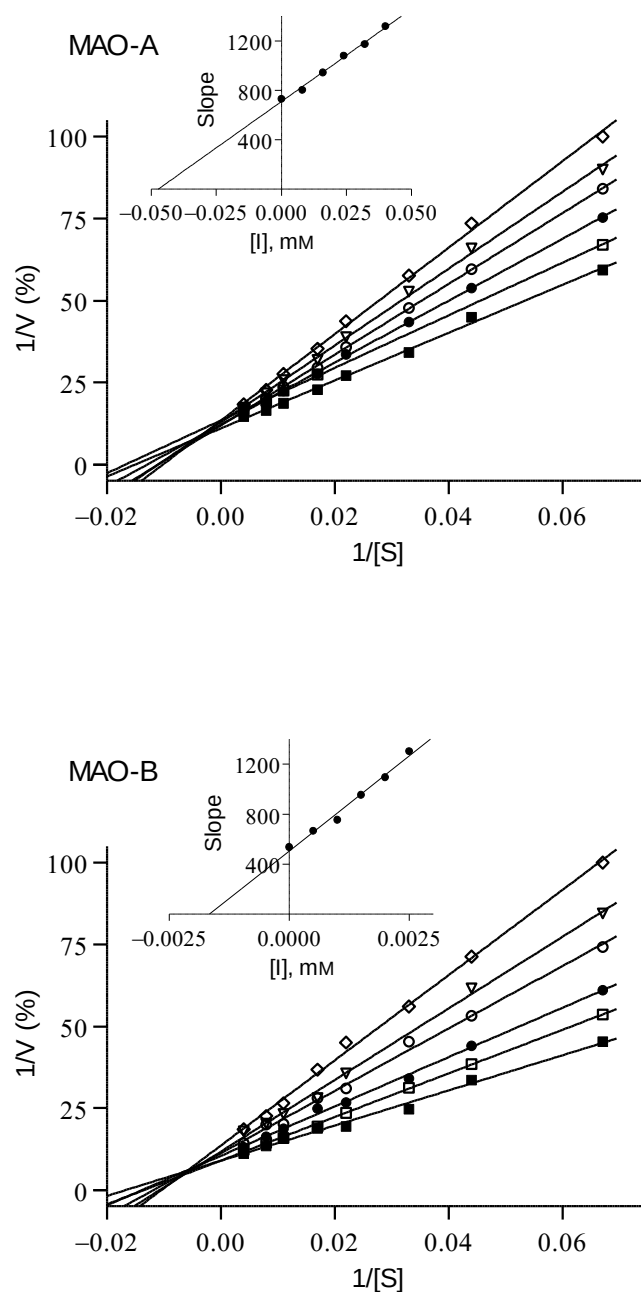


Figure 3: Lineweaver-Burk plots for human MAO-A and MAO-B in the absence (filled squares) and presence of various concentrations of **3d**. The concentrations of **3d** used were equal to $\frac{1}{4} \times IC_{50}$, $\frac{1}{2} \times IC_{50}$, $\frac{3}{4} \times IC_{50}$, $1 \times IC_{50}$ and $1\frac{1}{4} \times IC_{50}$. The insets are graphs of the slopes of the Lineweaver-Burk plots versus inhibitor concentration [K_i (MAO-A) = 0.047 μ M; K_i (MAO-B) = 0.0017 μ M].

4.2.4 Molecular modelling

Molecular docking experiments often provide insight into potential binding orientations and interactions of inhibitors with the active sites of enzymes. Such information may assist in the future design of inhibitors, as well as to explain the inhibition properties of existing inhibitors. A molecular docking study was thus performed in an attempt to determine potential modes of binding of the 1-indanone (**2**, **3**) and indane (**4**) derivatives to MAO-A and MAO-B. Representative compounds of each series, compounds **2a**, **3a** and **4a**, were selected to determine if major differences in the orientations and interactions with the MAOs between the three series exist. For the modelling studies, the protein models of human MAO-A co-crystallised with harmine (PDB: 2Z5X)^[28] and human MAO-B co-crystallised with safinamide (PDB: 2V5Z)^[29] were employed. The preparation of the models and the docking calculations were carried out with the Windows-based Discovery Studio 3.1 modelling software,^[30] according to the literature procedure.^[31] Docking of the inhibitors into the MAO models was performed with the CDOCKER protocol and the structural integrity of the highest ranked docking solutions was further examined with short (500 ps) molecular dynamics simulations of the resulting enzyme–inhibitor complexes. Frames of the production run were recorded at each 1 ps, to yield 500 frames. Since the CDOCKER protocol has the ability to correctly dock the co-crystallised inhibitors, harmine and safinamide, into the MAO active sites, this protocol appears to be suitable for predicting the binding orientations of inhibitors in the active sites of MAO-A and MAO-B.^[31] For harmine and safinamide, the 10 best ranked docked orientations exhibit root-mean-square deviation (RMSD) values of only 1.91 ± 0.93 Å and 1.48 ± 0.26 Å from the orientations of the co-crystallised ligands, respectively.

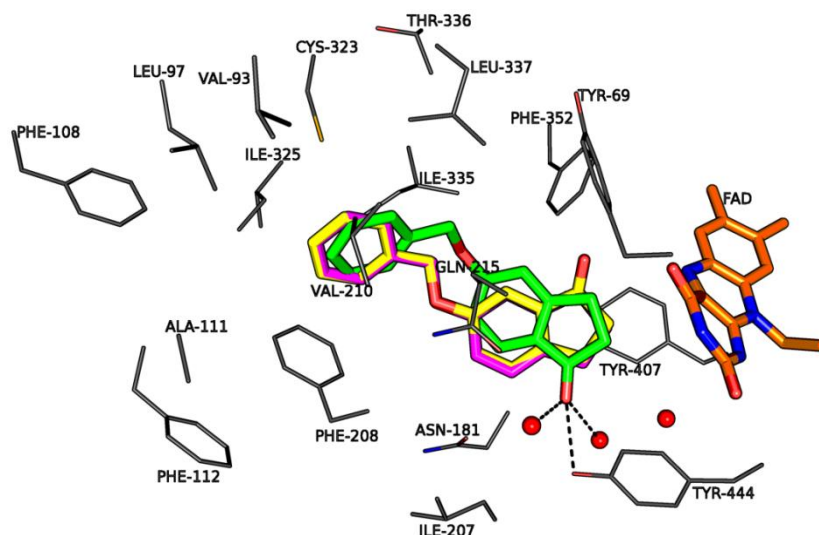


Figure 4: The best ranked docking solutions of **2a** (green), **3a** (yellow) and **4a** (magenta) for binding to the active site of MAO-A.

The best ranked solutions for the binding of compounds **2a**, **3a** and **4a** to the active site of MAO-A are shown in Figure 4. The results show that the 1-indanone and indane moieties of these inhibitors bind in the vicinity of the FAD cofactor of MAO-A, while the C5 or C6 substituents project towards the entrance of the active site cavity. While the 1-indanone moiety of **3a** and the indane moiety of **4a** exhibit similar orientations, the 1-indanone moiety of **2a** is rotated by approximately 180 ° with the carbonyl group projecting to the region in MAO-A lined by Tyr-444 and Tyr-407. The carbonyl group of compound **3a**, in turn, projects in the opposite direction towards the space lined by Tyr-69, Phe-352 and the FAD. These differing binding modes of the 1-indanone moieties of **2a** and **3a** are most likely guided by the optimal placement of the C5 and C6 substituents, which occupies almost the same space in the MAO-A active site. An analysis of the trajectories of **2a**, **3a** and **4a** shows that their orientations and placement in the MAO-A active site is relatively stable with average root mean square deviations (RMSDs) from the initial docked orientation of 0.83 ± 0.36 Å, 1.57 ± 0.51 Å and 1.75 ± 0.62 Å, respectively. For compound **2a**, hydrogen bonding occurs between the 1-indanone carbonyl oxygen and the phenolic hydrogen of Tyr-444. This interaction appears to play a relatively large role in the stabilization of this inhibitor in MAO-A since it is present in 429 frames (of a total of 500 frames). Compound **2a** also undergoes hydrogen bonding with two active site water molecules, interactions which are present in 296 and 223 frames (of a total of 500 frames) for the two waters HOH-739 and HOH-718, respectively. Compound **3a**, in turn, undergoes hydrogen bonding with one active site water molecule (HOH-710), an interaction which is only present in 84 frames (of a total of 500 frames). No hydrogen bonding is observed between **4a** and MAO-A. The hydrogen bond interactions contribute to 9.7% and 4.3% of the total interaction energy between **2a** and **3a**, respectively, and the MAO-A active site. The most prominent Van der Waals interactions of **2a**, **3a** and **4a** with MAO-A occur with Ile-180, Asn-181, Phe-208, Gln-215, Ile-335 and Tyr-407. These interactions contribute to 48.5%, 47.6% and 51.7% of the total interaction energy between **2a**, **3a** and **4a**, respectively, and the MAO-A active site. For **2a**, a significant Van der Waals interaction also occurs with Tyr-444, which contributes 6.0% of the total interaction energy. Based on this analysis it may be concluded that **2a**, **3a** and **4a** are stabilized to similar extents by Van der Waals interactions, and mostly interacts with the same residues in the MAO-A active site (Table 5). For compounds **3a** and **4a**, the inhibitory potencies are thus principally due to stabilization by Van der Waals interactions. In spite of this, **3a** and other C6-substituted indanones are, in general, more potent MAO-A inhibitors than **4a** and the other indane derivatives. A possible molecular explanation for the improved inhibitory potencies of the C6-substituted indanones (**3**) compared to the indane derivatives (**4**) may be that, although limited, the former undergoes hydrogen bonding with the MAO-A active site while the indane derivatives do not. An explanation for the improved inhibitory potency of **3a** compared to **2a**, however, is not apparent, particularly when considering that **2a** undergoes more extensive hydrogen bonding than **3a**.

Table 5: The interaction energies between key MAO-A residues and compounds **2a**, **3a** and **4a**.

	2a	3a	4a
Ile-180	-3.32 ± 0.63	-3.08 ± 0.52	-2.89 ± 0.41
Asn-181	-2.30 ± 0.67	-2.02 ± 0.50	-1.92 ± 0.46
Phe-208	-3.49 ± 1.10	-3.25 ± 1.15	-4.29 ± 0.86
Gln-215	-4.79 ± 0.63	-4.65 ± 0.77	-4.25 ± 0.60
Ile-335	-2.28 ± 0.51	-2.02 ± 0.42	-2.23 ± 0.66
Tyr-407	-1.89 ± 0.62	-1.96 ± 0.67	-2.13 ± 0.67

All values are expressed as the mean $\pm$ SD of 500 frames.

The best ranked solutions for the binding of compounds **2a**, **3a** and **4a** to the active site of MAO-B are shown in Figure 5. These inhibitors bind with their 1-indanone and indane moieties in the substrate cavity of MAO-B, in close proximity to the FAD cofactor, while the C5 or C6 substituents project towards the entrance cavity. The trajectory analysis for **2a**, **3a** and **4a** shows that their orientations and placements in MAO-B are stable with RMSDs of 0.76 ± 0.17 Å, 0.76 ± 0.17 Å and 0.71 ± 0.18 Å, respectively. The most significant Van der Waals interactions occur with Phe-168, Leu-171, Ile-198, Ile-199, Gln-206 and Tyr-326, which together contribute to 57.3%, 58.0% and 61.1% of the total interaction energy between **2a**, **3a** and **4a**, respectively, and the MAO-B active site. These interactions remain significant throughout the production run (20.3–82.5% of the total interaction energy). As in MAO-A, the 1-indanone moiety of **3a** and the indane moiety of **4a** exhibit similar orientations, while the 1-indanone moiety of **2a** is rotated by approximately 180 °. The carbonyl group of **2a** thus projects towards the space lined by Tyr-398 and Tyr-435. The carbonyl group of compound **3a**, in turn, projects in the opposite direction, towards a niche in the substrate cavity of MAO-B, lined by Tyr-60, Tyr-326 and Gln-206.^[29] The result of these differing binding modes is that the carbonyl oxygen of **2a** is able to undergo hydrogen bonding with waters HOH1155, HOH1351 and HOH1170. The interaction with HOH1155 appears to be important since it is observed in 362 of 500 frames, while hydrogen bonding with HOH1351 and HOH 1170 is only present in 53 and 40 frames. Hydrogen bonding also occurs between **2a** and Tyr-435 (133 of 500 frames). No hydrogen bonding is observed between the MAO-B active site and **3a** and **4a**. From these results it may be concluded that hydrogen bonding cannot explain the enhanced MAO-B inhibition potency of **3a** compared to **2a** and **4a**. Similarly, differing Van der Waals interactions also do not explain the differences between the inhibition potencies of **2a**, **3a** and **4a** since the binding orientations and interactions with key residues are highly comparable. As shown in table 6, the

interaction energies between key MAO-B residues and **2a**, **3a** and **4a** are similar. An explanation for the improved inhibitory potency of **3a** compared to **2a** and **4a** is thus not apparent. It is interesting to note that, in crystal structures of rasagiline in complex with MAO-B, the indane moiety of rasagiline adopts a similar binding orientation to that of indane derivative **4a**. For both structures, the indane moieties are located in the substrate cavity. For rasagiline, the C2 of the five-membered ring of the indane moiety is, however, directed towards the top of the substrate cavity, towards the niche lined by Tyr-60, Tyr-326 and Gln-206. In contrast, C2 of **4a** is directed towards the FAD. These differing binding modes are most likely guided by the placement of the benzyloxy side chain of **4a** in the entrance cavity and the formation of a covalent flavocyanyne adduct between rasagiline and MAO-B.^[20]

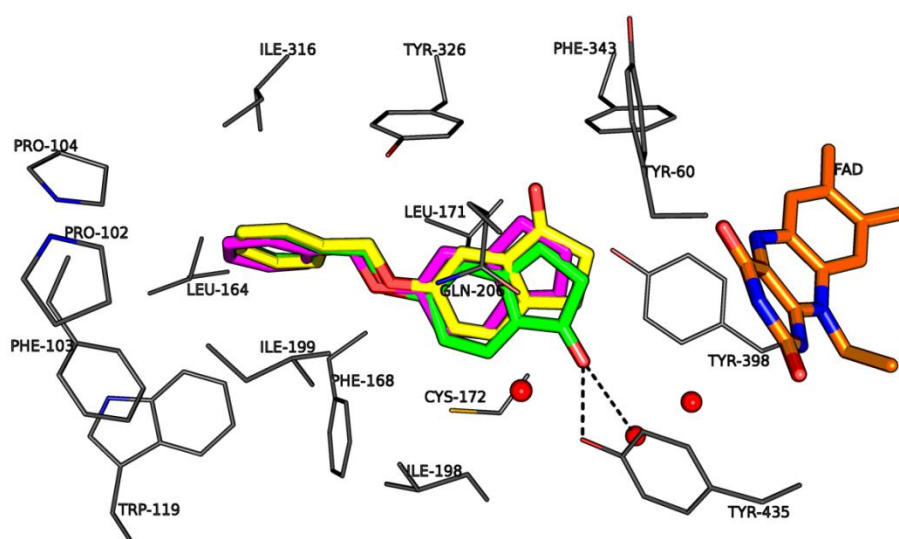


Figure 5: The best ranked docking solutions of **2a** (green), **3a** (yellow) and **4a** (magenta) for binding to the active site of MAO-B.

Table 6: The interaction energies between key MAO-B residues and compounds **2a**, **3a** and **4a**.

	2a	3a	4a
Phe-168	-2.49 ± 0.47	-2.31 ± 0.45	-2.48 ± 0.47
Leu-171	-4.16 ± 0.79	-4.61 ± 0.69	-4.29 ± 0.78
Ile-198	-2.43 ± 0.29	-2.36 ± 0.32	-2.25 ± 0.27
Ile-199	-5.28 ± 0.64	-5.29 ± 0.62	-5.26 ± 0.60
Gln-206	-3.36 ± 0.52	-4.06 ± 0.40	-3.11 ± 0.47
Tyr-326	-3.68 ± 0.39	-4.04 ± 0.45	-3.71 ± 0.36

All values are expressed as the mean $\pm$ SD of 500 frames.

4.3 Conclusion

In conclusion, the present study shows that C6-substituted 1-indanones (**3**) and to a lesser extent the C5-substituted homologues (**2**) are highly potent inhibitors of the human MAOs. All compounds from these two series proved to be selective MAO-B inhibitors with compound **3i** being the most selective (SI = 1348). In conjunction with L-dopa, selective MAO-B inhibitors are appropriate treatment for Parkinson's disease. While a clear explanation for the selectivity of the study compounds for MAO-B over the MAO-A isoform, is not apparent, it should be noted that MAO-B accommodates larger inhibitors better than MAO-A. The reason for this is that the entrance and substrate cavities of MAO-B may fuse after rotation of the side chain of Ile-199 from its normal position in the cavity. In contrast, the increased size of Phe-208, the residue in MAO-A that corresponds to Ile-199 in MAO-B, prevents it from occupying an alternative conformation, and this residue may hinder larger inhibitors from binding to MAO-A.^[32] The 1-indanones and indanes may thus possess selectivity for MAO-B, in part because their relatively larger structures are not well accommodated in the MAO-A active site. As exemplified by compounds **3b**, **3d** and **3e**, certain 1-indanones also are potent MAO-A/B non-selective inhibitors, drugs which may find application in the treatment of both Parkinson's disease and depression. In this regard, MAO-A/B non-selective inhibitors may be useful where Parkinson's disease patients also suffer from depression.^[33] As discussed, an important consideration for MAO-A inhibitors is the reversibility of inhibition. This study shows that a representative inhibitor, compound **3d**, is a reversible MAO-A inhibitor, a desirable property since reversible MAO-A inhibitors do not cause tyramine-induced hypertension.^[17,18] Interestingly, MAO-B inhibition by **3d** is not readily reversed by dialysis. While the molecular basis for this behaviour is not apparent, it is unlikely that this would be of concern from a drug safety point of view since clinically used irreversible MAO-B inhibitors are reported to possess excellent safety profiles.^[34] A similar result has been obtained with the previously reported α -tetralone derivatives,^[19] which served as leads for the current study. α -Tetralones also act as reversible MAO-A inhibitors, while MAO-B inhibition by α -tetralones is partially reversible. The C6-substituted 1-indanones (**3**) may be considered to be more potent MAO-B inhibitors compared to the previously reported α -tetralones derivatives.^[19] For example, the α -tetralone derivatives possess IC₅₀ values for the inhibition of MAO-B of 0.0045–0.078 μ M, while compounds **3a–k** exhibit IC₅₀ values of 0.001–0.030 μ M. The α -tetralone derivatives also are promising MAO-A inhibitors with one compound in particular, 6-(3-cyanobenzyloxy)-3,4-dihydro-2*H*-naphthalen-1-one (**8**) possessing an IC₅₀ value of 0.024 μ M (Figure 6). This compound also is a MAO-A/B non-selective inhibitor since it also inhibits MAO-B with an IC₅₀ value of 0.078 μ M. In this study it was shown that several compounds, such as **3b**, **3d** and **3e**, are high potency MAO-A inhibitors and excellent candidates for MAO-A/B non-selective inhibition. Based on this study, it may be concluded that 1-indanones are promising MAO inhibitors for the treatment of depression and Parkinson's disease.

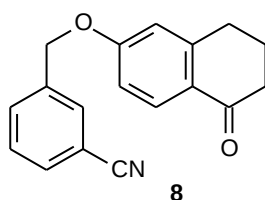


Figure 6: The structure of the α -tetralone derivative, 6-(3-cyanobenzoyloxy)-3,4-dihydro-2H-naphthalen-1-one (**8**).

4.4 Experimental Section

4.4.1 Chemicals and instrumentation

Unless otherwise noted, all starting materials and reagents were obtained from Sigma-Aldrich and were used without further purification. Proton (^1H) and carbon (^{13}C) NMR spectra were recorded with a Bruker Avance III 600 spectrometer at frequencies of 600 MHz and 150 MHz, respectively, with CDCl_3 as the NMR solvent. All chemical shifts are reported in parts per million (δ) downfield from the internal standard, tetramethylsilane. Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), qn (quintet), sept (septet) or m (multiplet). High resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF-Q II mass spectrometer in atmospheric-pressure chemical ionization (APCI) mode. Melting points (mp) were determined with a Buchi M-545 melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was carried out using silica gel 60 (Merck) with UV_{254} fluorescent indicator. Fluorescence spectrophotometry was carried out with a Varian Cary Eclipse fluorescence spectrophotometer. Microsomes from insect cells containing recombinant human MAO-A and MAO-B (5 mg/mL) and kynuramine.2HBr were from Sigma-Aldrich. Slide-A-Lyzer[®] dialysis cassettes with a molecular weight cut-off of 10 000 and a sample volume capacity of 0.5–3 mL were from Thermo Scientific. To determine the purities of the synthesised compound, HPLC analyses were carried out. HPLC analyses were performed with 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 $\times$ 150 mm, 5 μm) was used 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 254 nm.

4.4.2 The synthesis of 1-indanone (2, 3) and indane (4) derivatives

Commercially available 5-hydroxy-1-indanone (**5**), 6-hydroxy-1-indanone (**6**) or 5-indanol (**7**) (3.375 mmol) was dissolved in DMF (5 mL) and the reaction was placed under an atmosphere of argon. To the reaction solution was added K_2CO_3 (7.424 mmol) followed by the appropriately substituted alkyl bromide (3.712 mmol). After stirring the reaction for 5–24 h at room temperature, ethyl acetate (30 mL) was added to the reaction and the resulting mixture was washed with water (30 mL) and brine (2 × 30 mL). The ethyl acetate phase was dried over $MgSO_4$ and removed under reduced pressure. The crude obtained after evaporation of the solvent was purified by recrystallization from a mixture of cyclohexane (40 mL) and ethyl acetate (3 mL).^[21]

5-Benzoyloxy-2,3-dihydro-1H-inden-1-one (2a): Beige crystals; yield: 560 mg, 70%; mp: 107 °C; ^1H NMR (600 MHz, $CDCl_3$): δ =2.65 (t, J =5.8 Hz, 2H), 3.05 (t, J =5.8 Hz, 2H), 5.12 (s, 2H), 6.95 (m, 2H), 7.32–7.34 (m, 1H), 7.37–7.42 (m, 4H), 7.67 ppm (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ =25.8, 36.4, 70.2, 110.7, 115.8, 125.3, 127.4, 128.2, 128.7, 130.6, 136.0, 158.1, 164.3, 205.2 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $C_{16}H_{15}O_2$: 239.1072, found 239.1068; purity [HPLC]: 99%.

5-(3-Chlorobenzoyloxy)-2,3-dihydro-1H-inden-1-one (2b): Beige crystals solid; yield: 487 mg, 53%; mp: 116 °C; ^1H NMR (600 MHz, $CDCl_3$): δ =2.65 (t, J =6.0 Hz, 2H), 3.05 (t, J =6.0 Hz, 2H), 5.08 (s, 2H), 6.93 (m, 2H), 7.27–7.31 (m, 3H), 7.41 (s, 1H), 7.66 ppm (d, J =8.3 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ =25.8, 36.4, 69.3, 110.8, 115.7, 125.3, 125.4, 127.3, 128.3, 130.0, 130.8, 134.6, 138.1, 158.1, 164.0, 205.2 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $C_{16}H_{14}O_2Cl$: 273.0682, found 273.0682; purity [HPLC]: 96%.

5-(3-Bromobenzoyloxy)-2,3-dihydro-1H-inden-1-one (2c): Beige crystals solid; yield: 900 mg, 85%; mp: 121.5 °C; ^1H NMR (600 MHz, $CDCl_3$): δ =2.65 (t, J =5.8 Hz, 2H), 3.06 (t, J =5.8 Hz, 2H), 5.08 (s, 2H), 6.93 (m, 2H), 7.23–7.26 (m, 1H), 7.32 (d, J =7.9 Hz, 1H), 7.45 (d, J =7.9 Hz, 1H), 7.57 (s, 1H), 7.67 ppm (d, J =7.9 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ =25.8, 36.4, 69.3, 110.8, 115.7, 122.8, 125.4, 125.8, 130.2, 130.8, 131.3, 138.3, 158.1, 163.9, 205.2 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $C_{16}H_{14}O_2Br$: 317.0177, found 317.0155; purity [HPLC]: 97%.

5-(4-Chlorobenzoyloxy)-2,3-dihydro-1H-inden-1-one (2d): Beige solid; yield: 579 mg, 63%; mp: 127 °C; ^1H NMR (600 MHz, $CDCl_3$): δ =2.64 (t, J =6.0 Hz, 2H), 3.05 (t, J =6.0 Hz, 2H), 5.08 (s, 2H), 6.93 (m, 2H), 7.34 (s, 4H), 7.67 ppm (d, J =8.3 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ =25.8, 36.4, 69.4, 110.7, 115.8, 125.4, 128.7, 128.9, 130.7, 134.1, 134.2, 158.0, 164.0, 205.2 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $C_{16}H_{14}O_2Cl$: 273.0682, found 273.0664; purity [HPLC]: 99%.

5-(4-Bromobenzyloxy)-2,3-dihydro-1H-inden-1-one (2e): Beige crystals; yield: 503 mg, 47%; mp: 128.5 °C; ¹H NMR (600 MHz, CDCl₃): δ=2.64 (t, *J*=6.0 Hz, 2H), 3.05 (t, *J*=6.0 Hz, 2H), 5.06 (s, 2H), 6.92 (m, 2H), 7.29 (d, *J*=8.3 Hz, 2H), 7.49 (d, *J*=8.3 Hz, 2H), 7.66 ppm (d, *J*=8.3 Hz, 1H); ¹³C NMR (150 MHz, CDCl₃): δ=25.8, 36.4, 66.4, 110.7, 115.8, 122.2, 125.4, 129.0, 130.8, 131.8, 135.1, 158.0, 164.0, 205.2 ppm; APCI-HRMS *m/z* [*M*+H]⁺ calcd for C₁₆H₁₄O₂Br: 317.0177, found 317.0145; purity [HPLC]: 99%.

5-(2-Cyclohexylethoxy)-2,3-dihydro-1H-inden-1-one (2f): Beige crystals; yield: 315 mg, 36%; mp: 65.3 °C; ¹H NMR (600 MHz, CDCl₃): δ=0.93-0.98 (m, 2H), 1.12-1.25 (m, 3H), 1.45-1.51 (m, 1H), 1.62-1.74 (m, 7H), 2.63 (t, *J*=6.0 Hz, 2H), 3.04 (t, *J*=6.0 Hz, 2H), 4.03 (t, *J*=6.8 Hz, 2H), 6.86 (m, 2H), 7.63 ppm (d, *J*=9.0 Hz, 1H); ¹³C NMR (150 MHz, CDCl₃): δ=25.8, 26.2, 26.4, 33.2, 34.5, 36.4, 36.4, 66.4, 110.2, 115.6, 125.3, 130.1, 158.1, 164.8, 205.2 ppm; APCI-HRMS *m/z* [*M*+H]⁺ calcd for C₁₇H₂₃O₂: 259.1698, found 259.1686; purity [HPLC]: 99%.

5-(3-Phenylpropoxy)-2,3-dihydro-1H-inden-1-one (2g): Beige solid; yield: 273 mg, 30%; mp: 74 °C; ¹H NMR (600 MHz, CDCl₃): δ=2.13 (qn, *J*=7.5 Hz, 2H), 2.65 (t, *J*=6.0 Hz, 2H), 2.81 (t, *J*=7.5 Hz, 2H), 3.05 (t, *J*=6.0 Hz, 2H), 4.01 (t, *J*=6.4 Hz, 2H), 6.85 (s, 1H), 6.87 (dd, *J*=8.7, 2.3 Hz, 1H), 7.18-7.20 (m, 3H), 7.28 (t, *J*=7.5 Hz, 2H), 7.66 ppm (d, *J*=8.7 Hz, 1H); ¹³C NMR (150 MHz, CDCl₃): δ=25.8, 30.5, 32.0, 36.4, 67.2, 110.2, 115.6, 125.3, 126.0, 128.4, 128.4, 130.3, 141.1, 158.1, 164.7, 205.2 ppm; APCI-HRMS *m/z* [*M*+H]⁺ calcd for C₁₈H₁₉O₂: 267.1385, found 267.1360; purity [HPLC]: 97%.

5-[(4-Methylpentyl)oxy]-2,3-dihydro-1H-inden-1-one (2h): Orange oil; yield: 398 mg, 51%; ¹H NMR (600 MHz, CDCl₃): δ=0.90 (d, *J*=6.4 Hz, 6H), 1.31-1.33 (m, 2H), 1.56-1.61 (m, 1H), 1.77-1.81 (m, 2H), 2.64 (t, *J*=6.0 Hz, 2H), 3.04 (t, *J*=6.0 Hz, 2H), 3.98 (t, *J*=6.7 Hz, 2H), 6.85 (d, *J*=6.0 Hz, 2H), 7.63 ppm (d, *J*=9.4 Hz, 1H); ¹³C NMR (150 MHz, CDCl₃): δ=22.5, 25.8, 26.9, 27.8, 35.0, 36.4, 68.7, 110.2, 115.6, 125.2, 130.1, 158.1, 164.8, 205.2 ppm; APCI-HRMS *m/z* [*M*+H]⁺ calcd for C₁₅H₂₁O₂: 233.1542, found 233.1523; purity [HPLC]: 96%.

5-(2-Phenoxyethoxy)-2,3-dihydro-1H-inden-1-one (2i): Beige crystals; yield: 130 mg, 14%; mp: 36.7 °C; ¹H NMR (600 MHz, CDCl₃): δ=2.65 (t, *J*=6.0 Hz, 2H), 3.06 (t, *J*=6.0 Hz, 2H), 4.33 (t, *J*=4.0 Hz, 2H), 4.38 (t, *J*=4.0 Hz, 2H), 6.90-6.98 (m, 5H), 7.29 (t, *J*=7.9 Hz, 2H), 7.67 ppm (d, *J*=9.0 Hz, 1H); ¹³C NMR (150 MHz, CDCl₃): δ=25.8, 36.4, 66.1, 66.8, 110.5, 114.6, 115.6, 121.3, 125.3, 129.5, 130.7, 158.0, 158.4, 164.2, 205.2 ppm; APCI-HRMS *m/z* [*M*+H]⁺ calcd for C₁₇H₁₇O₃: 269.1178, found 269.1153; purity [HPLC]: 100%.

5-[2-(4-Chlorophenoxy)ethoxy]-2,3-dihydro-1H-inden-1-one (2j): Beige crystals; yield: 61 mg, 6%; mp: 145 °C; ^1H NMR (600 MHz, CDCl_3): δ =2.66 (t, J =6.0 Hz, 2H), 3.06 (t, J =6.0 Hz, 2H), 4.30 (t, J =5.0 Hz, 2H), 4.36 (t, J =5.0 Hz, 2H), 6.86 (d, J =9.0 Hz, 2H), 6.92 (m, 2H), 7.22 (m, 2H), 7.66 ppm (d, J =9.0 Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ =25.8, 36.4, 66.5, 66.7, 110.5, 115.9, 125.4, 126.1, 129.4, 130.8, 157.0, 158.0, 164.1, 205.2 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $\text{C}_{17}\text{H}_{16}\text{O}_3\text{Cl}$: 303.0788, found 303.0753; purity [HPLC]: 99%.

5-[2-(4-Bromophenoxy)ethoxy]-2,3-dihydro-1H-inden-1-one (2k): Beige solid; yield: 493 mg, 42%; mp: 148 °C; ^1H NMR (600 MHz, CDCl_3): δ =2.66 (t, J =6.0 Hz, 2H), 3.07 (t, J =6.0 Hz, 2H), 4.3 (t, J =4.3 Hz, 2H), 4.35 (t, J =4.9 Hz, 2H), 6.81 (d, J =8.7 Hz, 2H), 6.92 (m, 2H), 7.36 (d, J =9.0 Hz, 2H), 7.67 ppm (d, J =9.0 Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ =25.8, 36.4, 66.5, 66.7, 110.5, 113.5, 115.6, 116.4, 125.4, 130.8, 132.3, 157.5, 158.1, 164.1, 205.3 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $\text{C}_{17}\text{H}_{16}\text{O}_3\text{Br}$: 347.0283, found 347.0245; purity [HPLC]: 99%.

5-(Cyclohexylmethoxy)-2,3-dihydro-1H-inden-1-one (2l): Beige crystals; yield: 441 mg, 53%; mp: 128.5 °C; ^1H NMR (600 MHz, CDCl_3): δ =1.04-1.06 (m, 2H), 1.15-1.21 (m, 1H), 1.24-1.31 (m, 2H), 1.67-1.85 (m, 6H), 2.64 (t, J =6.0 Hz, 2H), 3.04 (t, J =6.0 Hz, 2H), 3.78 (d, J =6.4 Hz, 2H), 6.85 (m, 2H), 7.63 ppm (d, J =9.0 Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ =25.7, 25.8, 26.4, 29.8, 36.4, 37.5, 73.8, 110.2, 115.6, 125.2, 130.1, 158.1, 165.0, 205.2 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $\text{C}_{16}\text{H}_{21}\text{O}_2$: 245.1542, found 245.1534; purity [HPLC]: 100%.

6-(Benzyloxy)-2,3-dihydro-1H-inden-1-one (3a): Light beige solid; yield: 175 mg, 22%; mp: 96 °C; ^1H NMR (600 MHz, CDCl_3): δ =2.70 (t, J =6.0 Hz, 2H), 3.05 (t, J =6.0 Hz, 2H), 5.07 (s, 2H), 7.26 (m, 2H), 7.32 (t, J =7.5 Hz, 1H), 7.38 (m, 3H), 7.41 ppm (d, J =7.5 Hz, 2H); ^{13}C NMR (150 MHz, CDCl_3): δ =25.1, 36.9, 70.2, 106.1, 124.5, 127.4, 127.5, 128.1, 128.6, 136.4, 138.2, 148.2, 158.4, 206.9 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $\text{C}_{16}\text{H}_{15}\text{O}_2$: 239.1072, found 239.1045; purity [HPLC]: 97%.

6-(3-Chlorobenzyloxy)-2,3-dihydro-1H-inden-1-one (3b): Beige solid; yield: 267 mg, 29%; mp: 84.7 °C; ^1H NMR (600 MHz, CDCl_3): δ =2.70 (t, J =5.7 Hz, 2H), 3.05 (t, J =6.0 Hz, 2H), 5.03 (s, 2H), 7.21 (m, 1H), 7.23-7.25 (m, 1H), 7.26-7.31 (m, 3H), 7.36 (d, J =8.3 Hz, 1H), 7.41 ppm (s, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ =25.1, 37.0, 69.3, 106.0, 124.5, 125.3, 127.4, 127.5, 128.2, 129.9, 134.5, 138.2, 138.4, 148.4, 158.1, 206.8 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $\text{C}_{16}\text{H}_{14}\text{O}_2\text{Cl}$: 273.0682, found 273.0653; purity [HPLC]: 95%.

6-(3-Bromobenzyloxy)-2,3-dihydro-1H-inden-1-one (3c): Beige solid; yield: 257 mg, 24%; mp: 85 °C; ^1H NMR (600 MHz, CDCl_3): δ =2.75 (t, J =6.0 Hz, 2H), 3.09 (t, J =6.0 Hz, 2H), 5.07 (s, 2H), 7.26 (m, 1H), 7.27 (m, 2H), 7.36 (d, J =7.9 Hz, 1H), 7.41 (d, J =7.9 Hz, 1H), 7.47 (d, J =7.9 Hz, 1H), 7.61 ppm (s, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ =25.1, 36.9, 69.3, 106.0, 122.7, 124.5, 125.8, 127.6, 130.3, 130.1, 131.1, 138.2, 138.7, 148.4, 158.1, 206.9 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $\text{C}_{16}\text{H}_{14}\text{O}_2\text{Br}$: 317.0177, found 317.0144; purity [HPLC]: 96%.

6-(4-Chlorobenzyloxy)-2,3-dihydro-1H-inden-1-one (3d): Beige solid; yield: 381 mg, 41%; mp: 114.5 °C; ¹H NMR (600 MHz, CDCl₃): δ=2.69 (t, *J*=5.6 Hz, 2H), 3.04 (t, *J*=5.8 Hz, 2H), 5.02 (s, 2H), 7.21-7.23 (m, 2H), 7.32-7.36 ppm (m, 5H); ¹³C NMR (150 MHz, CDCl₃): δ=25.1, 36.9, 69.4, 106.0, 124.5, 127.5, 128.8, 128.8, 133.9, 134.9, 138.2, 148.3, 158.1, 206.8 ppm; APCI-HRMS *m/z* [*M*+H]⁺ calcd for C₁₆H₁₄O₂Cl: 273.0682, found 273.0681; purity [HPLC]: 95%.

6-(4-Bromobenzyloxy)-2,3-dihydro-1H-inden-1-one (3e): Beige solid; yield: 618 mg, 58%; mp: 120.8 °C; ¹H NMR (600 MHz, CDCl₃): δ=2.69 (t, *J*=5.6 Hz, 2H), 3.04 (t, *J*=5.6 Hz, 2H), 5.01 (s, 2H), 7.20-7.23 (m, 2H), 7.28 (d, *J*=8.3 Hz, 2H), 7.35 (d, *J*=7.9 Hz, 1H), 7.47 ppm (d, *J*=8.3 Hz, 2H); ¹³C NMR (150 MHz, CDCl₃): δ=25.1, 36.9, 69.4, 106.0, 122.0, 124.5, 127.5, 129.1, 131.7, 135.4, 138.2, 148.3, 158.1, 206.8 ppm; APCI-HRMS *m/z* [*M*+H]⁺ calcd for C₁₆H₁₄O₂Br: 317.0177, found 317.0176; purity [HPLC]: 100%.

6-(2-Cyclohexylethoxy)-2,3-dihydro-1H-inden-1-one (3f): Light beige solid; yield: 273 mg, 31%; mp: 91 °C; ¹H NMR (600 MHz, CDCl₃): δ=0.91-0.97 (m, 2H), 1.10-1.17 (m, 1H), 1.20-1.25 (m, 2H), 1.46 (m, 1H), 1.61-1.73 (m, 7H), 2.67 (t, *J*=5.6 Hz, 2H), 3.03 (t, *J*=5.6 Hz, 2H), 3.98 (t, *J*=6.8 Hz, 2H), 7.14-7.15 (m, 2H), 7.31-7.33 ppm (m, 1H); ¹³C NMR (150 MHz, CDCl₃): δ=25.1, 26.2, 26.5, 33.2, 34.5, 36.4, 37.0, 66.4, 105.5, 124.4, 127.2, 138.1, 147.7, 158.8, 207.1 ppm; APCI-HRMS *m/z* [*M*+H]⁺ calcd for 259.1698, found 259.1702; purity [HPLC]: 100%.

6-(3-Phenylpropoxy)-2,3-dihydro-1H-inden-1-one (3g): Light beige solid; yield: 293 mg, 33%; mp: 70 °C; ¹H NMR (600 MHz, CDCl₃): δ=2.10 (qn, *J*=7.2 Hz, 2H), 2.70 (t, *J*=5.7 Hz, 2H), 2.79 (t, *J*=7.5 Hz, 2H), 3.04 (t, *J*=5.7 Hz, 2H), 3.96 (t, *J*=6.4 Hz, 2H), 7.15 (m, 1H), 7.17-7.20 (m, 4H), 7.24 (t, *J*=7.2 Hz, 2H), 7.34 ppm (d, *J*=8.3 Hz, 1H); ¹³C NMR (150 MHz, CDCl₃): δ=25.1, 30.6, 32.0, 37.0, 67.2, 105.6, 124.3, 126.0, 127.3, 128.4, 128.4, 138.1, 141.3, 147.9, 158.7, 207.0 ppm; APCI-HRMS *m/z* [*M*+H]⁺ calcd for C₁₈H₁₉O₂: 267.1385, found 267.1360; purity [HPLC]: 100%.

6-[(4-Methylpentyl)oxy]-2,3-dihydro-1H-inden-1-one (3h): Orange oil; yield: 525 mg, 67%; ¹H NMR (600 MHz, CDCl₃): δ=0.88 (d, *J*=6.7 Hz, 6H), 1.29 (m, 2H), 1.57 (sept, *J*=6.7 Hz, 1H), 1.75 (m, 2H), 2.68 (t, *J*=5.6 Hz, 2H), 3.03 (t, *J*=5.8 Hz, 2H), 3.93 (t, *J*=6.8 Hz, 2H), 7.14-7.16 (m, 2H), 7.31 ppm (d, *J*=9.0 Hz, 1H); ¹³C NMR (150 MHz, CDCl₃): δ=22.5, 25.0, 27.0, 27.8, 35.0, 37.0, 68.7, 105.5, 124.4, 127.3, 138.1, 147.7, 158.8, 207.1 ppm; APCI-HRMS *m/z* [*M*+H]⁺ calcd for C₁₅H₂₁O₂: 233.1542, found 233.1557; purity [HPLC]: 98%.

6-(2-Phenoxyethoxy)-2,3-dihydro-1H-inden-1-one (3i): Light beige solid; yield: 288 mg, 32%; mp: 160.4 °C; ¹H NMR (600 MHz, CDCl₃): δ=2.70 (t, *J*=5.6 Hz, 2H), 3.05 (t, *J*=5.6 Hz, 2H), 4.33 (s, 4H), 6.93-7.00 (m, 3H), 7.21-7.24 (m, 2H), 7.28 (t, *J*=8.0 Hz, 2H), 7.35 ppm (d, *J*=7.5 Hz, 1H); ¹³C NMR (150 MHz, CDCl₃): δ=25.1, 37.0, 66.3, 66.9, 105.7, 114.6, 121.1, 124.6, 127.5, 129.5, 138.2, 148.3, 158.3, 158.5, 206.9 ppm; APCI-HRMS *m/z* [*M*+H]⁺ calcd for C₁₇H₁₇O₃: 269.1178, found 269.1153; purity [HPLC]: 95%.

6-[2-(4-Chlorophenoxy)ethoxy]-2,3-dihydro-1H-inden-1-one (3j): Beige solid; yield: 171 mg, 17%; mp: 127 °C; ^1H NMR (600 MHz, CDCl_3): δ =2.70 (t, J =5.6 Hz, 2H), 3.05 (t, J =5.6 Hz, 2H), 4.27-4.32 (m, 4H), 6.86 (m, 2H), 7.20-7.23 (m, 4H), 7.35 ppm (d, J =8.3 Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ =25.1, 37.0, 66.6, 66.7, 105.6, 116.0, 124.5, 126.0, 127.5, 129.3, 138.2, 148.4, 157.1, 158.2, 206.9 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $\text{C}_{17}\text{H}_{16}\text{O}_3\text{Cl}$: 303.0788, found 303.0772; purity [HPLC]: 99%.

6-[2-(4-Bromophenoxy)ethoxy]-2,3-dihydro-1H-inden-1-one (3k): Beige solid; yield: 165 mg, 14%; mp: 141.5 °C; ^1H NMR (600 MHz, CDCl_3): δ =2.71 (t, J =5.6 Hz, 2H), 3.05 (t, J =5.6 Hz, 2H), 4.27-4.32 (m, 4H), 6.80-6.81 (m, 2H), 7.20-7.22 (m, 2H), 7.35-7.37 ppm (m, 3H); ^{13}C NMR (150 MHz, CDCl_3): δ =25.1, 37.0, 66.6, 66.7, 105.6, 113.3, 116.5, 124.5, 127.5, 132.3, 138.2, 148.4, 157.6, 158.2, 206.9 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $\text{C}_{17}\text{H}_{16}\text{O}_3\text{Br}$: 347.0283, found 347.0254; purity [HPLC]: 98%.

5-Benzyloxy-2,3-dihydro-1H-indene (4a): Light orange oil; yield: 498 mg, 66%; ^1H NMR (600 MHz, CDCl_3): δ =2.08 (qn, J = 7.5 Hz, 2H), 2.86 (t, J =7.5 Hz, 2H), 2.89 (t, J =7.5 Hz, 2H), 5.05 (s, 2H), 6.79 (dd, J = 8.0, 2.3 Hz, 1H), 6.89 (s, 1H), 7.12 (d, J =8.3 Hz, 1H), 7.33 (t, J =7.2 Hz, 1H), 7.39 (t, J =7.9 Hz, 2H), 7.44 ppm (d, J =7.2 Hz, 2H); ^{13}C NMR (150 MHz, CDCl_3): δ =25.8, 32.0, 33.2, 70.2, 110.9, 112.8, 124.7, 127.4, 127.8, 128.5, 136.4, 137.4, 145.7, 157.7 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $\text{C}_{16}\text{H}_{17}\text{O}$: 225.1279, found 225.1253; purity [HPLC]: 95%.

5-(3-Chlorobenzyloxy)-2,3-dihydro-1H-indene (4b): Light orange oil; yield: 571 mg, 65%; ^1H NMR (600 MHz, CDCl_3): δ =2.07 (qn, 7.5 Hz, 2H), 2.84 (t, J =7.5 Hz, 2H), 2.88 (t, J =7.5 Hz, 2H), 5.0 (s, 2H), 6.75 (dd, J =7.9, 2.3 Hz, 1H), 6.85 (s, 1H), 7.11 (d, J =7.9 Hz, 1H), 7.29 (m, 3H), 7.44 ppm (s, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ =25.8, 32.0, 33.1, 69.4, 110.9, 112.8, 124.8, 125.3, 127.3, 127.9, 129.8, 134.4, 136.8, 139.5, 145.8, 157.4 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $\text{C}_{16}\text{H}_{16}\text{OCl}$: 259.0890, found 259.0851.

5-(3-Bromobenzyloxy)-2,3-dihydro-1H-indene (4c): Light beige oil; yield: 81 mg, 8%; ^1H NMR (600 MHz, CDCl_3): δ =2.07 (qn, J =7.5 Hz, 2H), 2.84 (t, J =7.5 Hz, 2H), 2.87 (t, J =7.5 Hz, 2H), 4.99 (s, 2H), 6.75 (dd, J =7.9, 2.3 Hz, 1H), 6.84 (s, 1H), 7.11 (d, J =8.3 Hz, 1H), 7.23 (m, 1H), 7.33 (d, J =8.3 Hz, 1H), 7.43 (d, J =7.9 Hz, 1H), 7.59 ppm (s, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ =25.8, 32.0, 33.1, 69.3, 110.9, 112.8, 122.6, 124.8, 125.8, 130.1, 130.2, 130.8, 136.8, 139.8, 145.8, 157.4 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $\text{C}_{16}\text{H}_{16}\text{OBr}$: 303.0385, found 303.0355; purity [HPLC]: 100%.

5-(4-Chlorobenzyloxy)-2,3-dihydro-1H-indene (4d): White crystals; yield: 137 mg, 16%; mp: 79 °C; ^1H NMR (600 MHz, CDCl_3): δ =2.08 (qn, J =7.5 Hz, 2H), 2.84 (t, J =7.5 Hz, 2H), 2.88 (t, J =7.5 Hz, 2H), 5.0 (s, 2H), 6.75 (dd, J =7.9, 1.9 Hz, 1H), 6.85 (s, 1H), 7.1 (d, J =7.9 Hz, 1H), 7.34-7.37 ppm (m, 4H); ^{13}C NMR (150 MHz, CDCl_3): δ =25.8, 32.0, 33.1, 69.4, 110.9, 112.8, 124.8, 128.7, 128.7, 133.5, 135.9, 136.7, 145.8, 157.4 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $\text{C}_{16}\text{H}_{16}\text{OCl}$: 259.0890, found 259.0869; purity [HPLC]: 100%.

5-(4-Bromobenzyloxy)-2,3-dihydro-1H-indene (4e): White crystals; yield: 420 mg, 41%; mp: 82 °C; ¹H NMR (600 MHz, CDCl₃): δ=2.06 (qn, *J*=7.5 Hz, 2H), 2.83 (t, *J*=7.5 Hz, 2H), 2.87 (t, *J*=7.5 Hz, 2H), 4.98 (s, 2H), 6.74 (dd, *J*=8.3, 2.3 Hz, 1H), 6.83 (s, 1H), 7.1 (d, *J*=8.3 Hz, 1H), 7.30 (d, *J*=8.3 Hz, 2H), 7.48 ppm (d, *J*=8.3 Hz, 2H); ¹³C NMR (150 MHz, CDCl₃): δ=25.8, 32.0, 33.1, 69.4, 110.9, 112.8, 121.6, 124.8, 129.0, 131.6, 136.4, 136.7, 145.8, 157.4 ppm; APCI-HRMS *m/z* [*M*+H]⁺ calcd for C₁₆H₁₆OBr: 303.0385, found 303.0367; purity [HPLC]: 100%.

5-(2-Cyclohexylethoxy)-2,3-dihydro-1H-indene (4f): Light yellow oil; yield: 598 mg, 73%; ¹H NMR (600 MHz, CDCl₃): δ=0.95-1.01 (m, 2H), 1.14-1.29 (m, 3H), 1.5 (m, 1H), 1.7-1.78 (m, 7H), 2.07 (qn, *J*=7.5 Hz, 2H), 2.84 (t, *J*=7.5 Hz, 2H), 2.88 (t, *J*=7.5 Hz, 2H), 3.97 (t, *J*=6.5 Hz, 2H), 6.70 (d, *J*=7.9 Hz, 1H), 6.80 (s, 1H), 7.10 ppm (d, *J*=8.3 Hz, 1H); ¹³C NMR (150 MHz, CDCl₃): δ=25.8, 26.3, 26.6, 32.0, 33.2, 33.3, 34.6, 36.7, 66.1, 110.5, 112.5, 124.6, 135.9, 145.6, 158.0 ppm; APCI-HRMS *m/z* [*M*+H]⁺ calcd for C₁₇H₂₅O: 245.1905, found 245.1916; purity [HPLC]: 95%.

5-(3-Phenylpropoxy)-2,3-dihydro-1H-indene (4g): Yellow oil; yield: 542 mg, 64%; ¹H NMR (600 MHz, CDCl₃): δ=2.05-2.13 (m, 4H), 2.82 (t, *J*=7.9 Hz, 2H), 2.85 (t, *J*=7.5 Hz, 2H), 2.88 (t, *J*=7.5 Hz, 2H), 3.95 (t, *J*=6.4 Hz, 2H), 6.71 (dd, *J*=7.9, 2.3 Hz, 1H), 6.8 (s, 1H), 7.11 (d, *J*=8.3 Hz, 1H), 7.19-7.23 (m, 3H), 7.3 ppm (t, *J*=7.5 Hz, 2H); ¹³C NMR (150 MHz, CDCl₃): δ=25.8, 30.9, 32.0, 32.2, 33.1, 67.0, 110.6, 112.6, 124.7, 125.8, 128.4, 128.5, 136.1, 141.6, 145.6, 157.9 ppm; APCI-HRMS *m/z* [*M*+H]⁺ calcd for C₁₈H₂₁O: 253.1592, found 253.1597; purity [HPLC]: 96%.

5-[(4-Methylpentyl)oxy]-2,3-dihydro-1H-indene (4h): Yellow oil; yield: 539 mg, 73%; ¹H NMR (600 MHz, CDCl₃): δ=0.92 (d, *J*=9.0 Hz, 6H), 1.34 (m, 2H), 1.61 (m, 1H), 1.78 (qn, *J*=8.2 Hz, 2H), 2.07 (qn, *J*=7.5 Hz, 2H), 2.84 (t, *J*=7.5 Hz, 2H), 2.88 (t, *J*=7.5 Hz, 2H), 3.92 (t, *J*=6.7 Hz, 2H), 6.69 (d, *J*=7.9 Hz, 1H), 6.79 (s, 1H), 7.10 ppm (t, *J*=7.9 Hz, 1H); ¹³C NMR (150 MHz, CDCl₃): δ=22.6, 25.8, 27.3, 27.8, 32.0, 33.2, 35.2, 68.5, 110.6, 112.5, 124.7, 135.9, 145.6, 158.0 ppm; APCI-HRMS *m/z* [*M*+H]⁺ calcd for C₁₅H₂₃O: 219.1749, found 219.1756; purity [HPLC]: 96%.

5-(2-Phenoxyethoxy)-2,3-dihydro-1H-indene (4i): White solid; yield: 507 mg, 59%; mp: 91.3 °C; ¹H NMR (600 MHz, CDCl₃): δ=2.09 (qn, *J*=7.5 Hz, 2H), 2.86 (t, *J*=7.5 Hz, 2H), 2.9 (t, *J*=7.5 Hz, 2H), 4.31 (s, 4H), 6.77 (dd, *J*=8.3, 2.3 Hz, 1H), 6.86 (s, 1H), 6.96-7.00 (m, 3H), 7.13 (d, *J*=8.3 Hz, 1H), 7.31 ppm (t, *J*=8.0 Hz, 2H); ¹³C NMR (150 MHz, CDCl₃): δ=25.8, 31.0, 33.1, 66.4, 66.7, 110.8, 112.7, 114.7, 121.0, 124.7, 129.4, 136.6, 145.7, 157.5, 158.6 ppm; APCI-HRMS *m/z* [*M*+H]⁺ calcd for C₁₇H₁₉O₂: 255.1385, found 255.1580; purity [HPLC]: 98%.

5-[2-(4-Chlorophenoxy)ethoxy]-2,3-dihydro-1H-indene (4j): White solid; yield: 162 mg, 17%; mp: 128.8 °C; ¹H NMR (600 MHz, CDCl₃): δ=2.12 (qn, *J*=7.5 Hz, 2H), 2.89 (t, *J*=7.5 Hz, 2H), 2.93 (t, *J*=7.5 Hz, 2H), 4.31 (m, 4H), 6.78 (dd, *J*=8.0, 1.9 Hz, 1H), 6.88 (s, 1H), 6.93 (d, *J*=9.0 Hz, 2H), 7.16 (d, *J*=7.9 Hz, 1H), 7.28 ppm (d, *J*=9.0 Hz, 2H); ¹³C NMR (150 MHz, CDCl₃): δ=25.8, 32.0, 33.1, 66.7, 66.9, 110.8, 112.7, 116.0, 124.8, 125.9, 129.3, 136.7, 145.8, 157.3, 157.4 ppm; APCI-HRMS *m/z* [*M*+H]⁺ calcd for C₁₇H₁₈O₂Cl: 289.0995, found 289.0987; purity [HPLC]: 100%.

5-[2-(4-Bromophenoxy)ethoxy]-2,3-dihydro-1H-indene (4k): White solid; yield: 765 mg, 68%; mp: 135.4 °C; ¹H NMR (600 MHz, CDCl₃): δ=2.07 (qn, *J*=7.5 Hz, 2H), 2.84 (t, *J*=7.5 Hz, 2H), 2.87 (t, *J*=7.5 Hz, 2H), 4.27 (m, 4H), 6.73 (dd, *J*=8.3, 2.3 Hz, 1H), 6.81-6.83 (m, 3H), 7.11 (d, *J*=8.3 Hz, 1H), 7.37 ppm (d, *J*=8.7 Hz, 2H); ¹³C NMR (150 MHz, CDCl₃): δ=25.8, 32.0, 33.1, 66.7, 66.9, 110.8, 112.7, 113.2, 116.5, 124.7, 132.2, 136.7, 145.8, 157.4, 157.8 ppm; APCI-HRMS *m/z* [*M*+H]⁺ calcd for C₁₇H₁₈O₂Br: 333.0490, found 333.0473; purity [HPLC]: 99%.

4.4.3 IC₅₀ values

IC₅₀ values for the inhibition of MAO-A and MAO-B were measured by using the non-selective MAO substrate, kynuramine, as enzyme substrate. The recombinant human MAOs served as enzyme sources.^[35] The reactions were carried out in white 96-well microtiter plates (Eppendorf) in potassium phosphate buffer (100 mM, made isotonic with KCl) at pH 7.4. The final volume of the reactions was 200 µL and contained the MAO-A/B mixed substrate kynuramine (50 µM) and different inhibitor concentrations spanning at least 3 orders of magnitude and each reaction also contained DMSO as co-solvent (4%). The reactions were initiated with the addition of MAO-A or MAO-B (0.0075 mg protein/mL) and incubated for 20 min at 37 °C in a convection oven. After termination with the addition of 80 µL NaOH (2 N), the concentrations of 4-hydroxyquinoline were measured by fluorescence spectrophotometry (λ_{ex} = 310; λ_{em} = 400 nm).^[22] A linear calibration curve was constructed using authentic 4-hydroxyquinoline (0.047–1.56 µM). The enzyme catalytic rates were calculated and fitted to the one site competition model incorporated into the Prism 5 software package (GraphPad). The IC₅₀ values were determined in triplicate and are expressed as mean ± standard deviation (SD).

4.4.4 Reversibility of the MAO inhibition

The reversibility of the MAO inhibition by **3d** was examined by dialysis.^[24] MAO-A or MAO-B (0.03 mg/mL) and the test inhibitor, at a concentration of 4 × IC₅₀ for the inhibition of the MAOs, were placed in the Slide-A-Lyzer[®] dialysis cassettes. These mixtures were prepared in potassium phosphate buffer (100 mM, pH 7.4, 5% sucrose) to a final volume of 0.8 mL, and contained 4% DMSO as co-solvent. These mixtures were preincubated for 15 min at 37 °C and subsequently dialyzed at 4 °C in 80 mL of dialysis buffer (100 mM potassium phosphate, pH 7.4, 5% sucrose). The dialysis buffer was replaced with fresh buffer at 3 h and 7 h after the start of dialysis. As controls, MAO-A and MAO-B were similarly preincubated in the presence of the irreversible inhibitors, pargyline and (R)-deprenyl, respectively, as well as in the absence of inhibitor. The concentrations of pargyline [IC₅₀(MAO-A) = 13 µM]^[36] and (R)-deprenyl [IC₅₀(MAO-B) = 0.079 µM]^[37] employed were equal to 4 × IC₅₀ for the inhibition of MAO-A and MAO-B, respectively. After 24 h of dialysis, samples of the dialysis mixtures (250 µL) were diluted twofold with the addition of kynuramine (250 µL, dissolved in potassium phosphate buffer, 100 mM, pH 7.4, made isotonic with KCl). This yielded a final inhibitor concentration of 2 × IC₅₀, and a final kynuramine concentration of

50 μ M. After incubation of these enzyme reactions for 20 min at 37 $^{\circ}$ C, the residual MAO activities were subsequently measured by fluorescence spectrophotometry as described above. For comparison, undialyzed mixtures of the MAOs and the test inhibitor were maintained at 4 $^{\circ}$ C over the same time period. All reactions were carried out in triplicate and the residual enzyme catalytic rates were expressed as mean $\pm$ SD.

4.4.5 Lineweaver-Burk plots and K_i value calculations

For the inhibition of MAO-A and MAO-B by **3d**, sets consisting of six Lineweaver-Burk plots were constructed. The first plot was constructed in the absence of inhibitor while the remaining five plots were constructed in the presence of different concentrations of the test inhibitor: $\frac{1}{4} \times IC_{50}$, $\frac{1}{2} \times IC_{50}$, $\frac{3}{4} \times IC_{50}$, $1 \times IC_{50}$ and $1\frac{1}{4} \times IC_{50}$. The enzyme substrate, kynuramine, was used at concentrations ranging from 15 to 250 μ M while the final enzyme concentration was 0.015 mg/mL. All incubations were carried out in 1.5 mL microcentrifuge tubes to a volume of 500 μ L. The MAO catalytic activities were measured by fluorescence spectrophotometry as described for the IC_{50} value determination.^[24] K_i values were estimated from plots of the slopes of the Lineweaver-Burk plots versus inhibitor concentration, where the x-axis intercept equals $-K_i$.

4.4.6 Molecular docking and dynamics simulations

All modelling calculations were carried out with the Windows-based Discovery Studio 3.1 software package.^[30] Unless otherwise specified, all the applications within Discovery Studio were set to their default values. The X-ray crystal structures of human MAO-A (PDB code 2Z5X)^[28] and human MAO-B (PDB code 2V5Z)^[29] were obtained from the Brookhaven Protein Data Bank. The pKa values and protonation states of the ionizable amino acids were calculated and hydrogen atoms were added at pH 7.4 to the protein models. When necessary, the valences of the FAD cofactors (oxidized state) and co-crystallised ligands were corrected, the protein models were automatically typed with the Momany and Rone CHARMM forcefield and a fixed atom constraint was applied to the backbone. The models were subsequently energy minimized using the Smart Minimizer algorithm with the maximum amount of steps set to 50000. The implicit generalized Born solvation model with molecular volume was used during the minimization procedure. The co-crystallised ligands, waters and the backbone constraints were removed from the models and the binding sites were identified from an analysis of the enzyme cavities. In each model, three active site waters are considered to be conserved and were retained. These waters are HOH 710, 718 and 739 in the MAO-A active site, and HOH 1155, 1170 and 1351 in the A-chain of the MAO-B active site. The structures of compounds **2a**, **3a** and **4a** were drawn in Discovery Studio, the geometries were briefly optimized using a Dreiding-like forcefield (5000 iterations) and the structures were submitted to the Prepare Ligands protocol. Atom potential types and partial charges were subsequently assigned to the structures with the Momany and Rone CHARMM forcefield. Docking of **2a**, **3a** and **4a** into the MAO models was carried out with the CDOCKER algorithm. For this purpose, ten

random ligand conformations were generated, the heating target temperature was set to 700 K and full potential mode was employed. The docking solutions were finally refined using in situ ligand minimization with the Smart Minimizer algorithm.

The highest ranked docking solutions were subjected to a short (500 ps) molecular dynamics simulation. Fixed atom constraints were applied to the backbone of the protein models and the residues that are further than 5 Å from the docked ligand. The models were automatically typed with the Momany and Rone CHARMM forcefield. The standard dynamics cascade was used for this study and consisted of a steepest descent minimization (maximum steps, 2500) followed by a conjugate gradient minimization (maximum steps, 2500; RMS gradient, 0.01). Heating was subsequently carried out in 2000 steps (time step, 0.001 ps) from 50 K to 300 K, and equilibration of the system was allowed for 10000 steps (time step, 0.001 ps). The production runs were carried out for 500 000 steps (time step, 0.001 ps) in the NVT ensemble, applying the SHAKE algorithm to all bonds involving hydrogen atoms. The equations for motion were integrated with the Leapfrog Verlet algorithm. The implicit generalized Born solvation model with simple switching was used during the dynamics calculations. The conformation of the complex was saved every 1 ps, yielding 500 frames. The illustrations were generated with PyMOL and represent the docked orientations prior to the molecular dynamics simulation.^[38]

4.5 Acknowledgements

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4.6 References

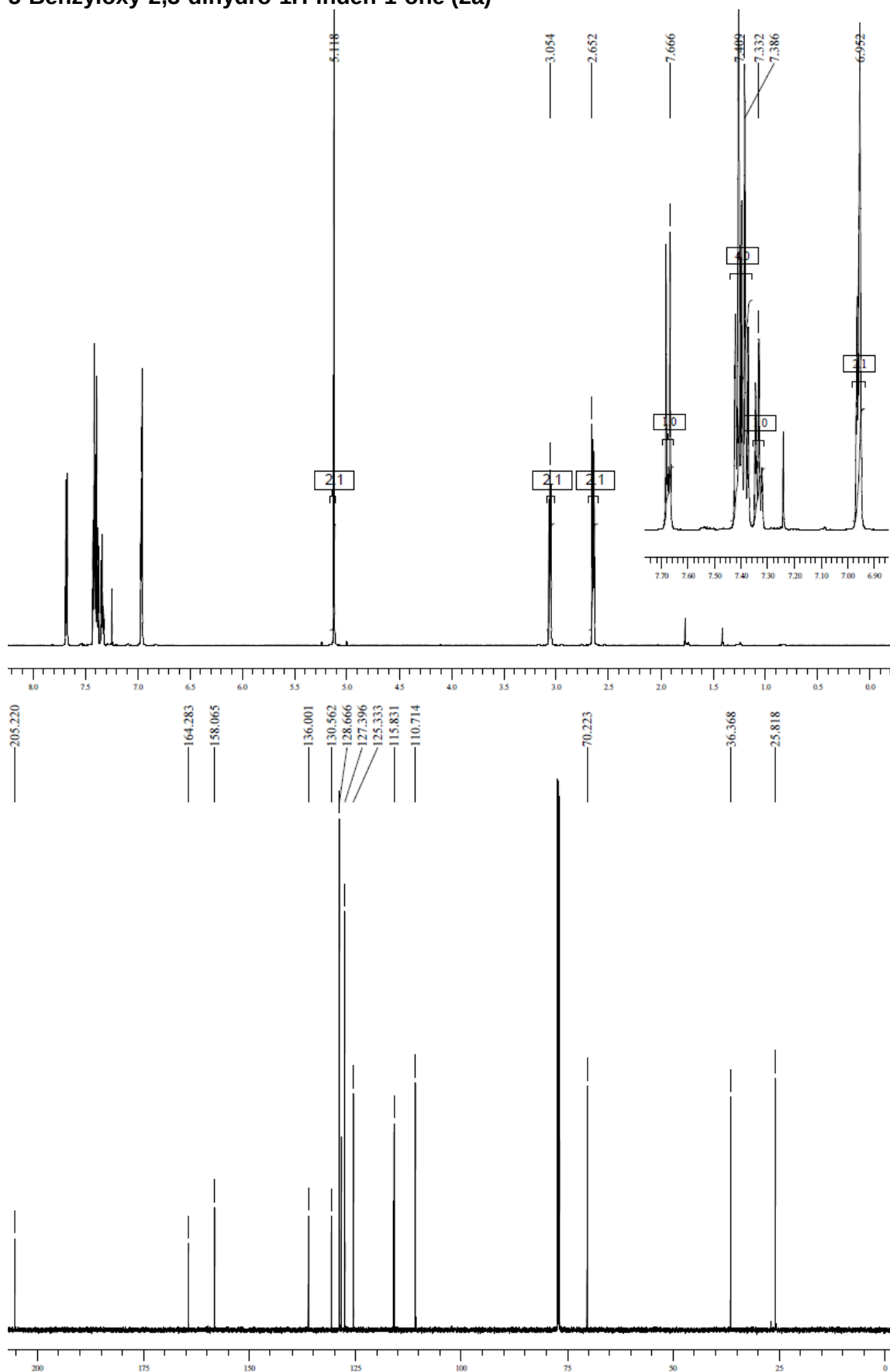
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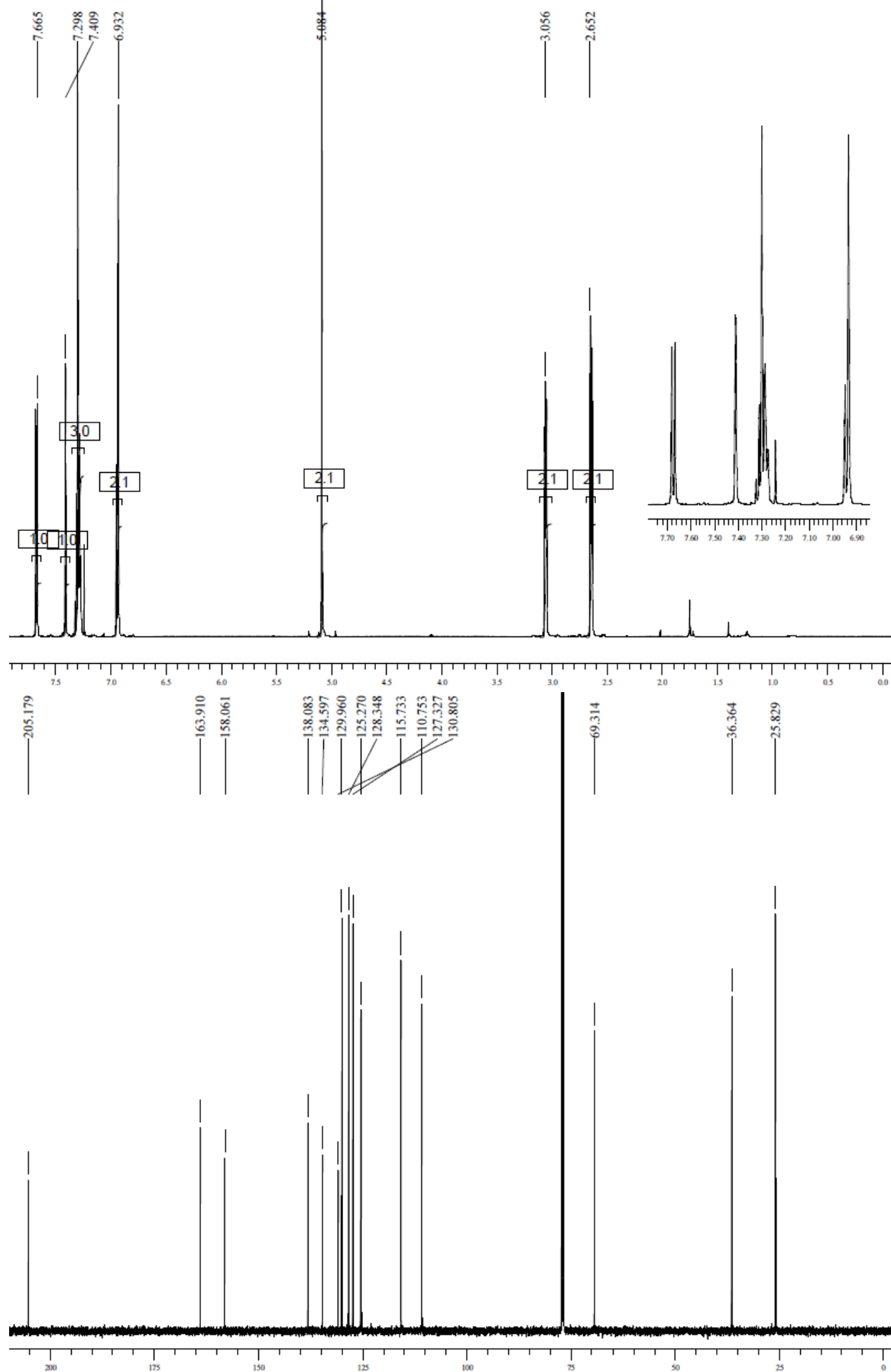
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Supplementary Material

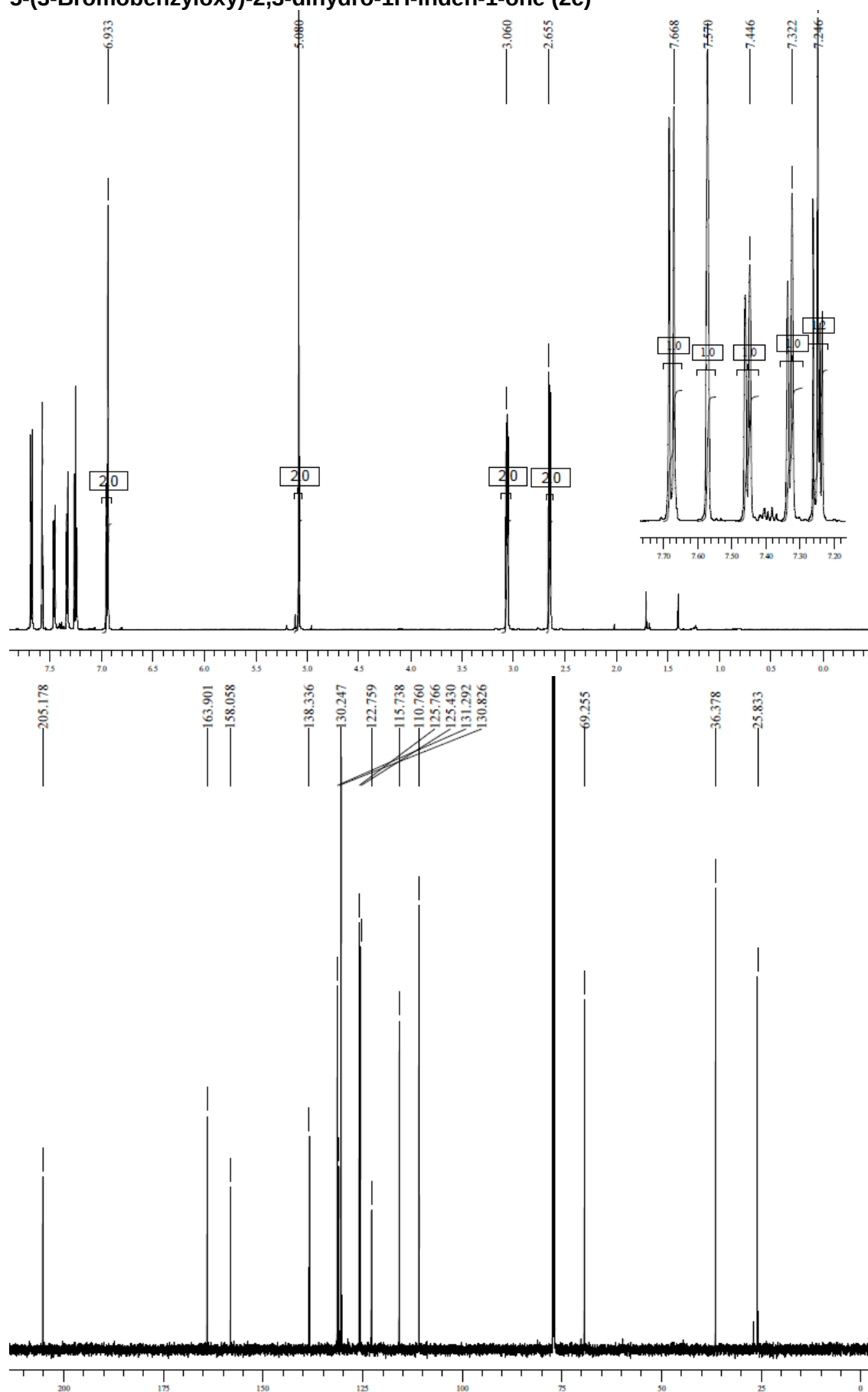
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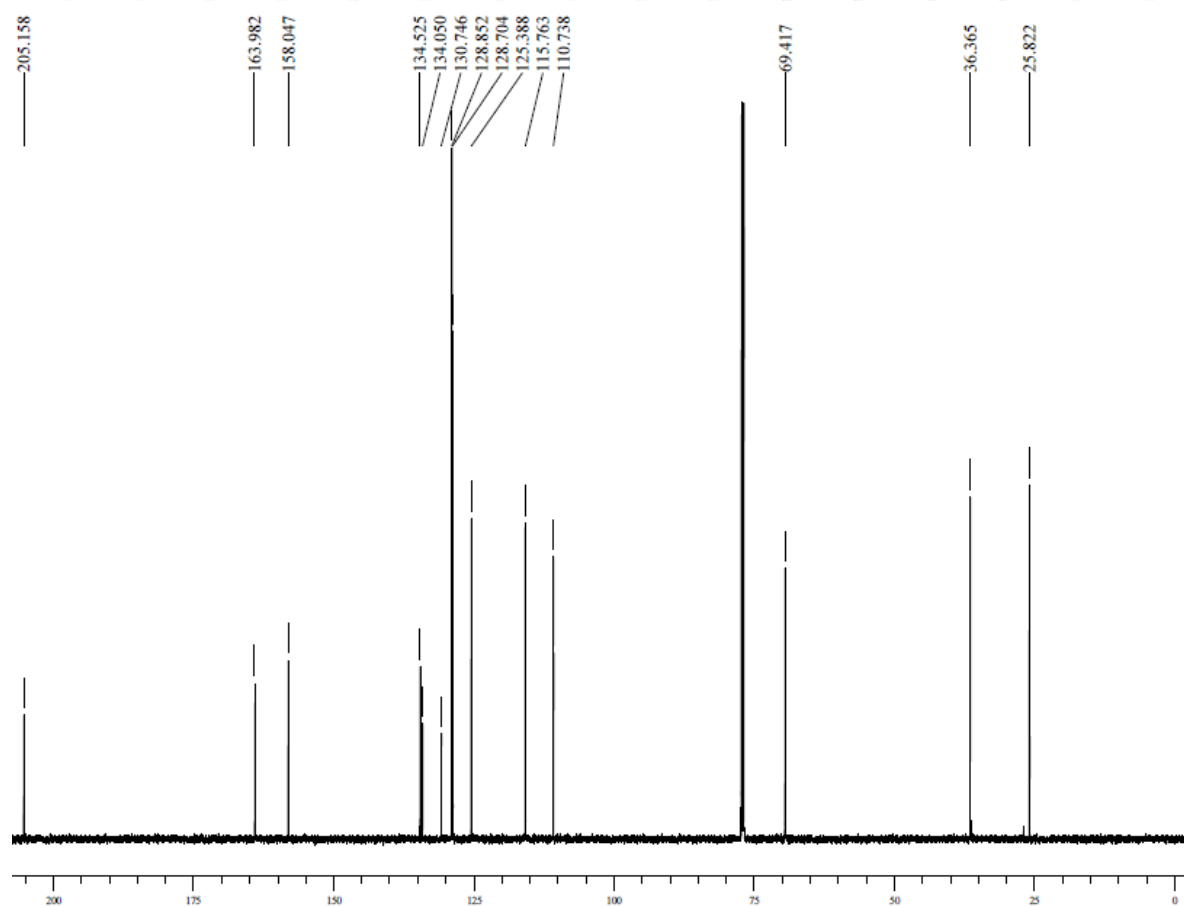
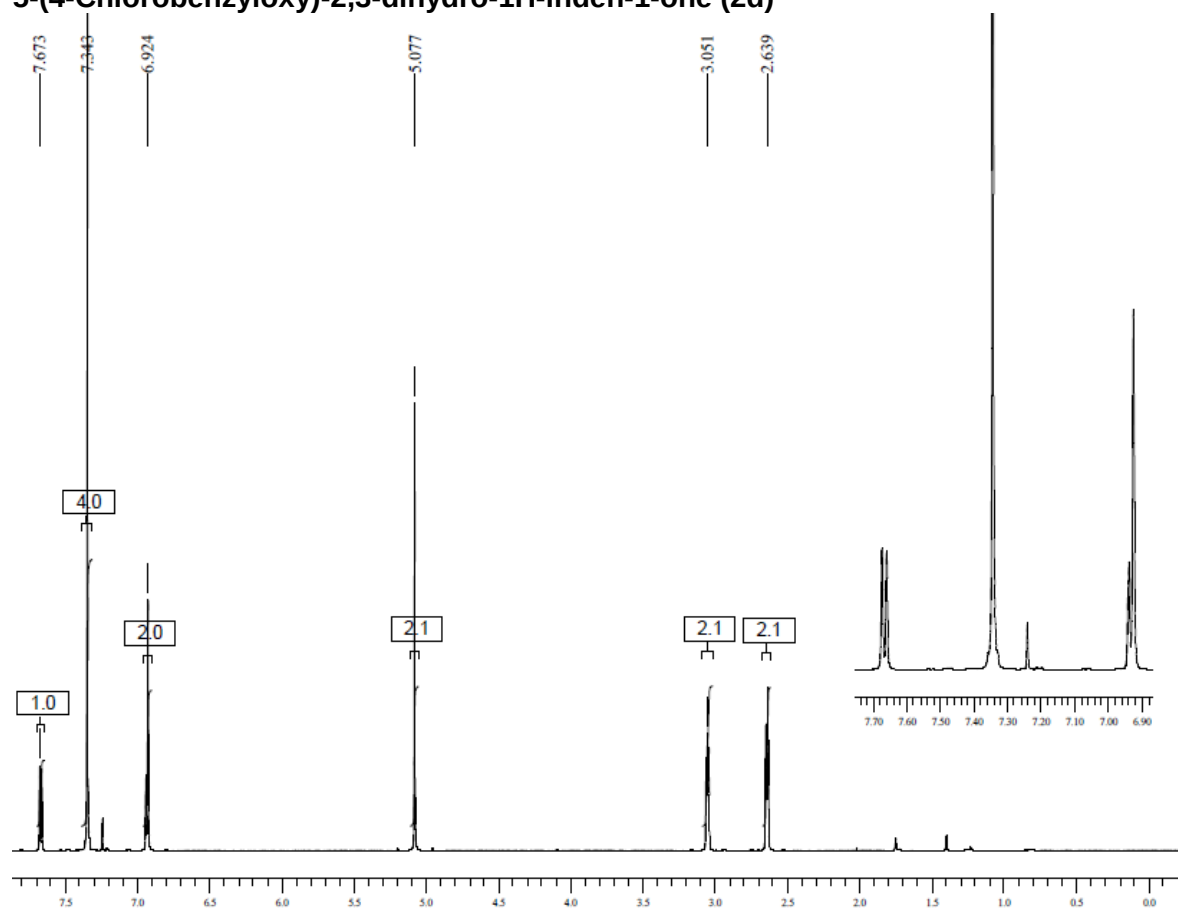
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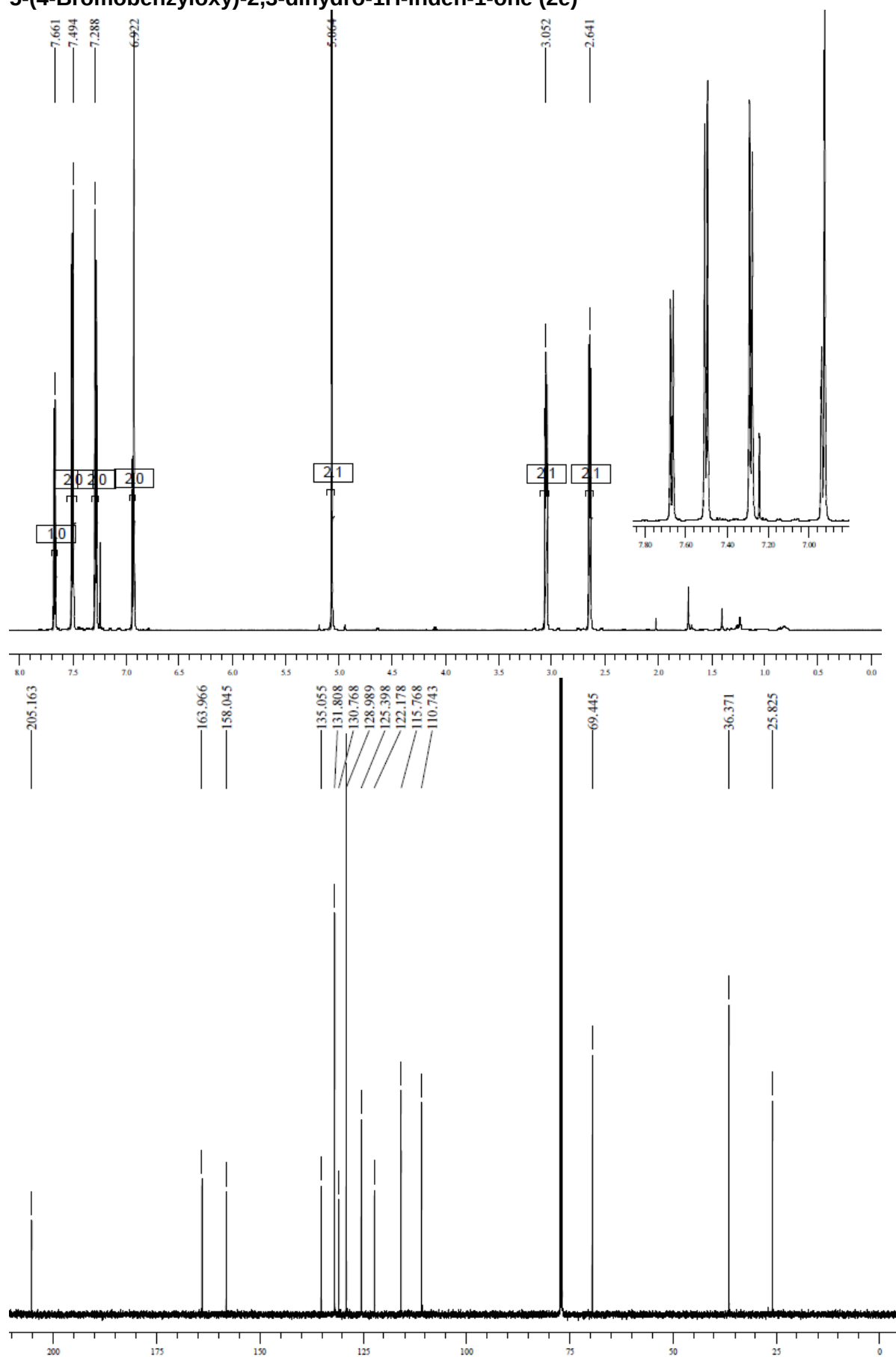
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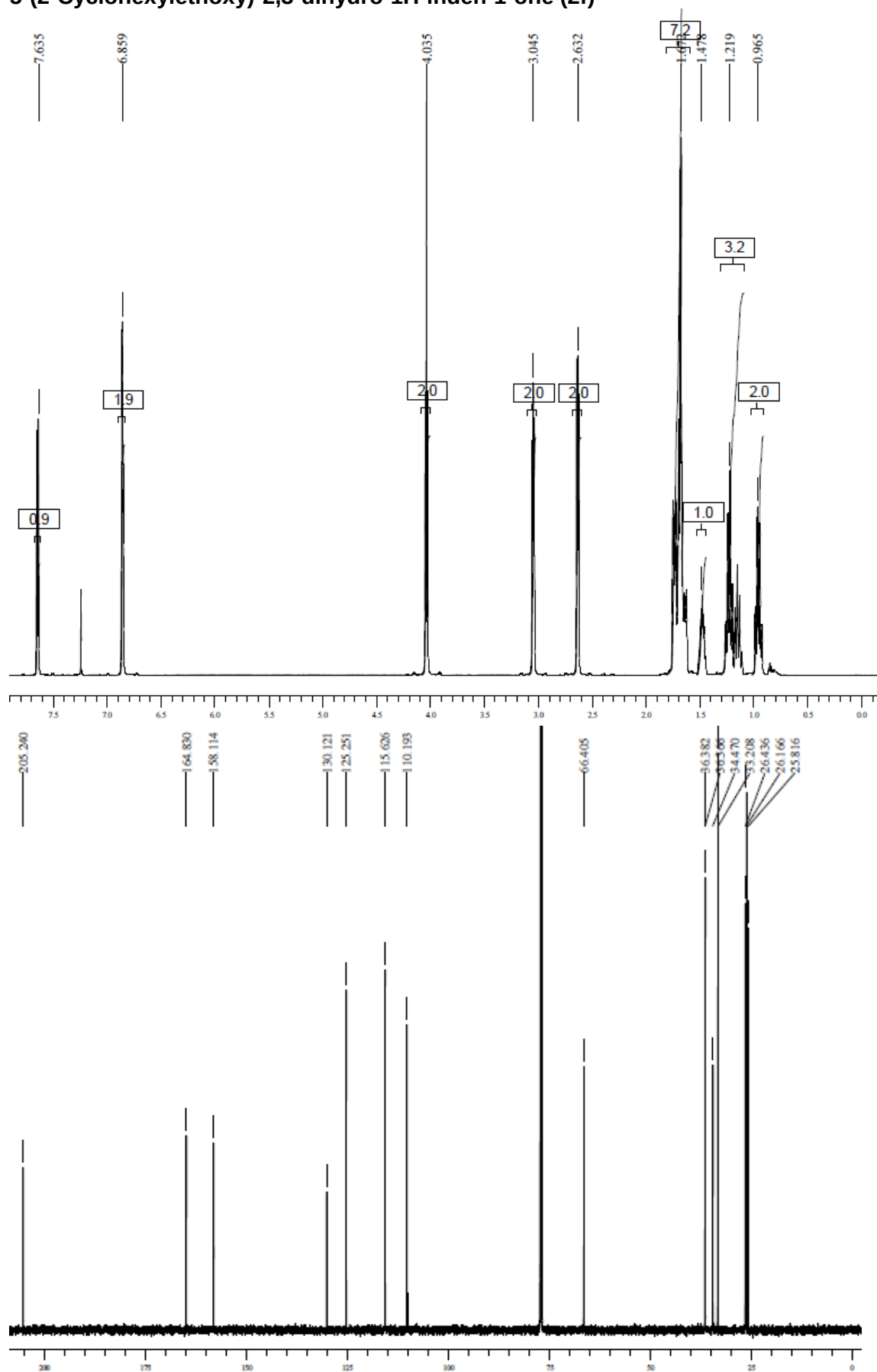
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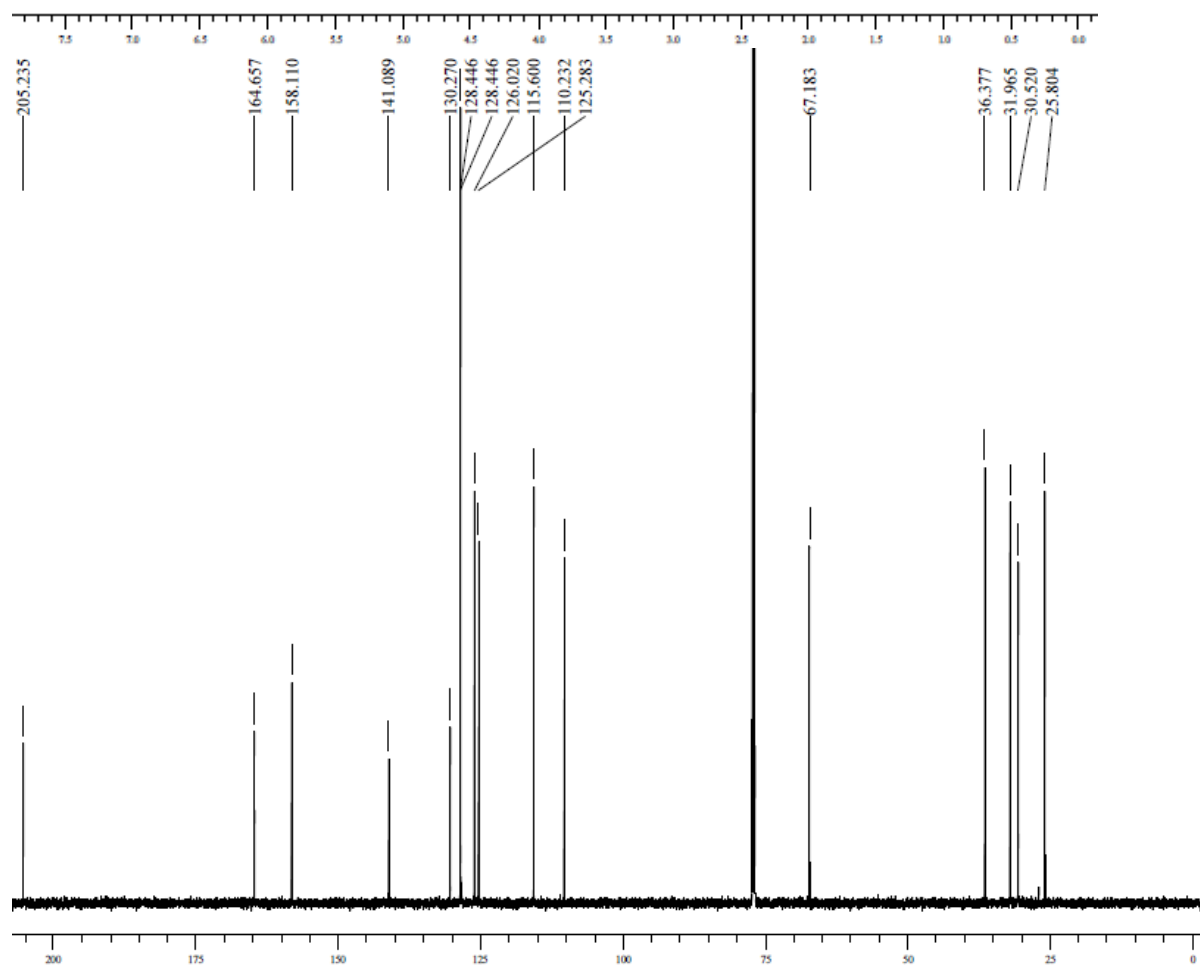
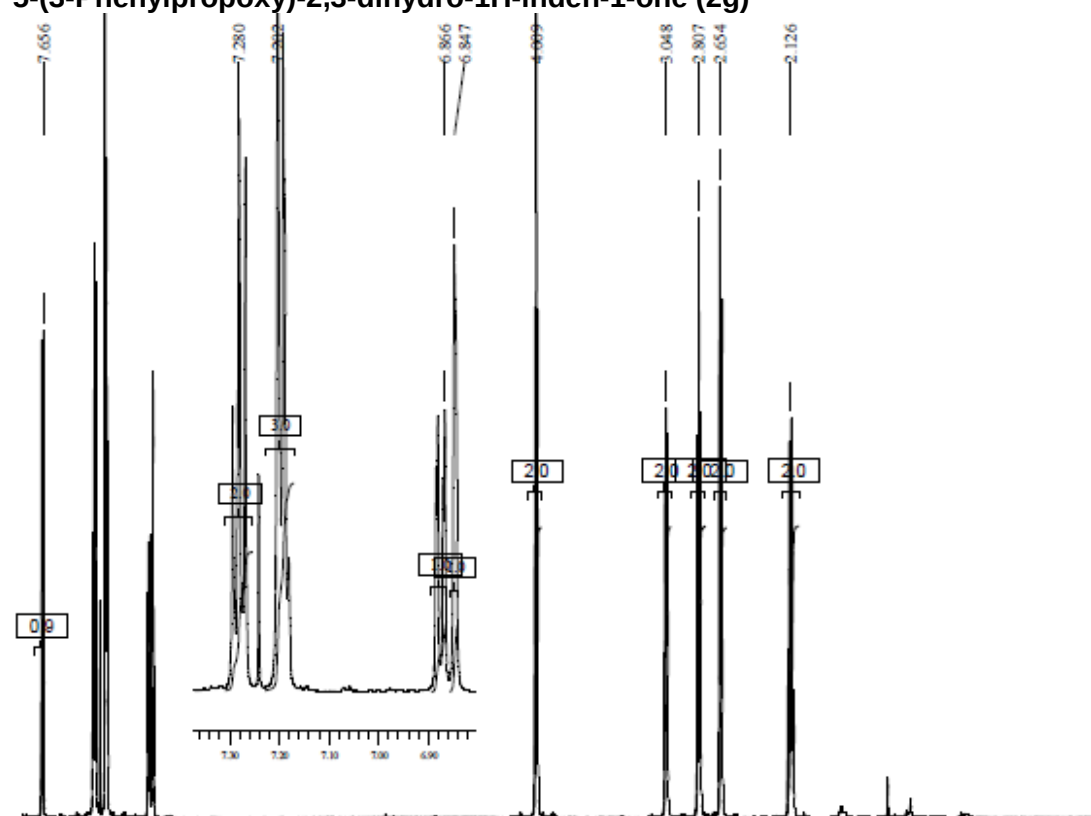
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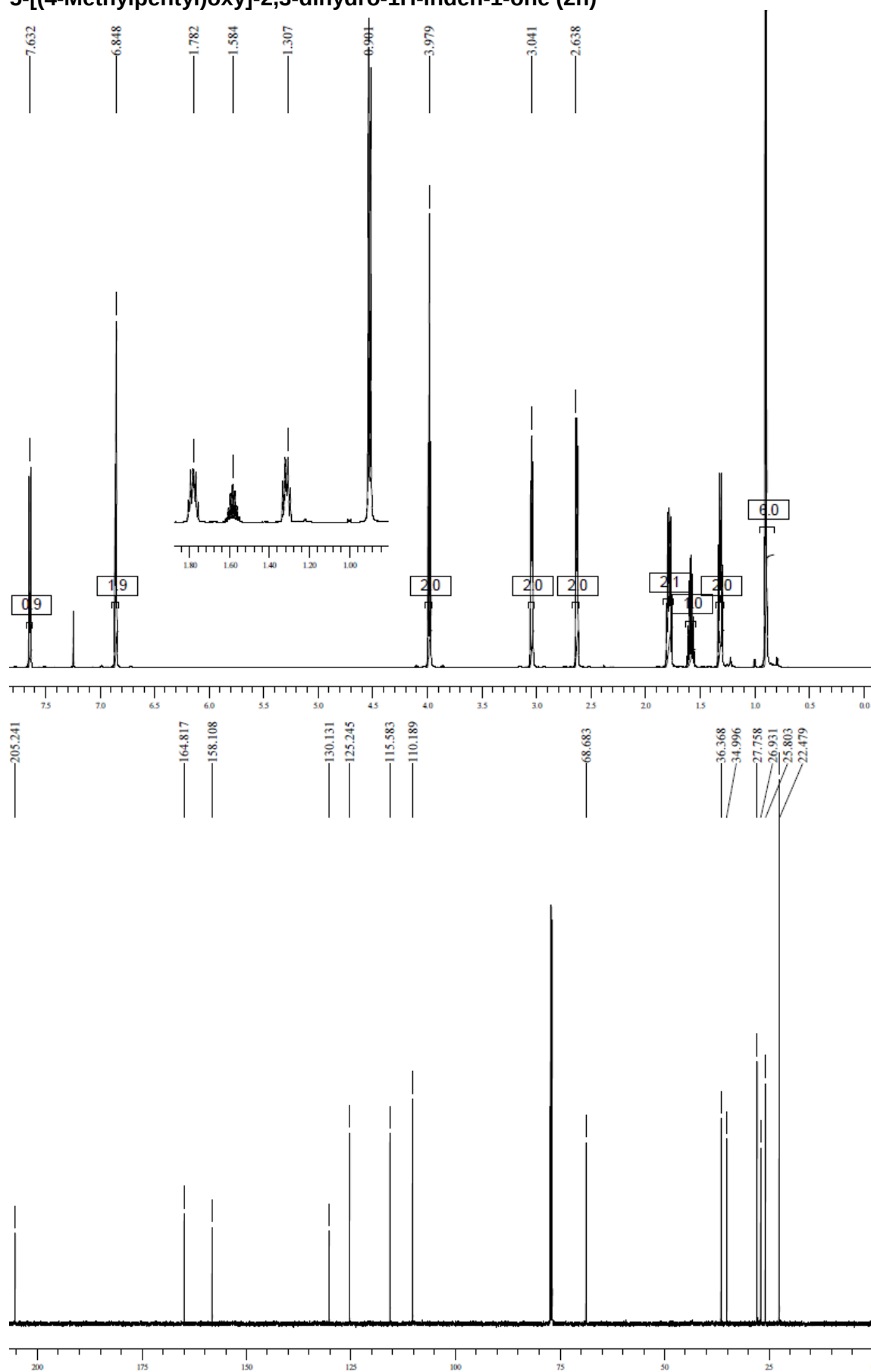
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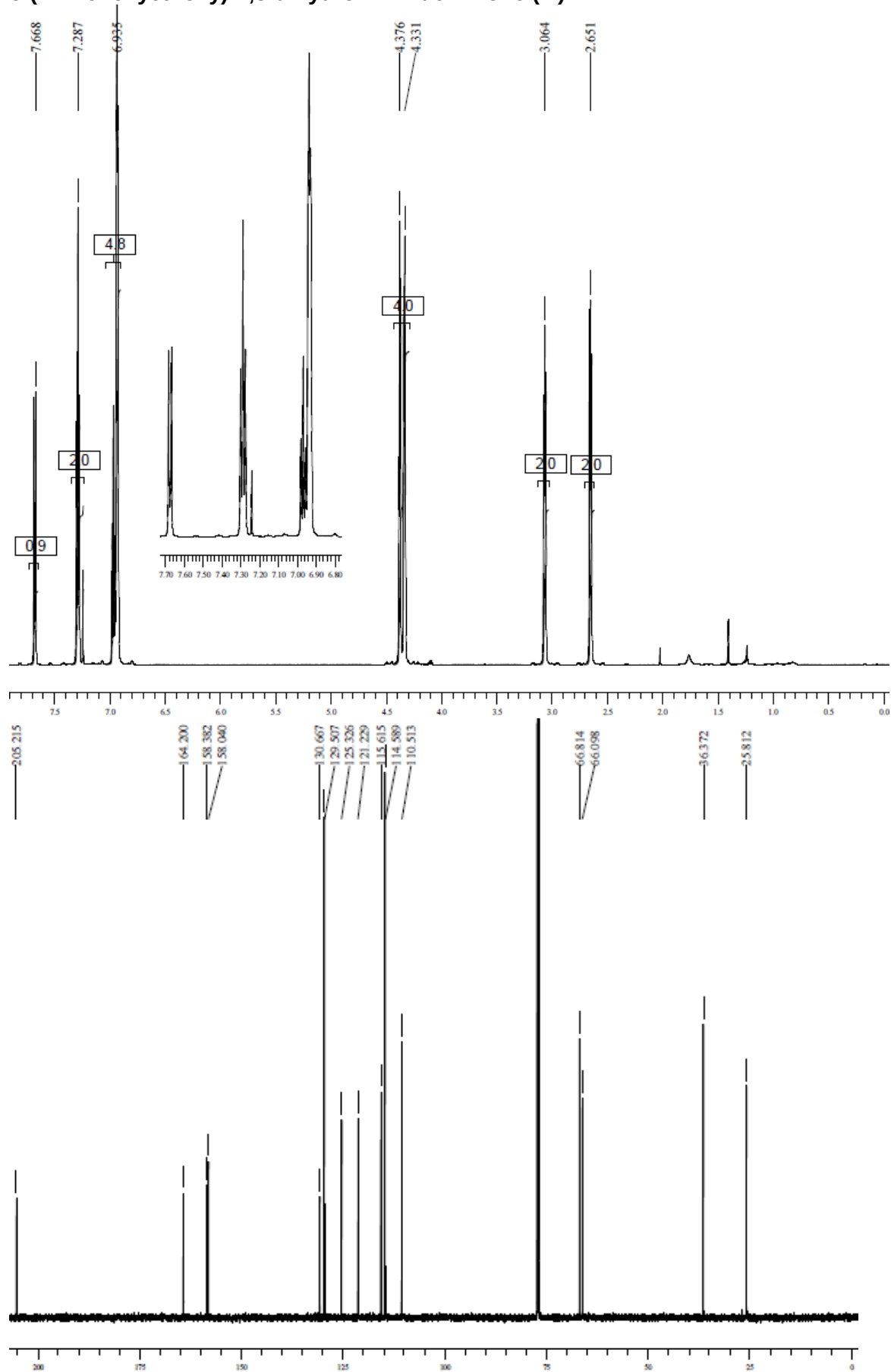
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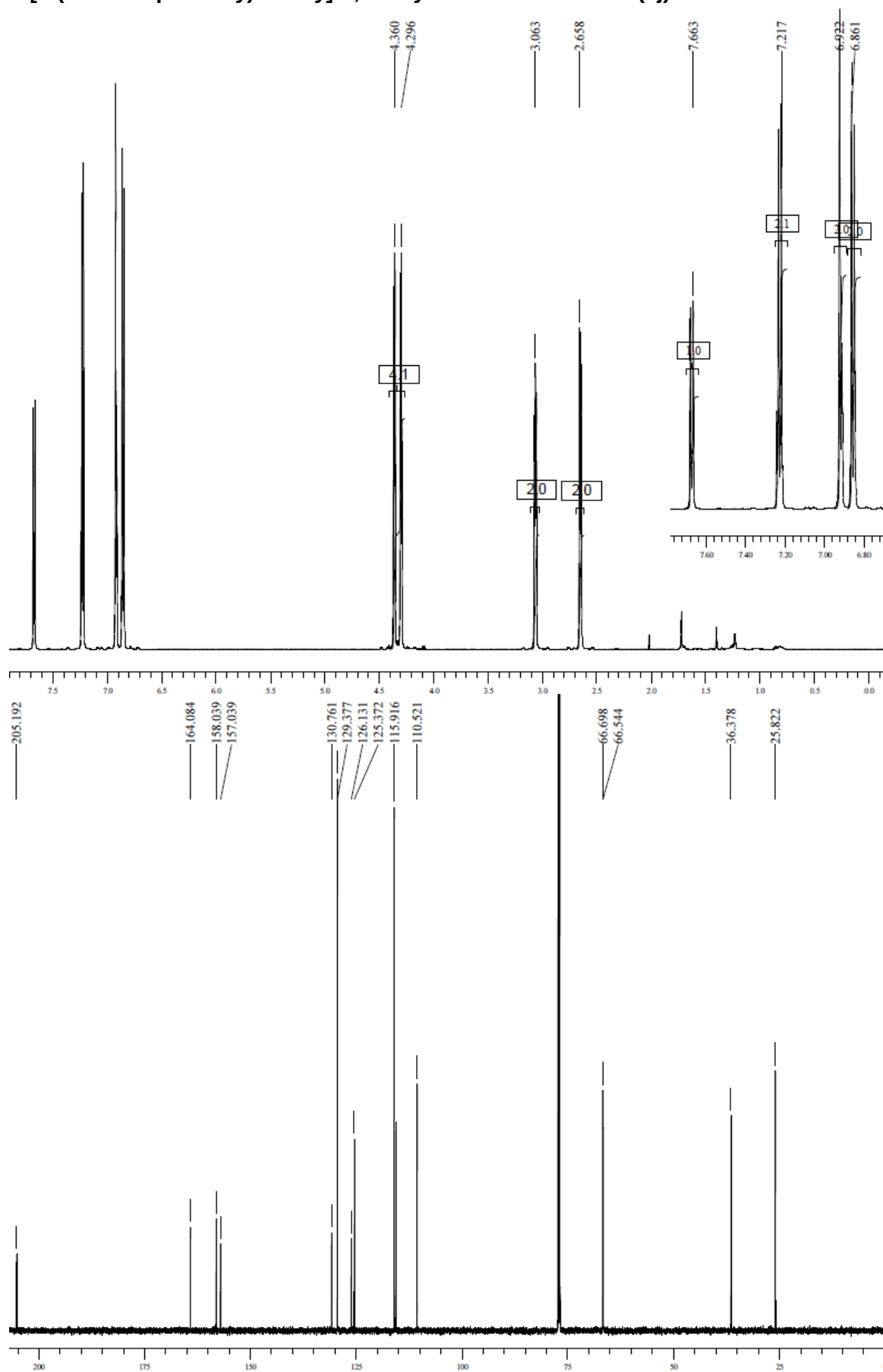
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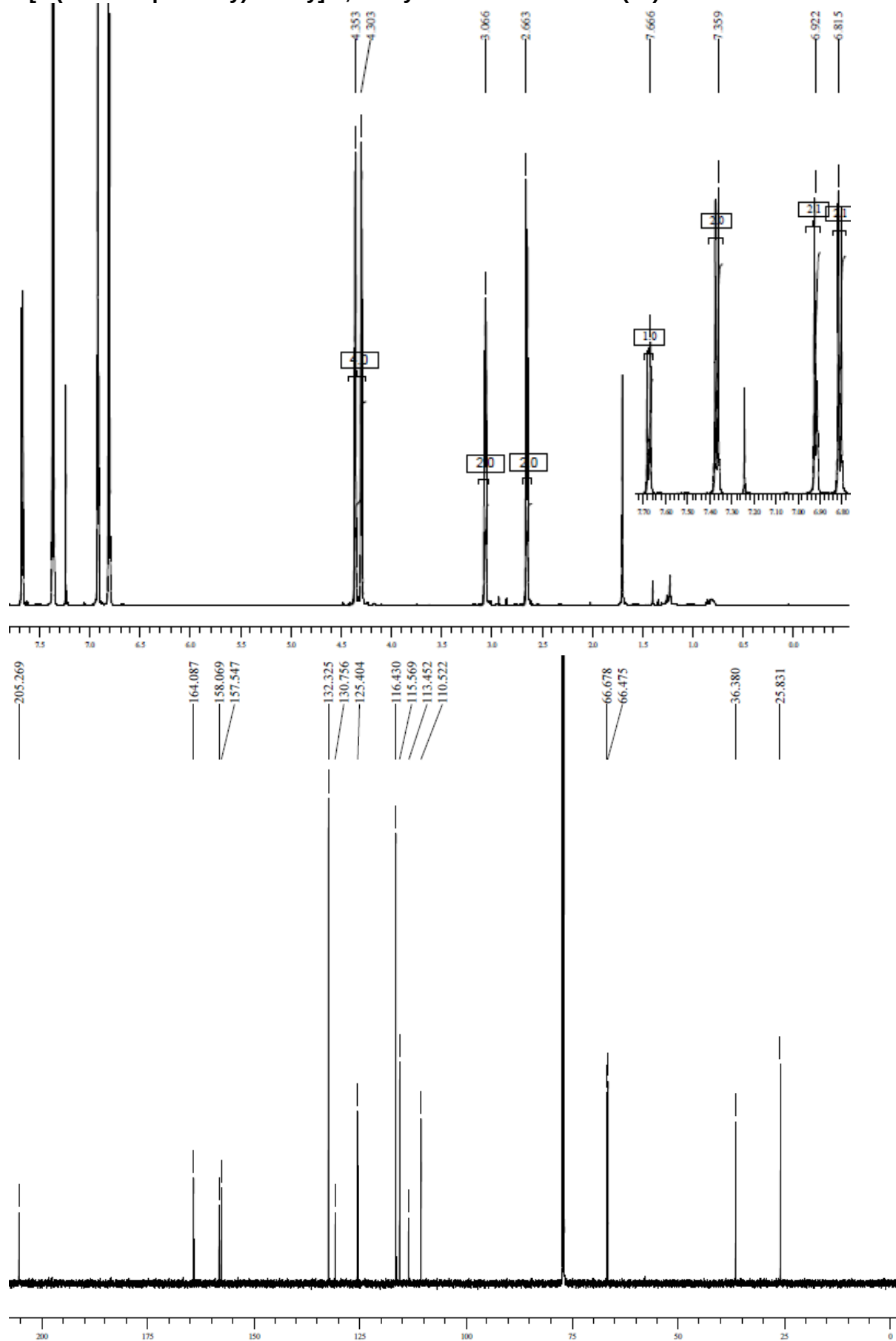
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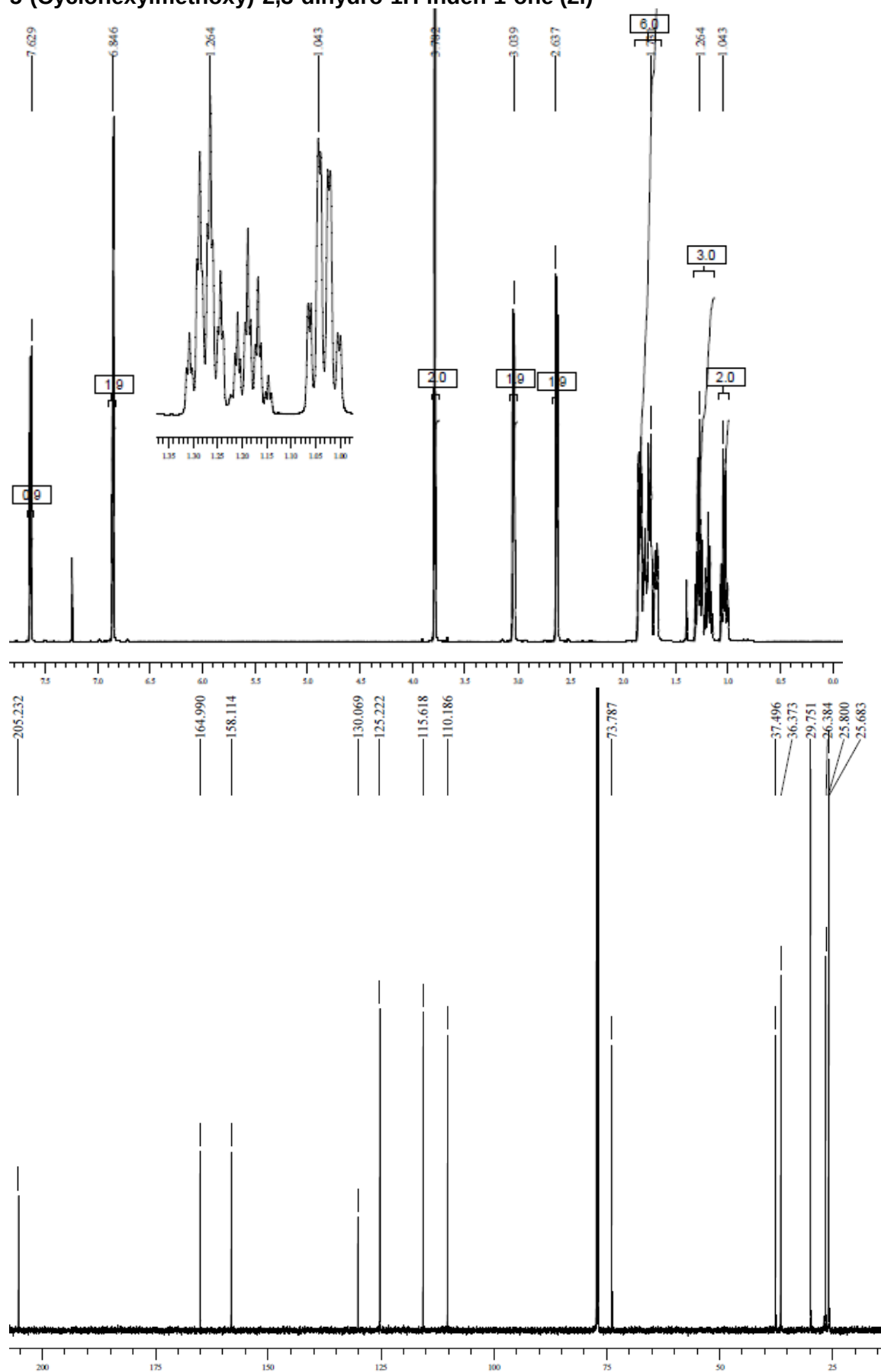
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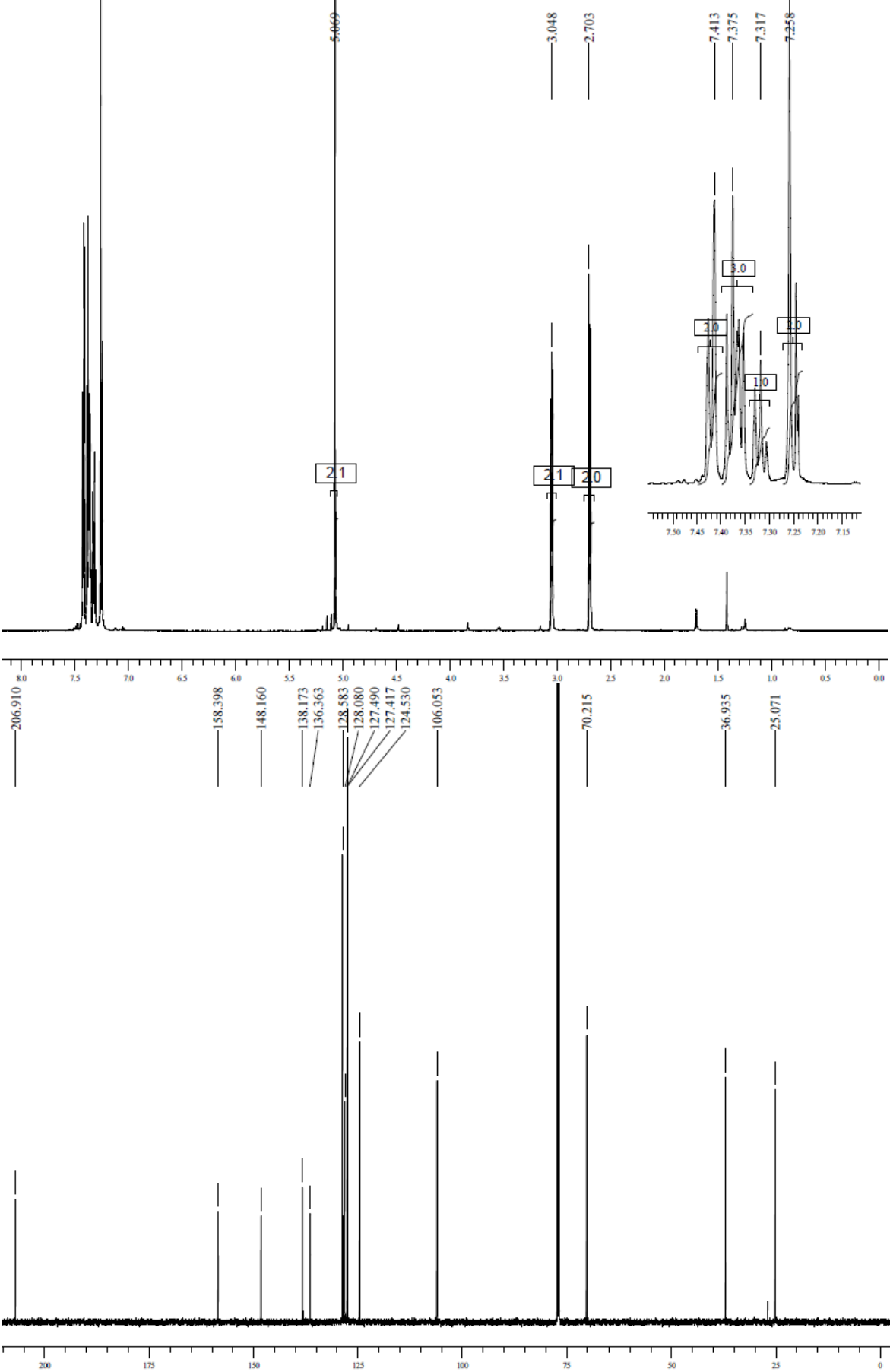
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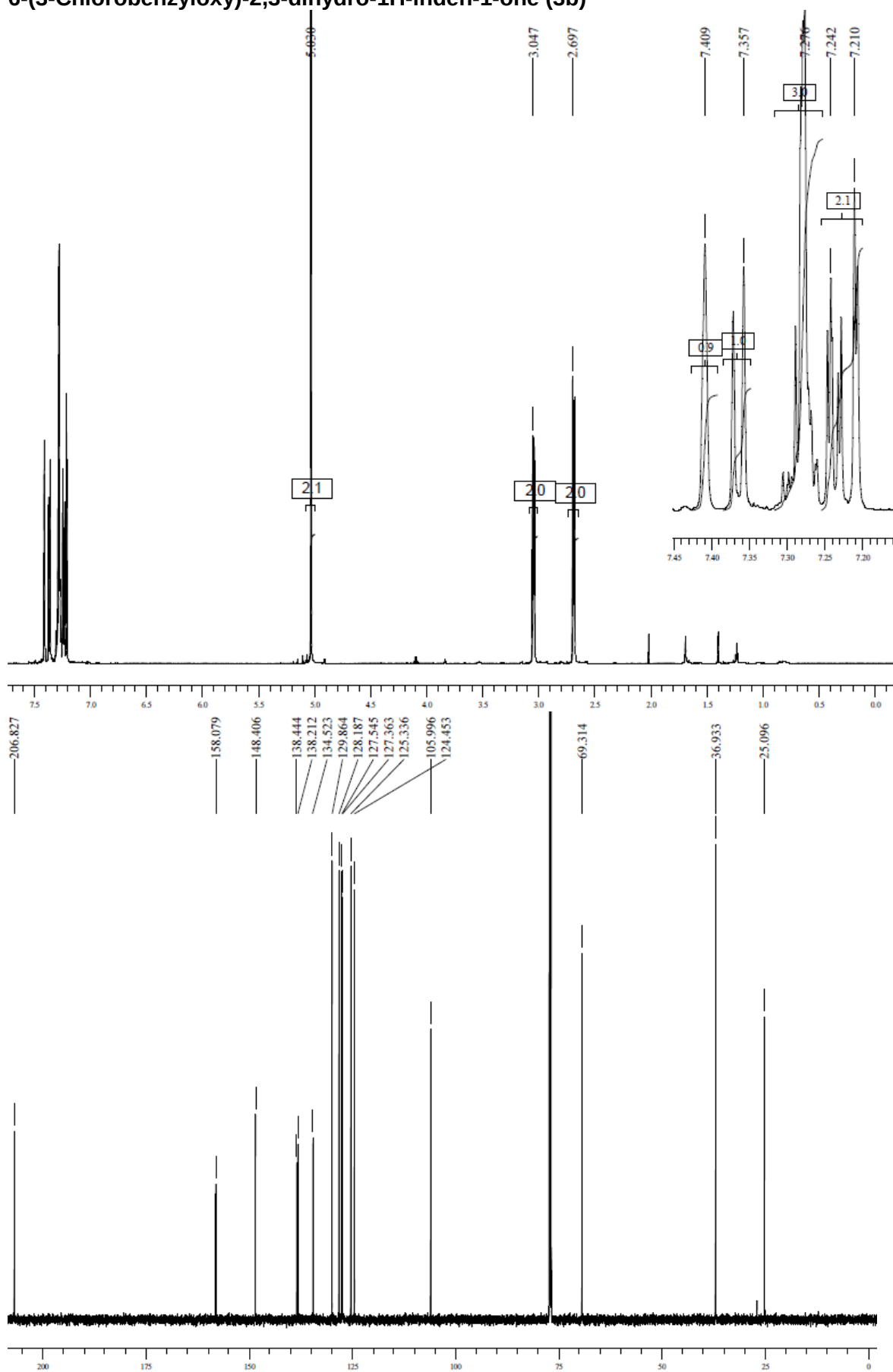
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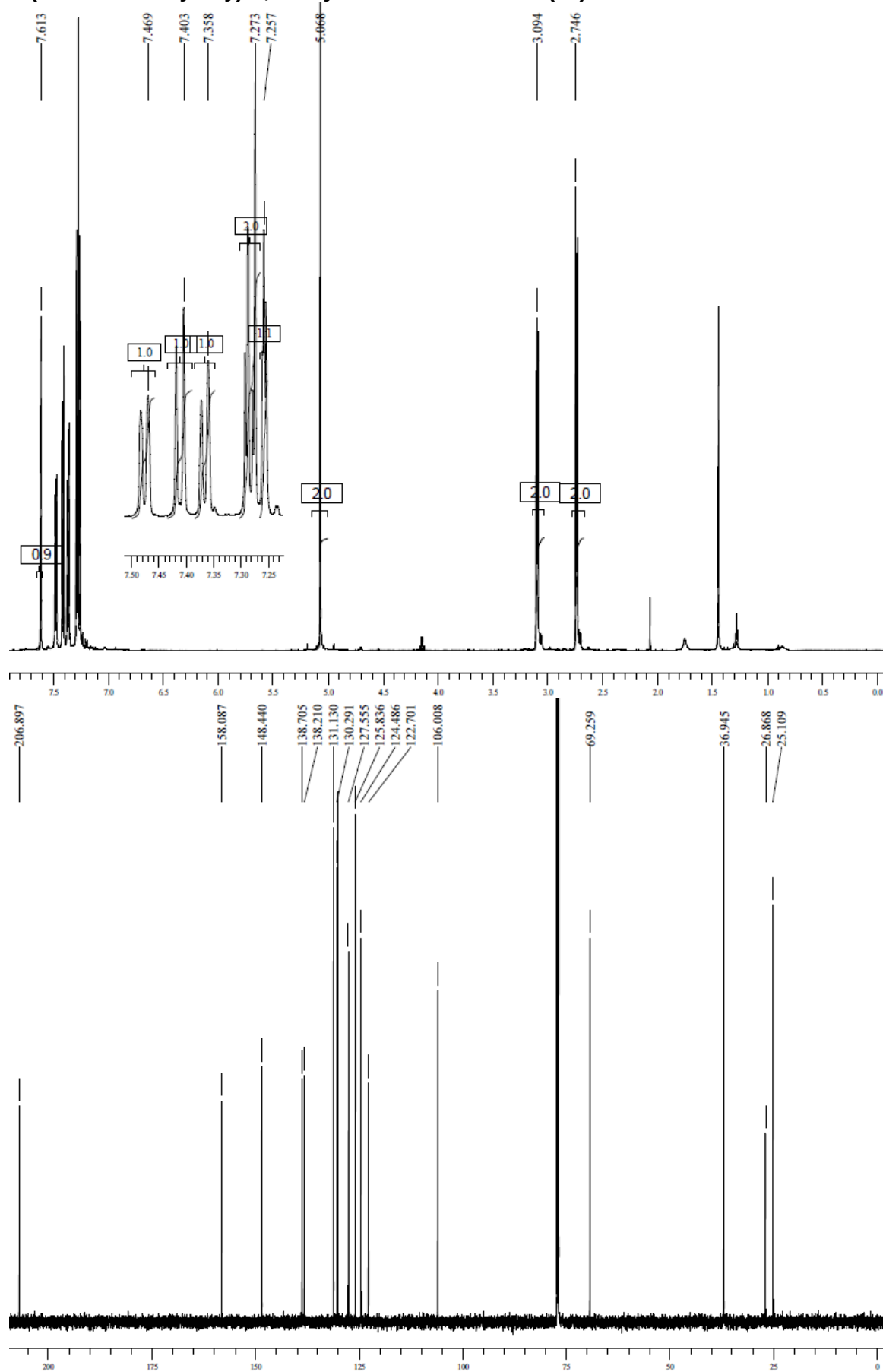
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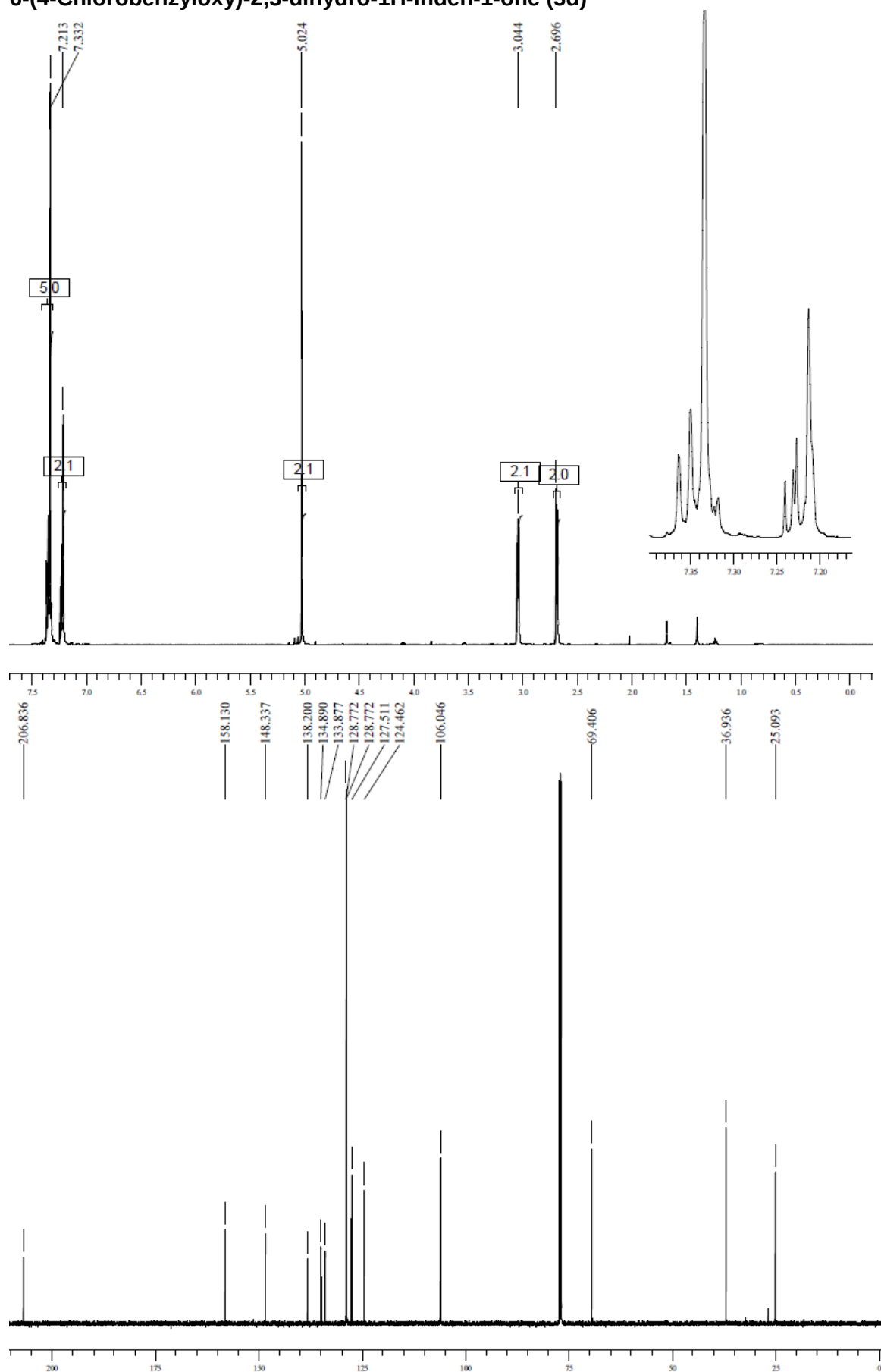
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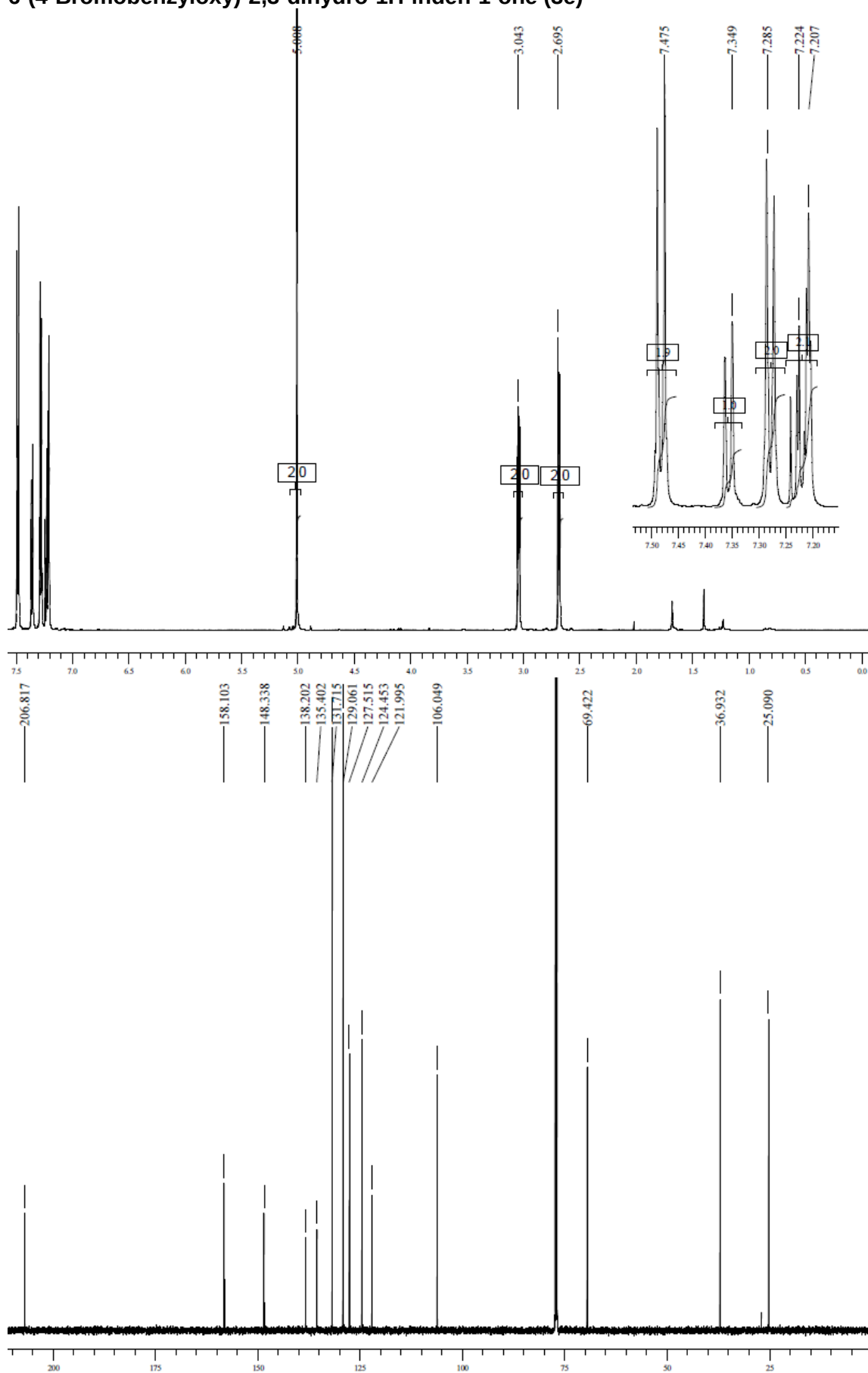
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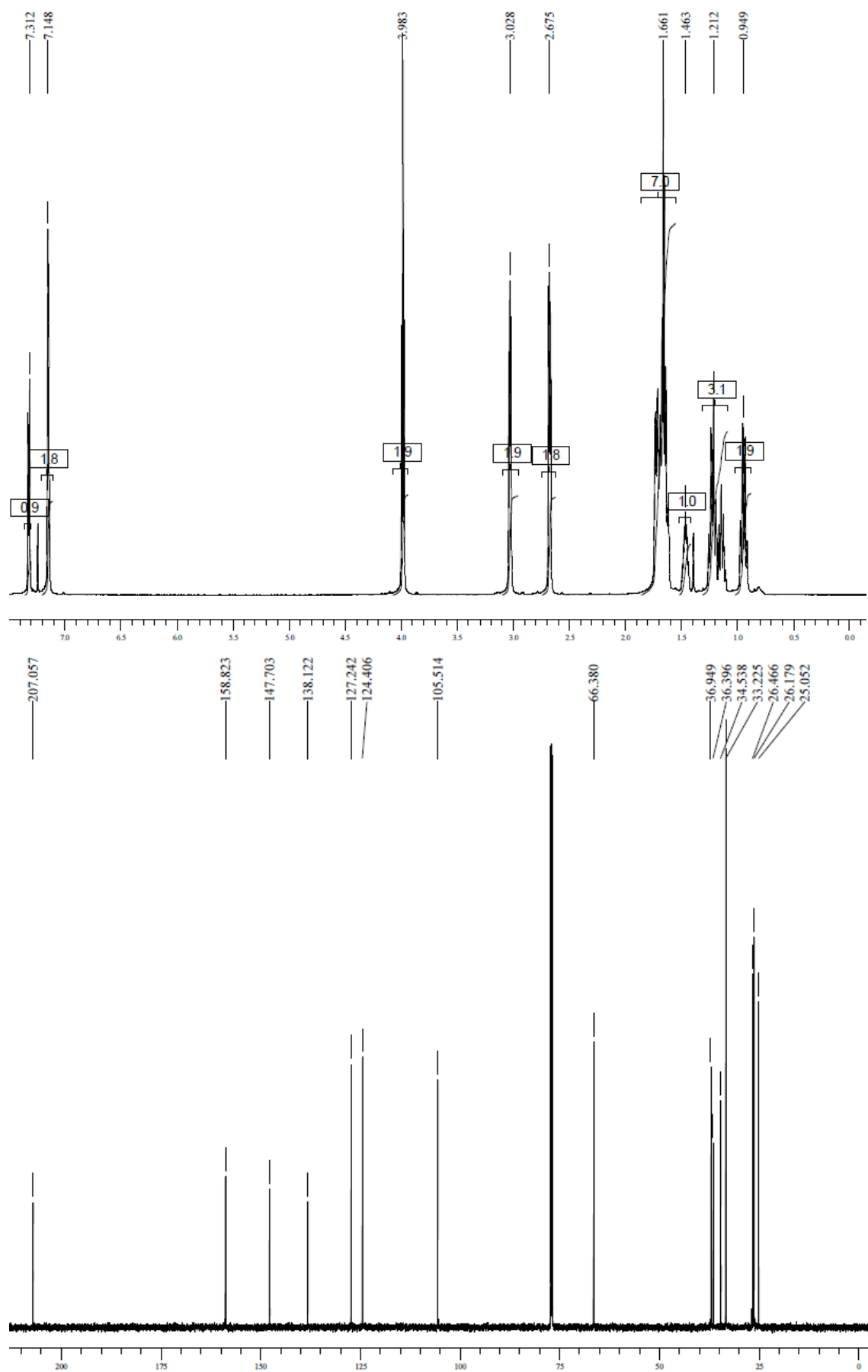
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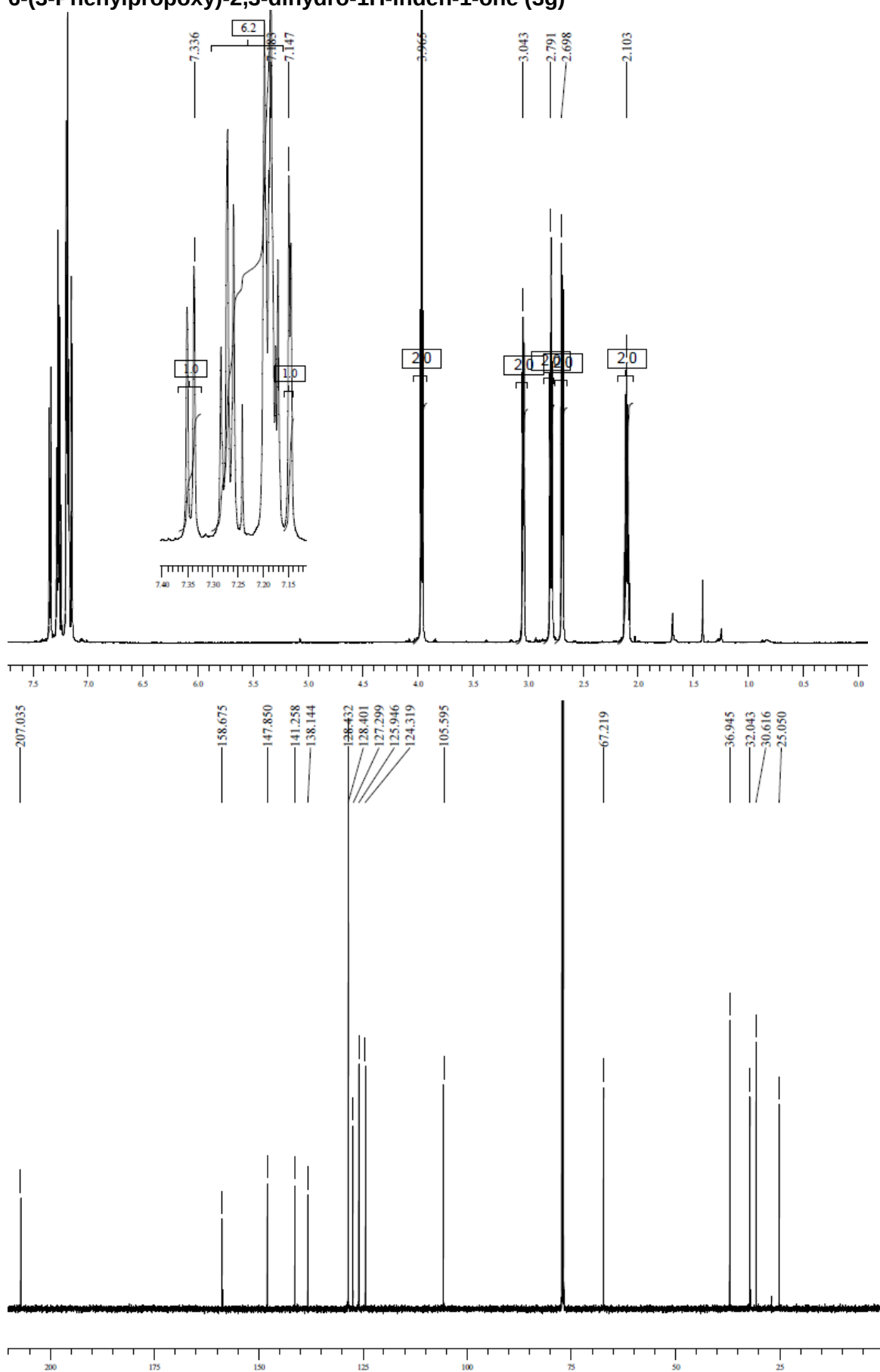
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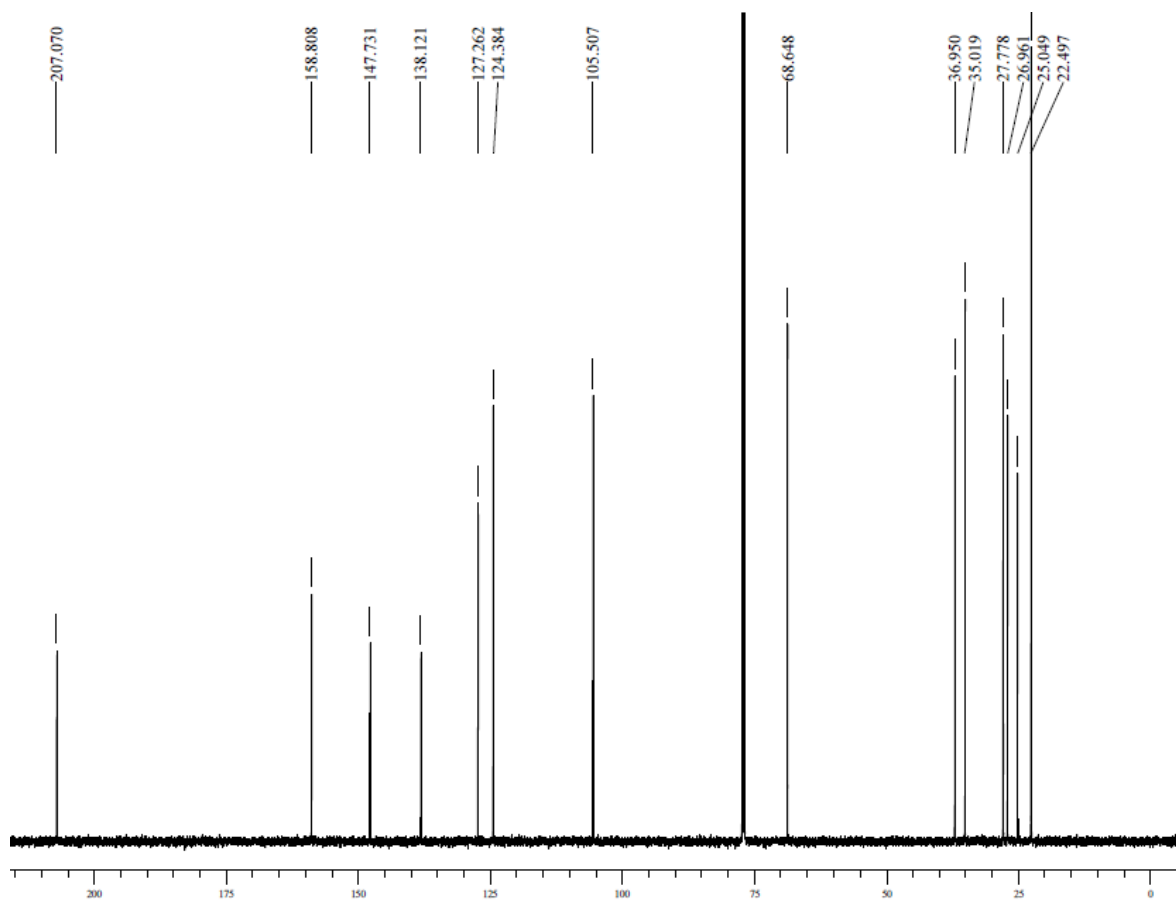
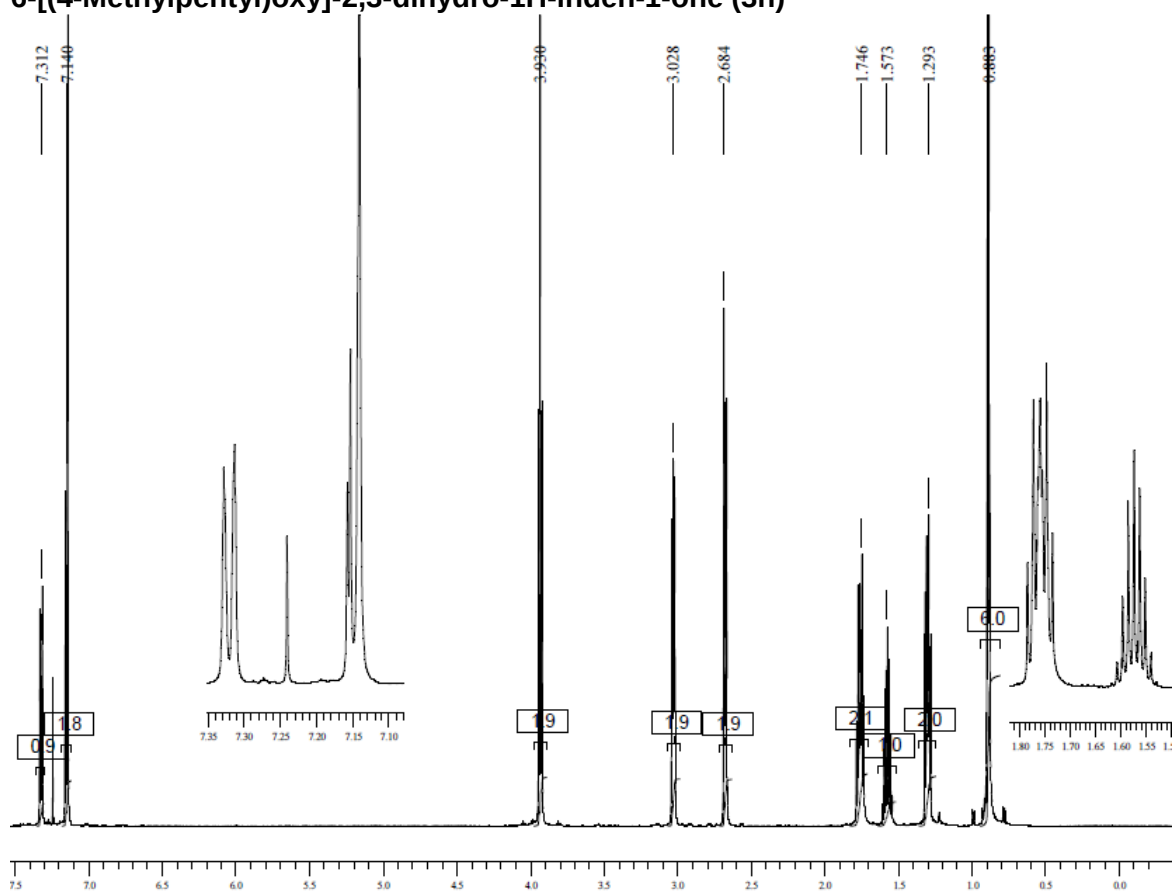
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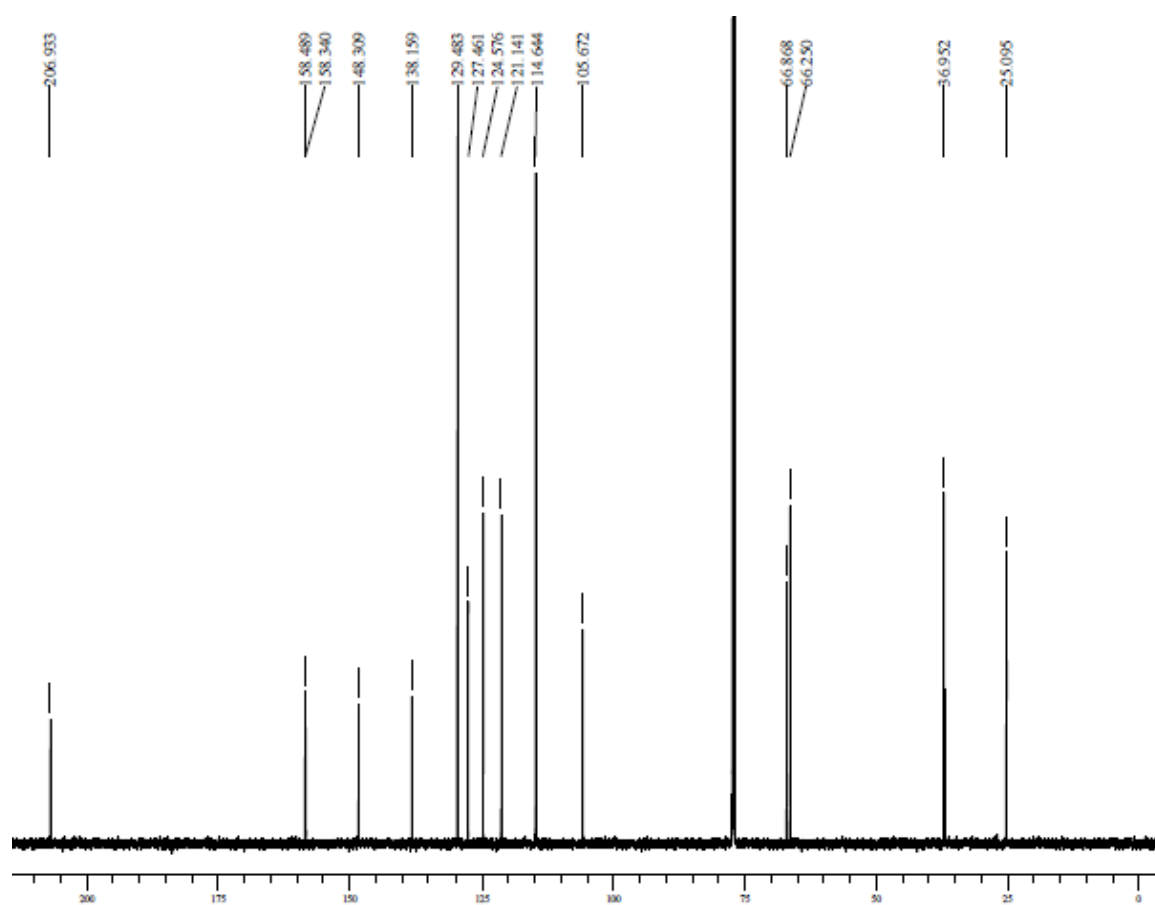
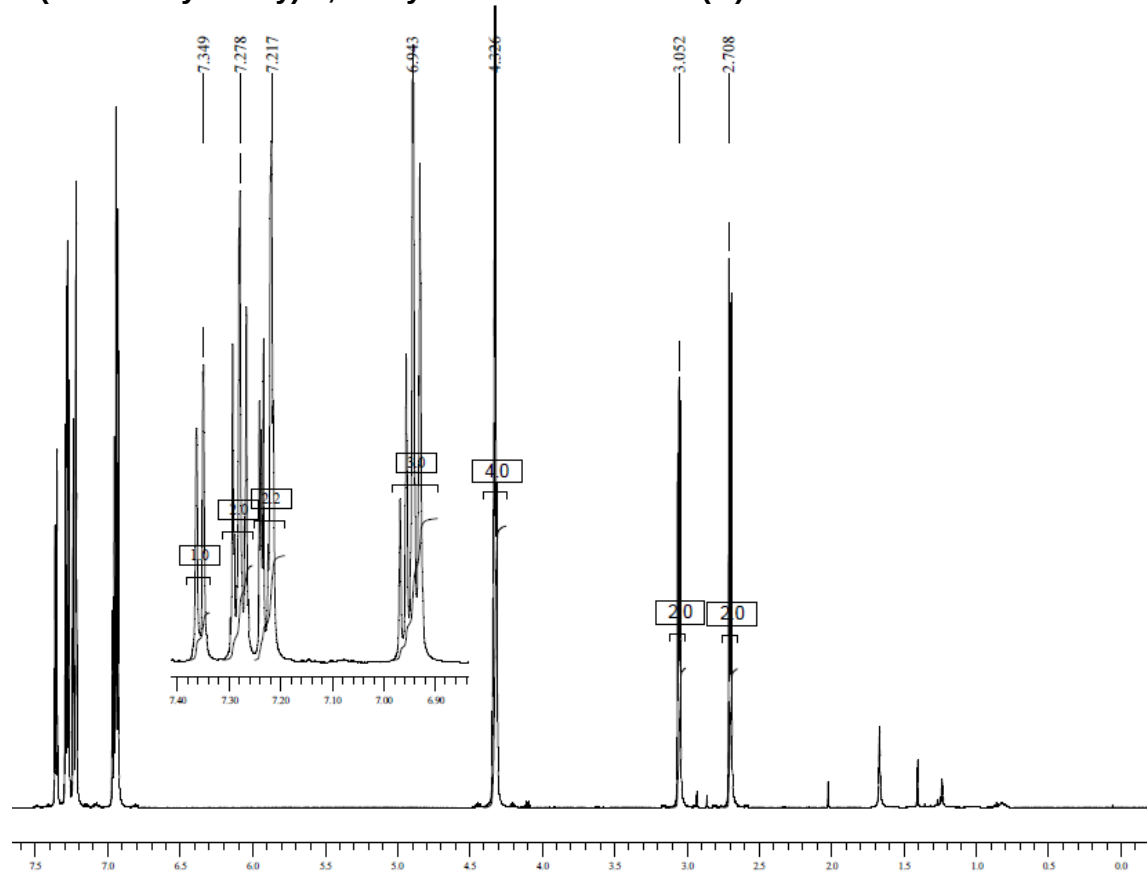
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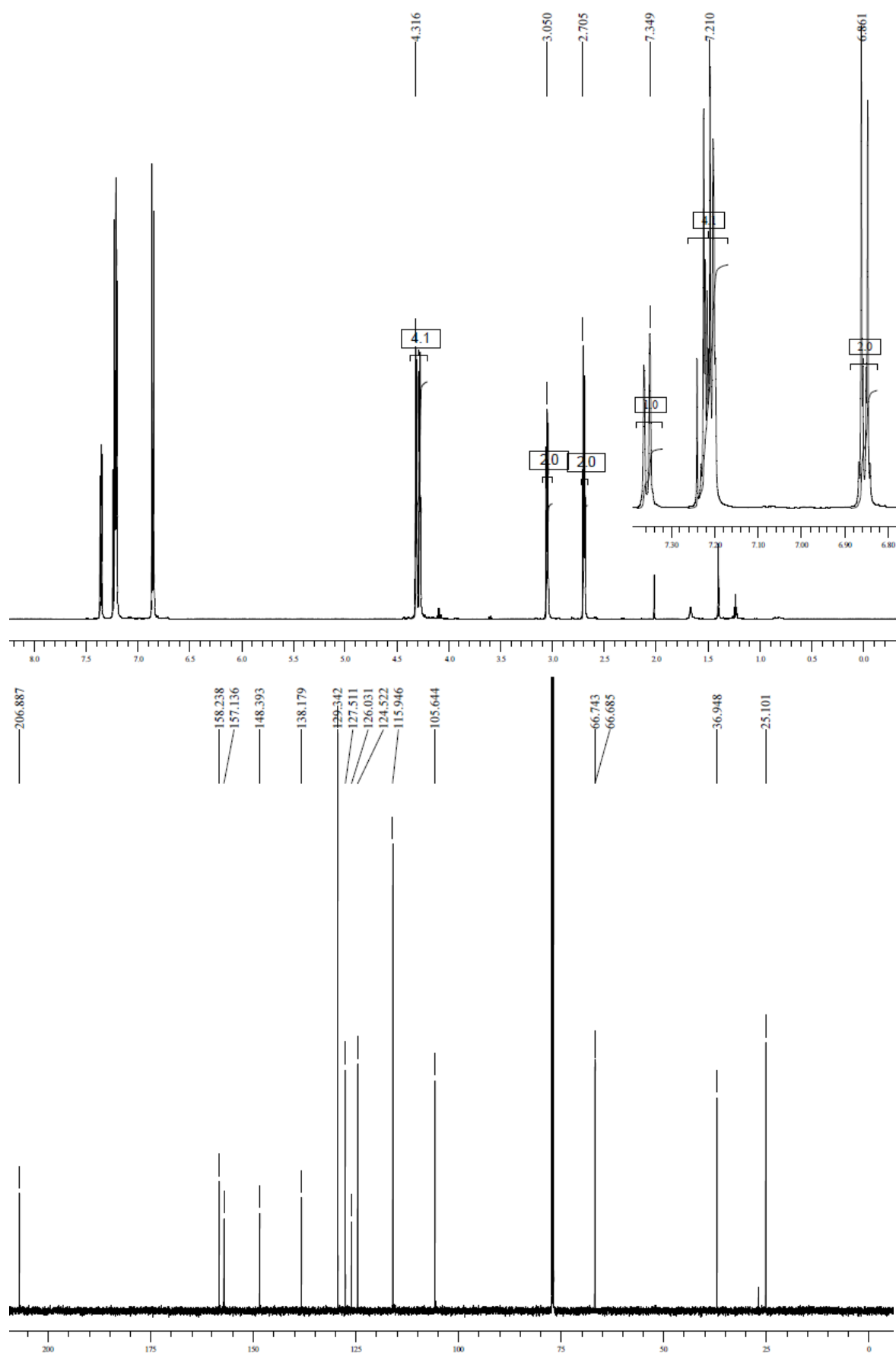
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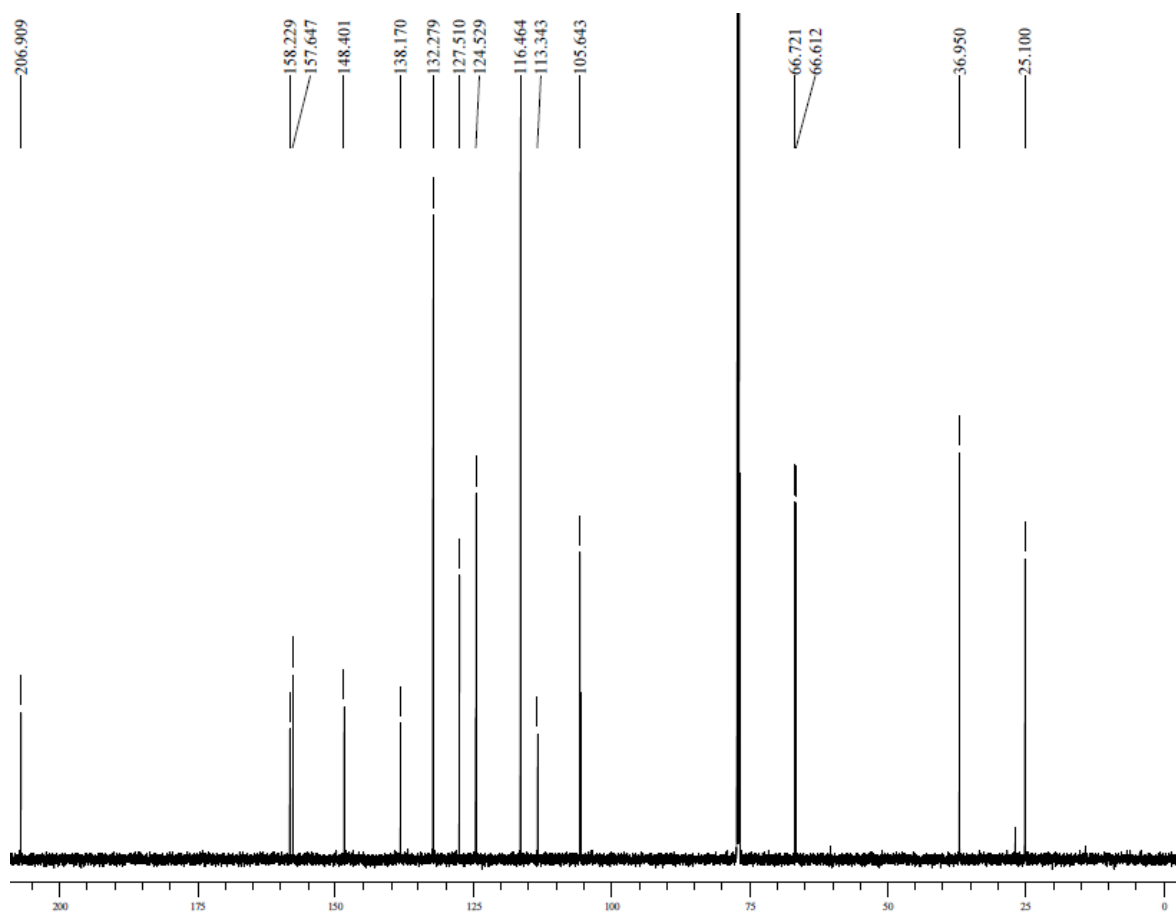
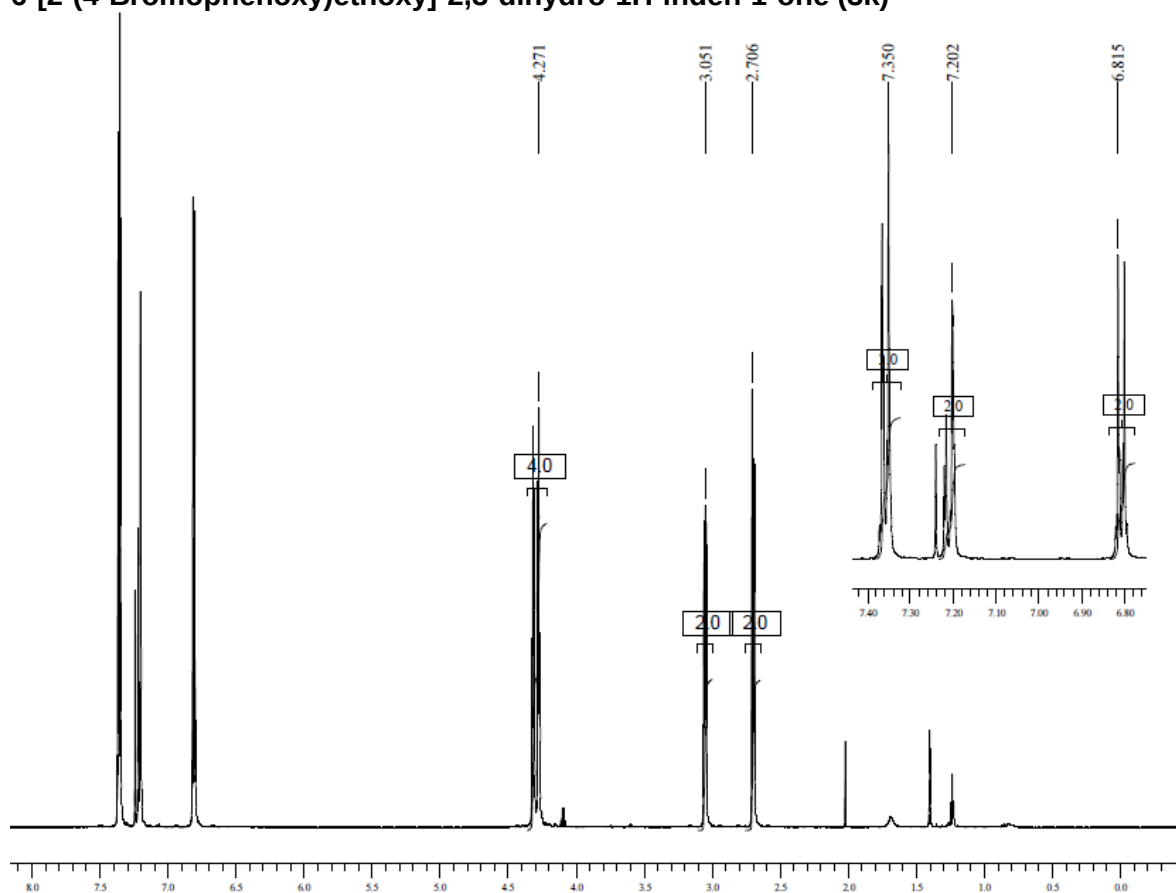
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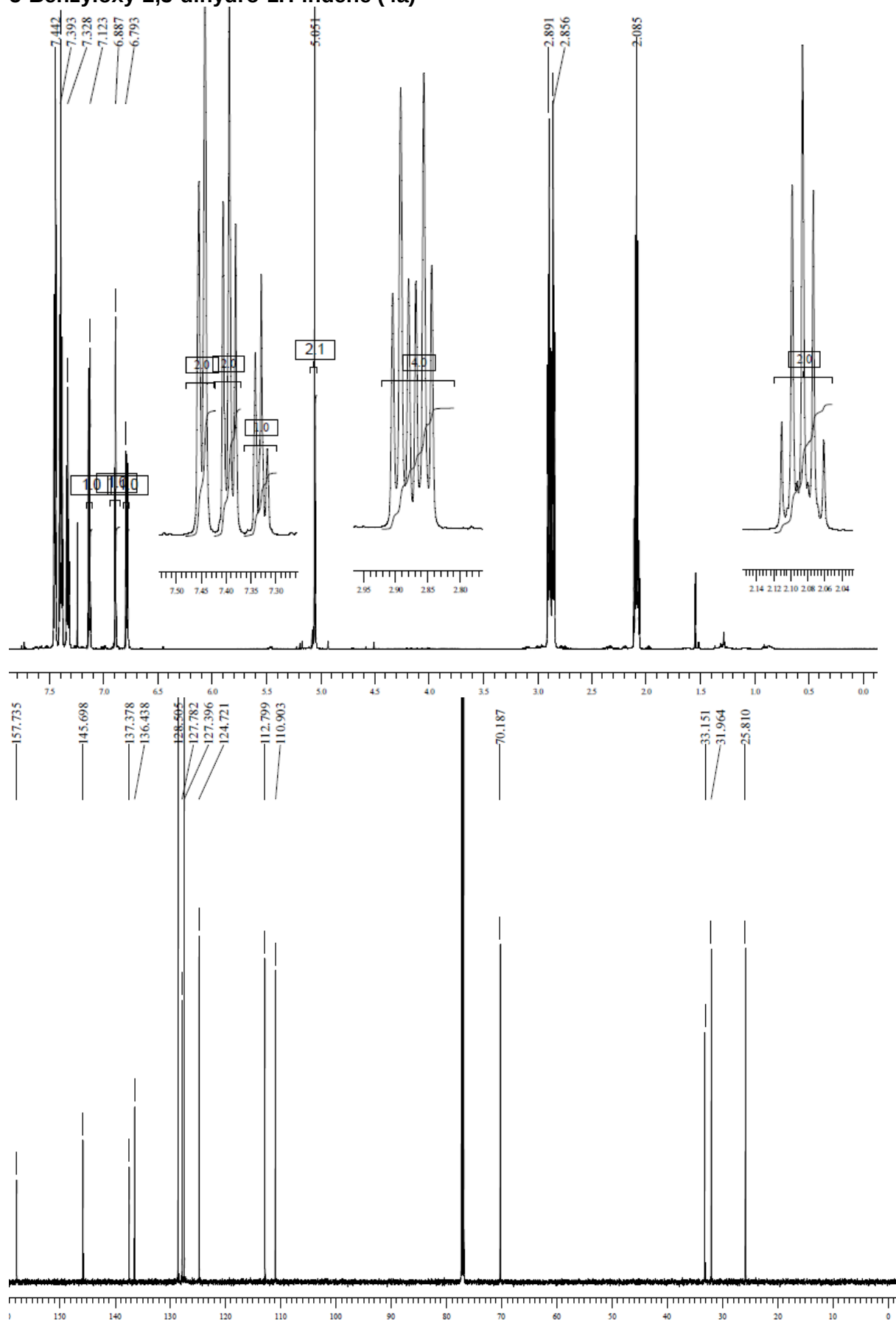
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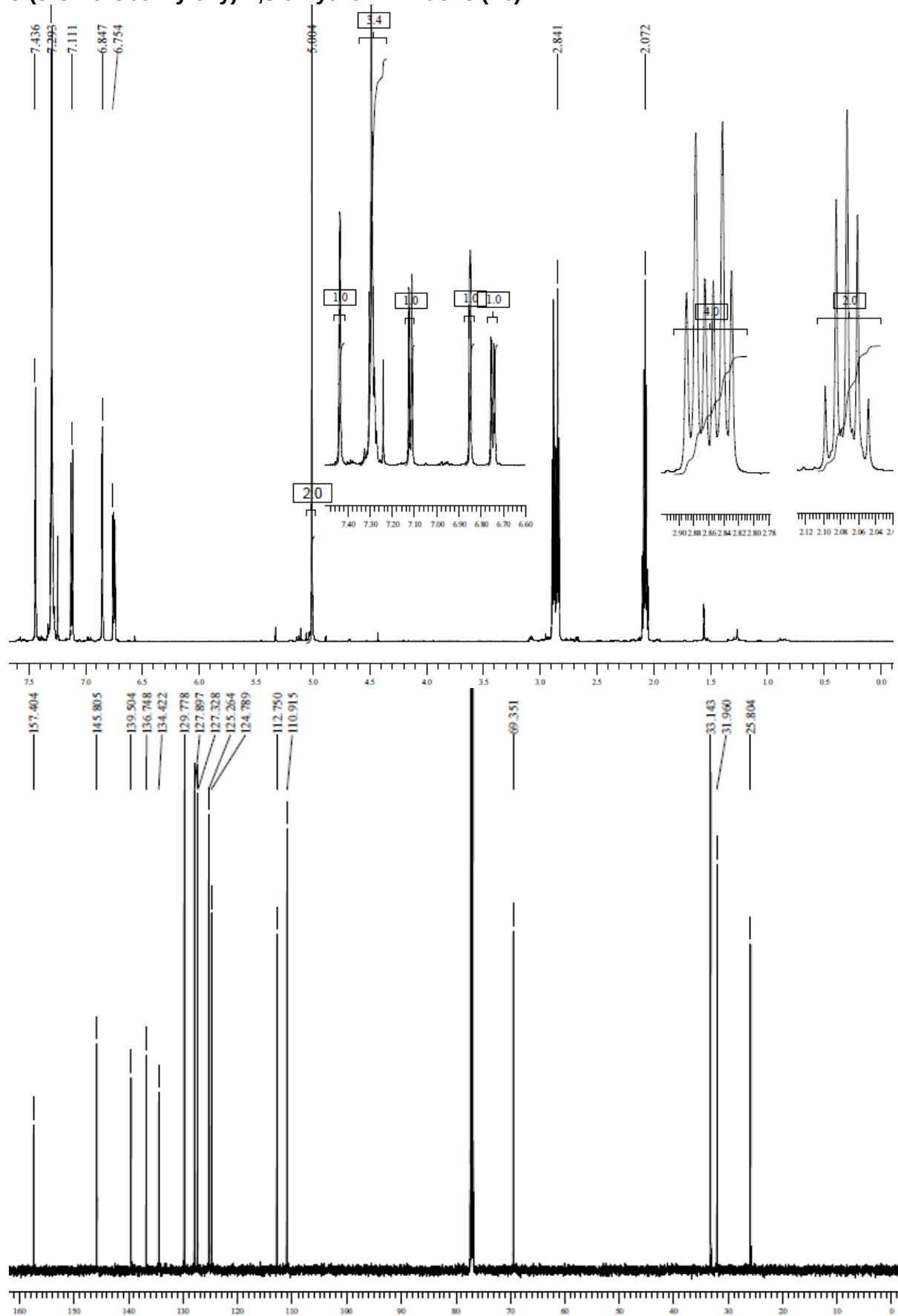
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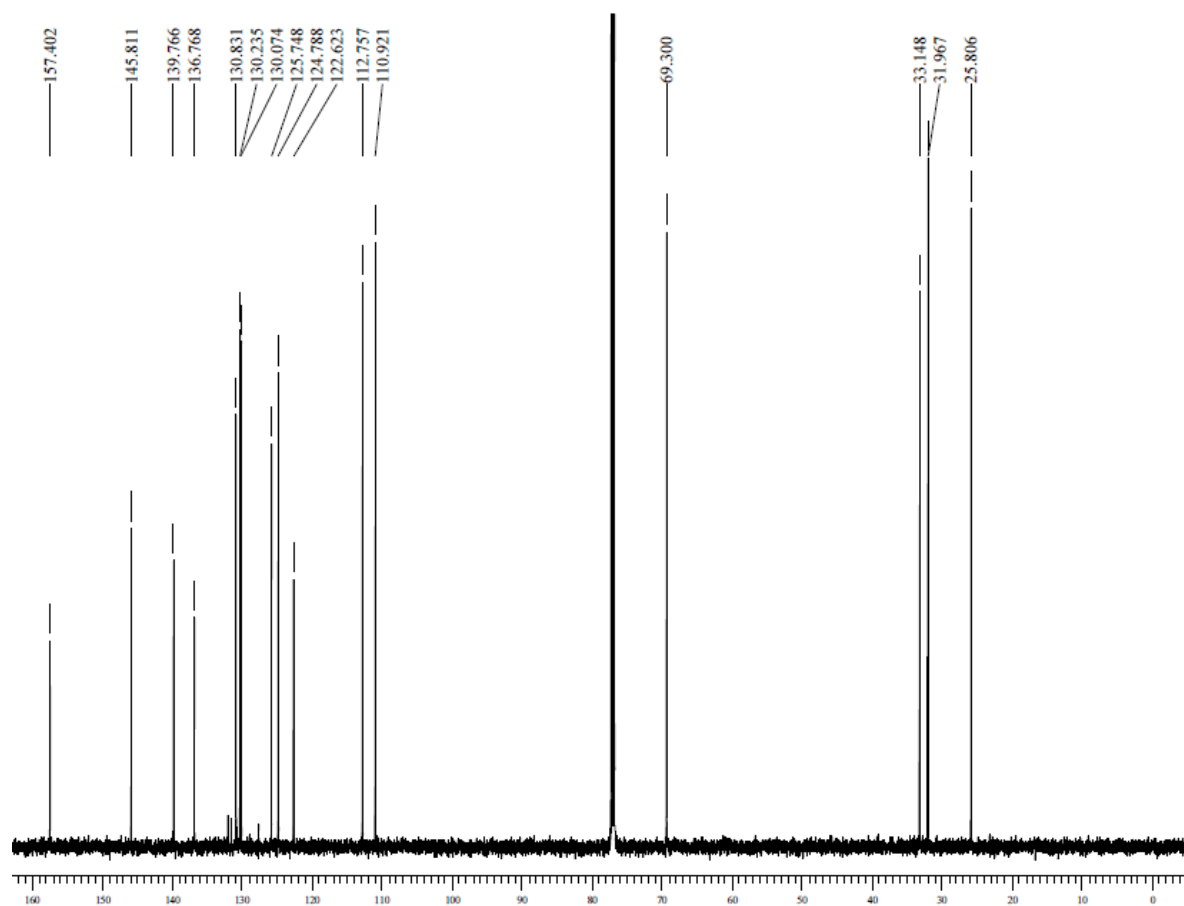
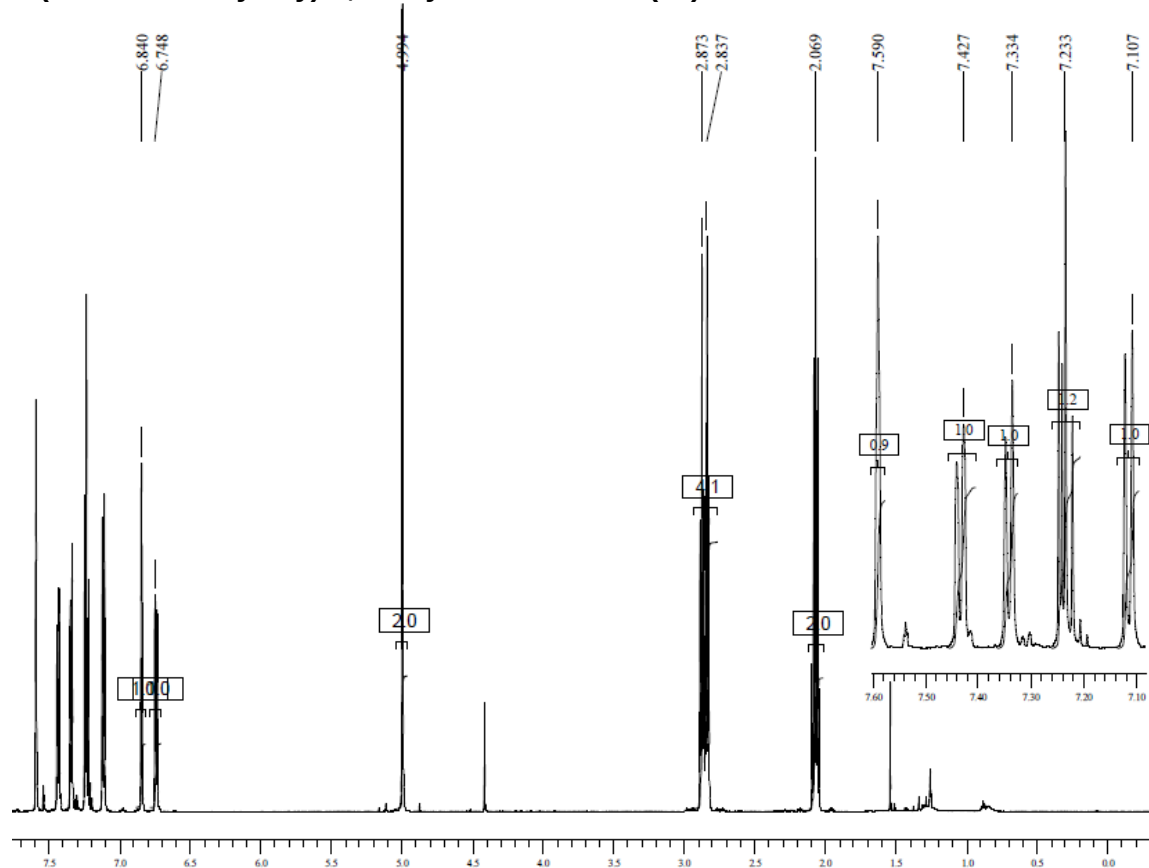
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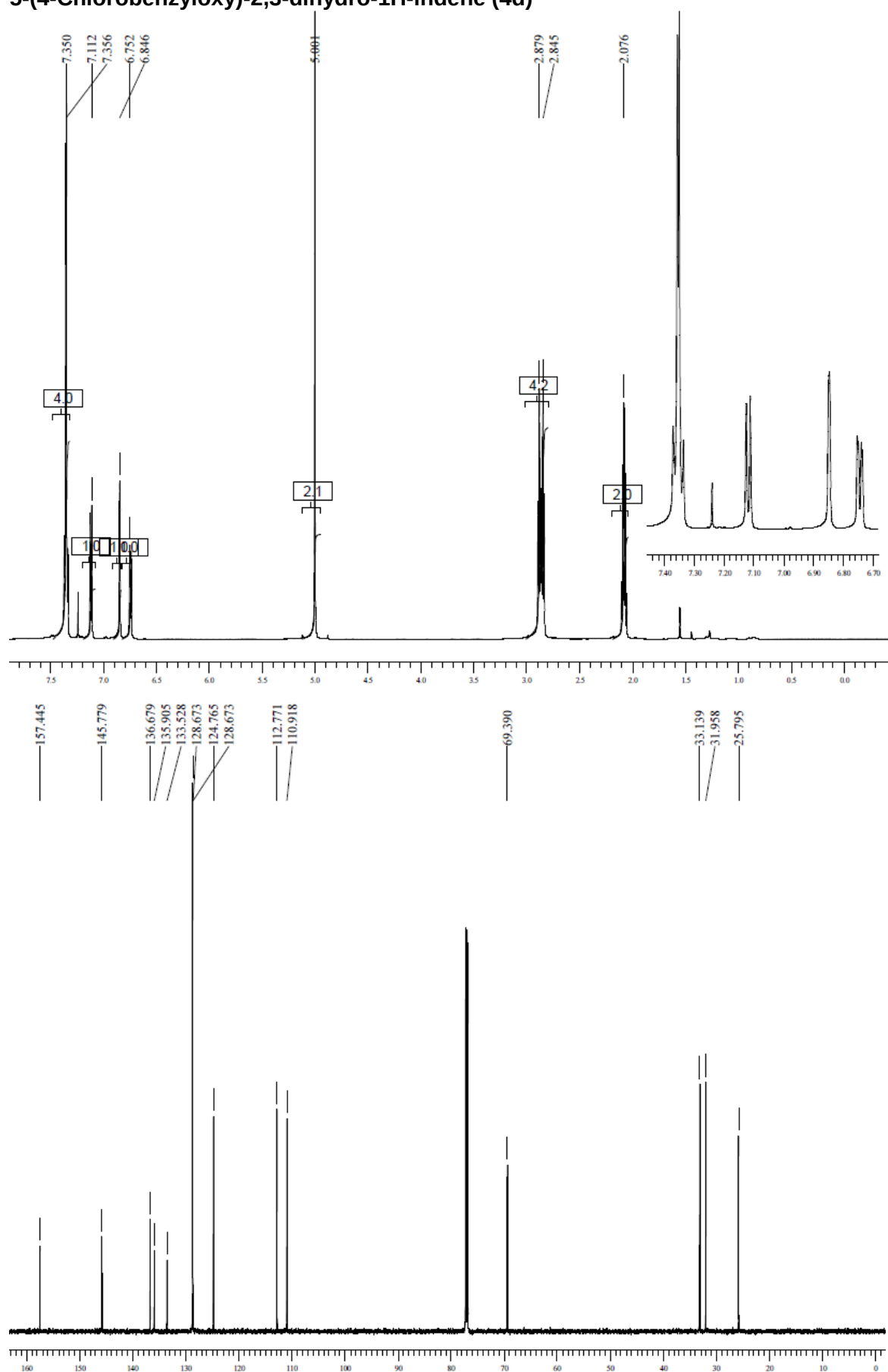
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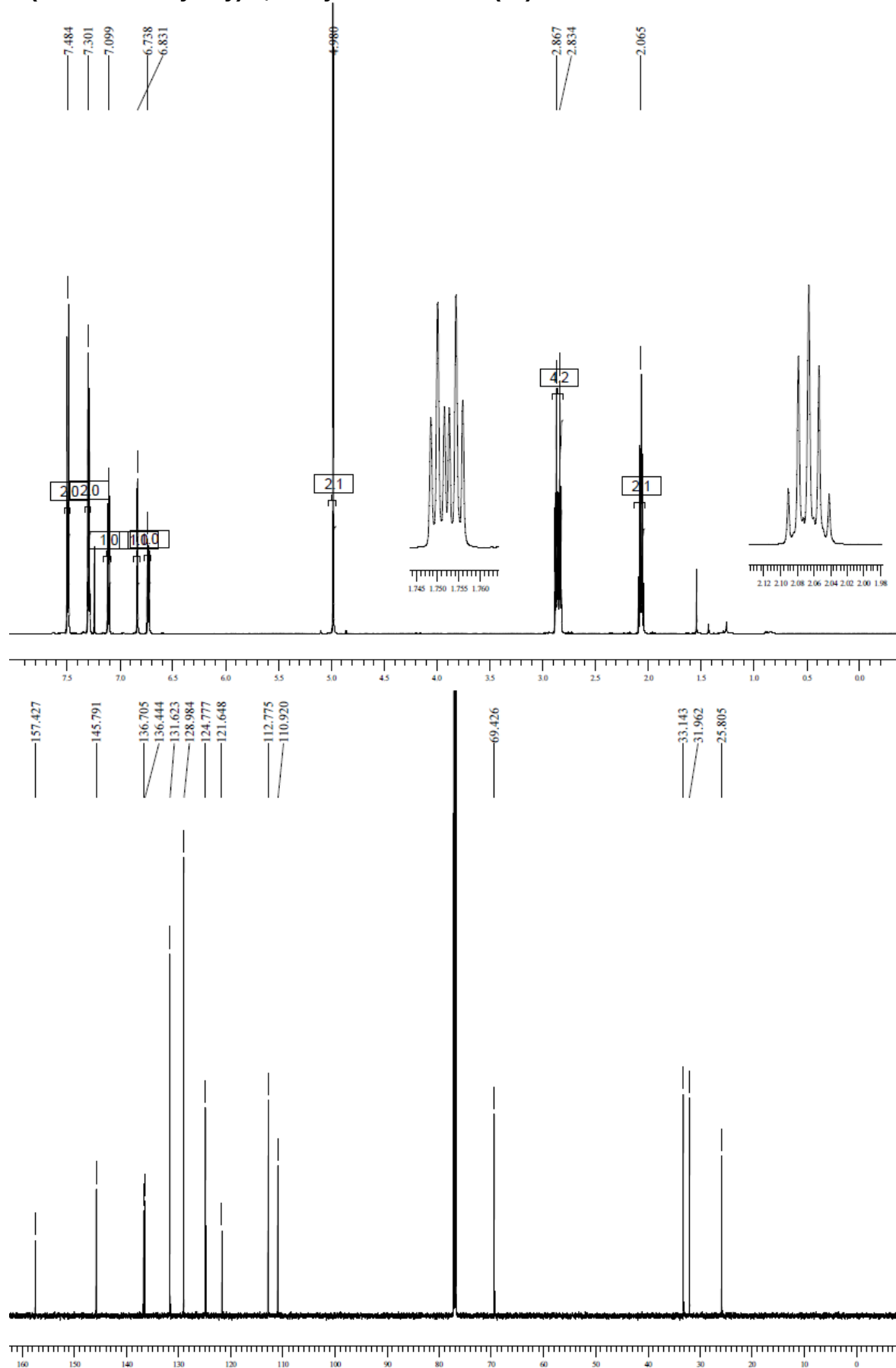
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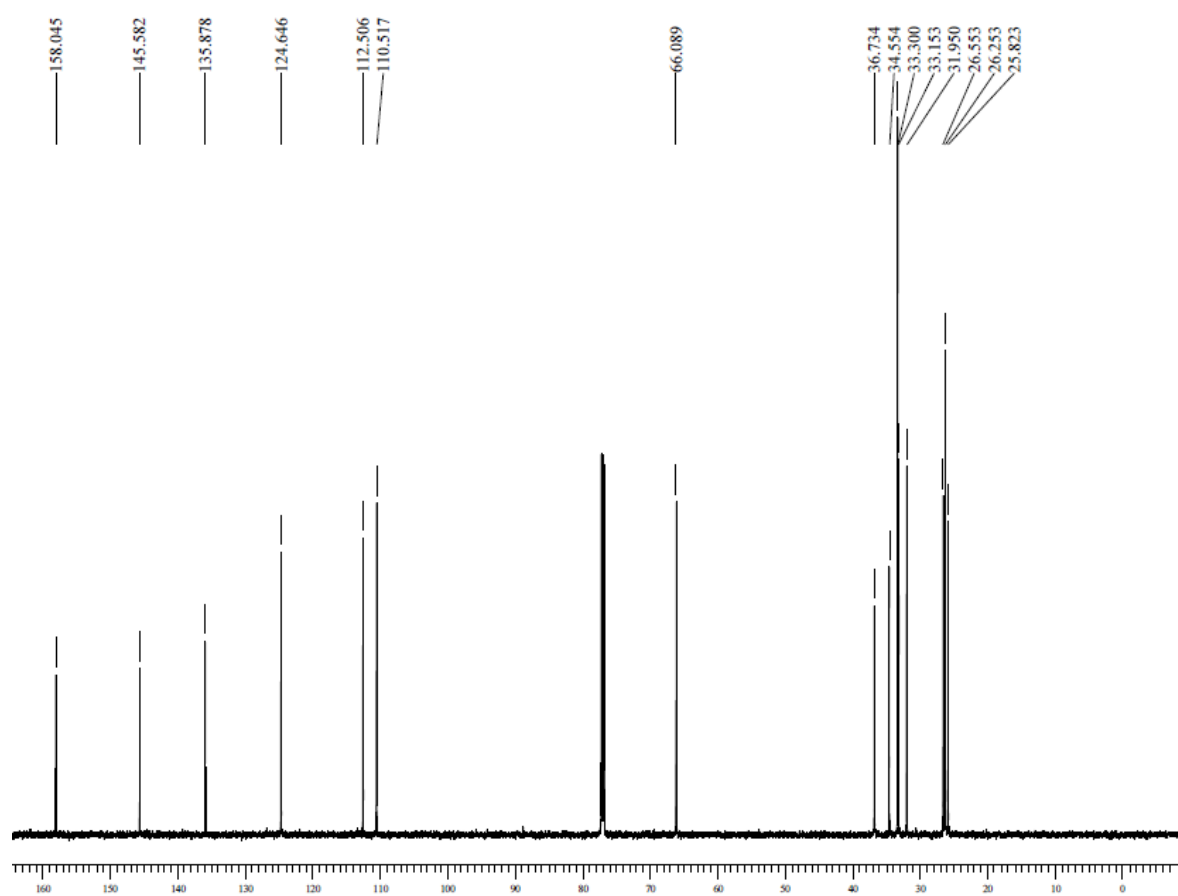
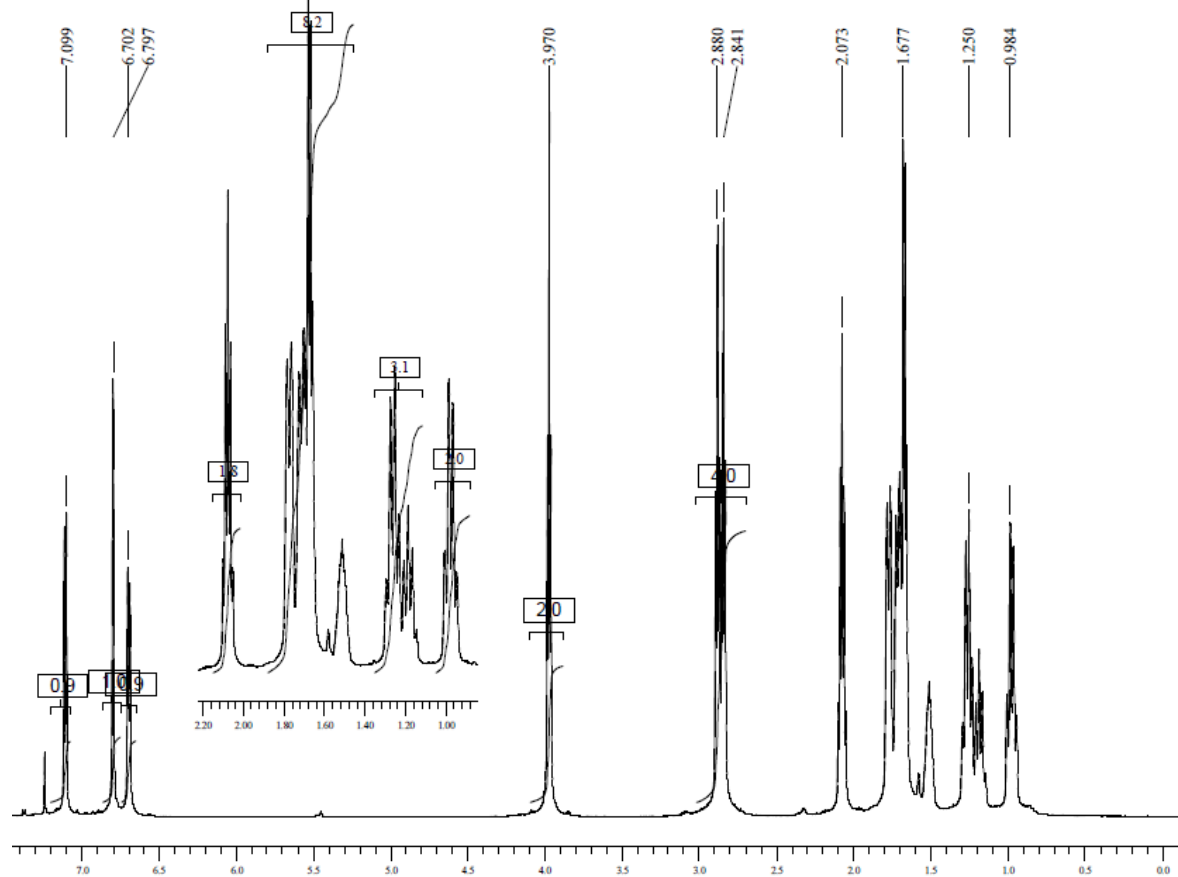
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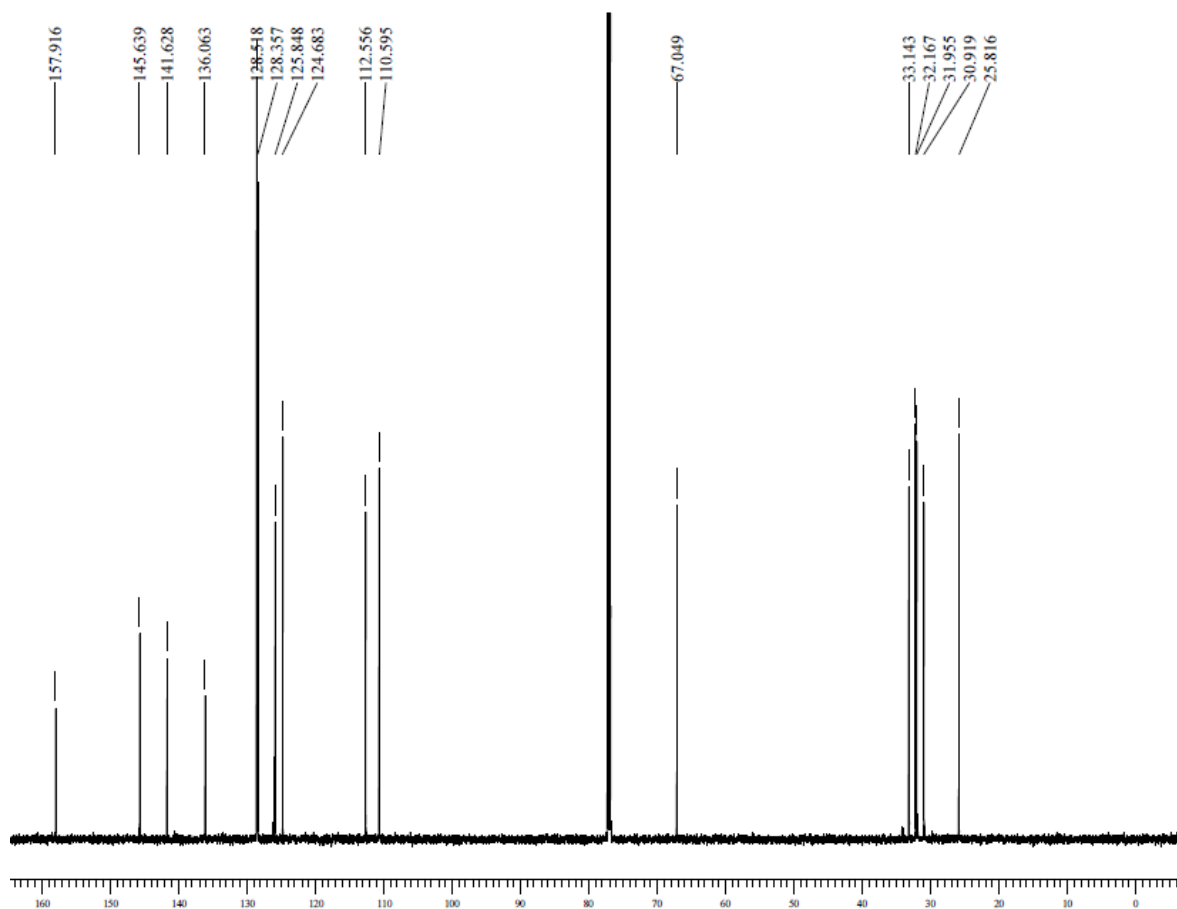
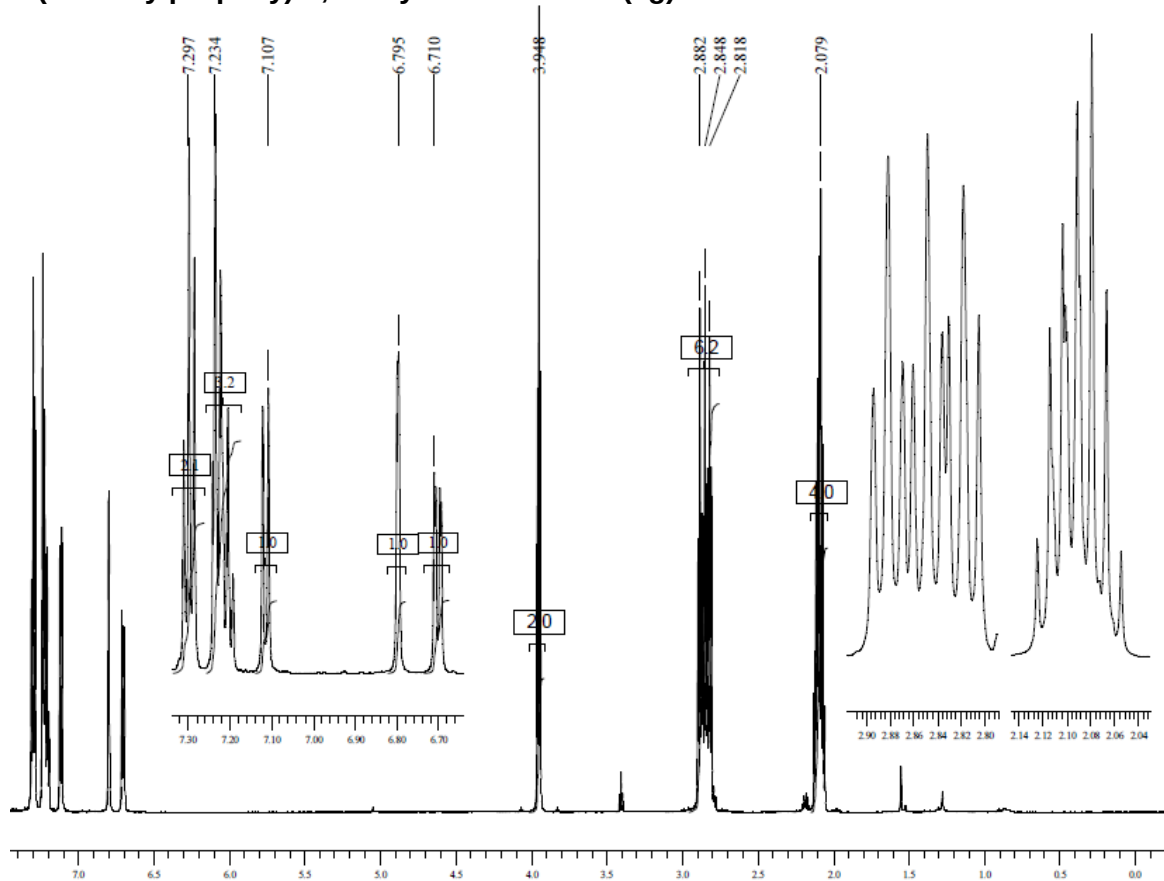
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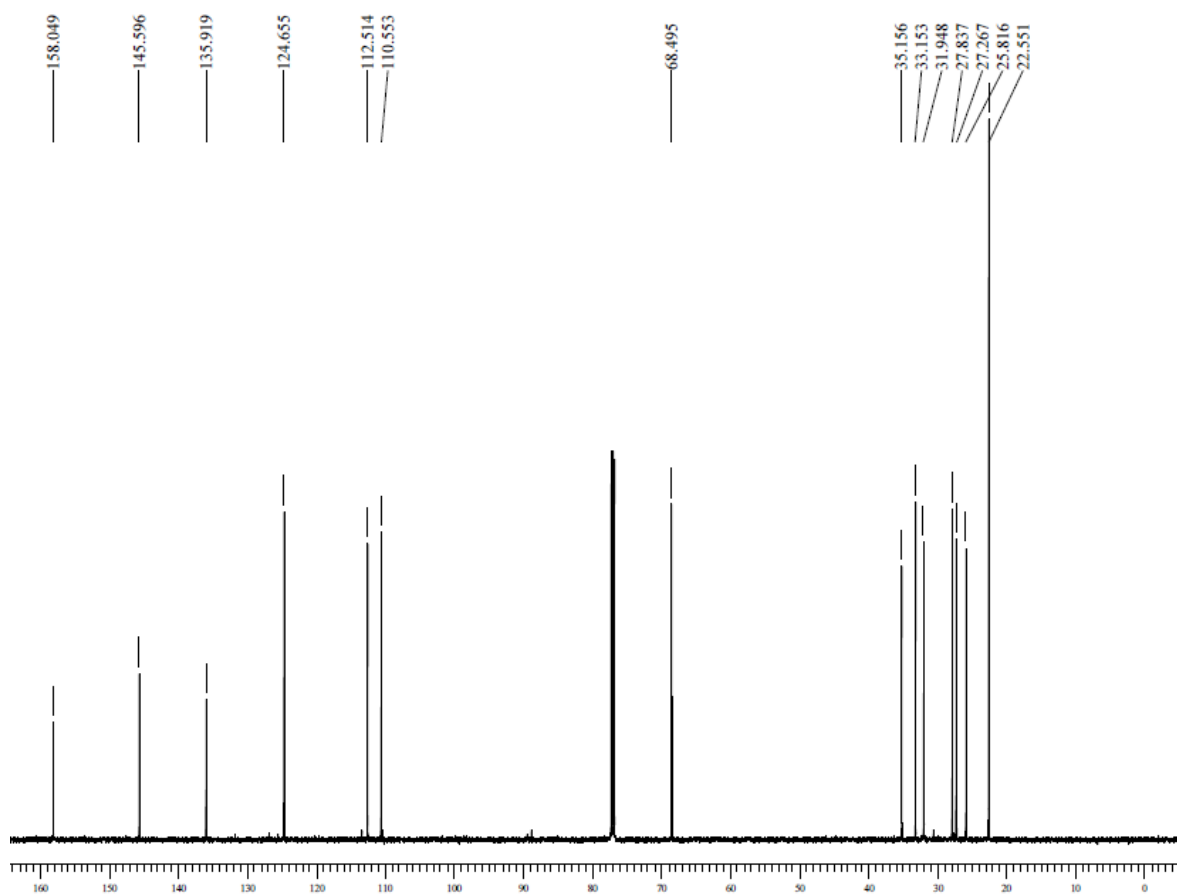
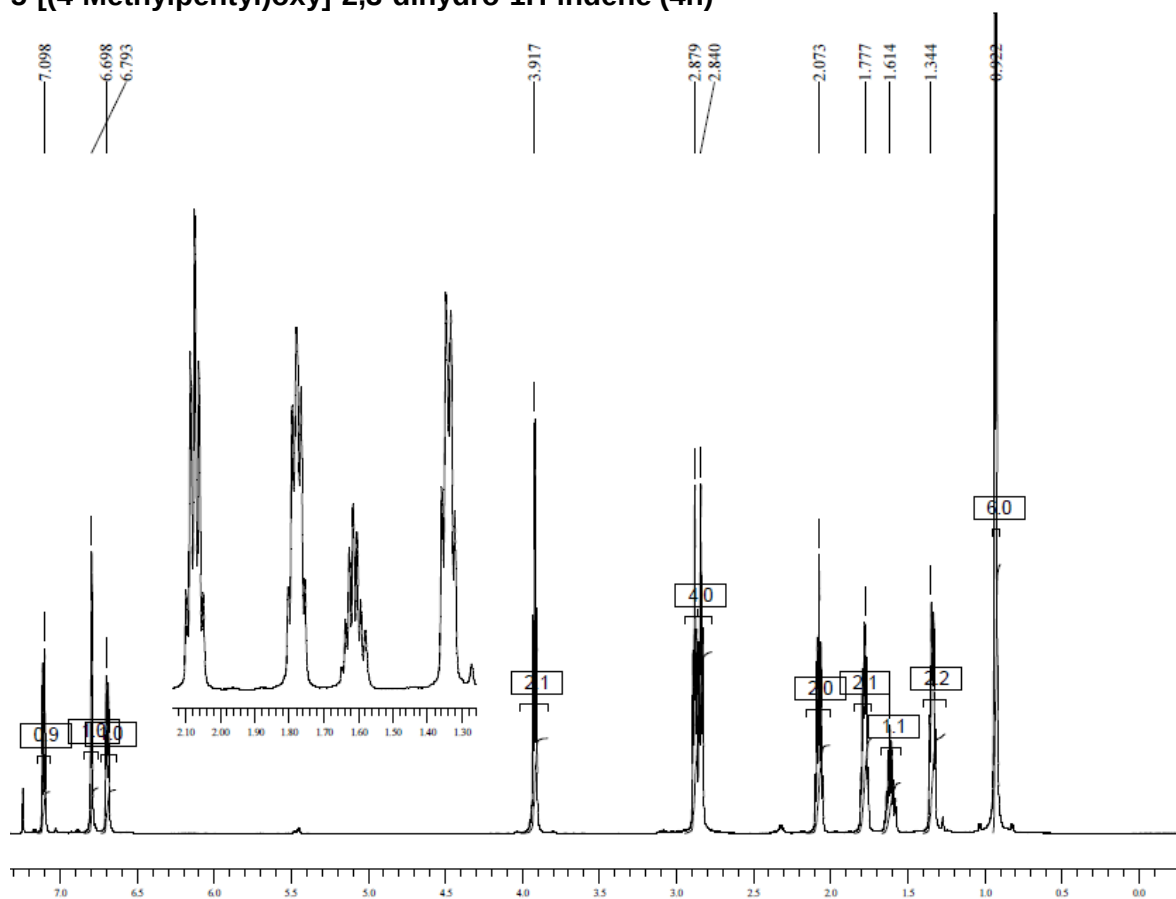
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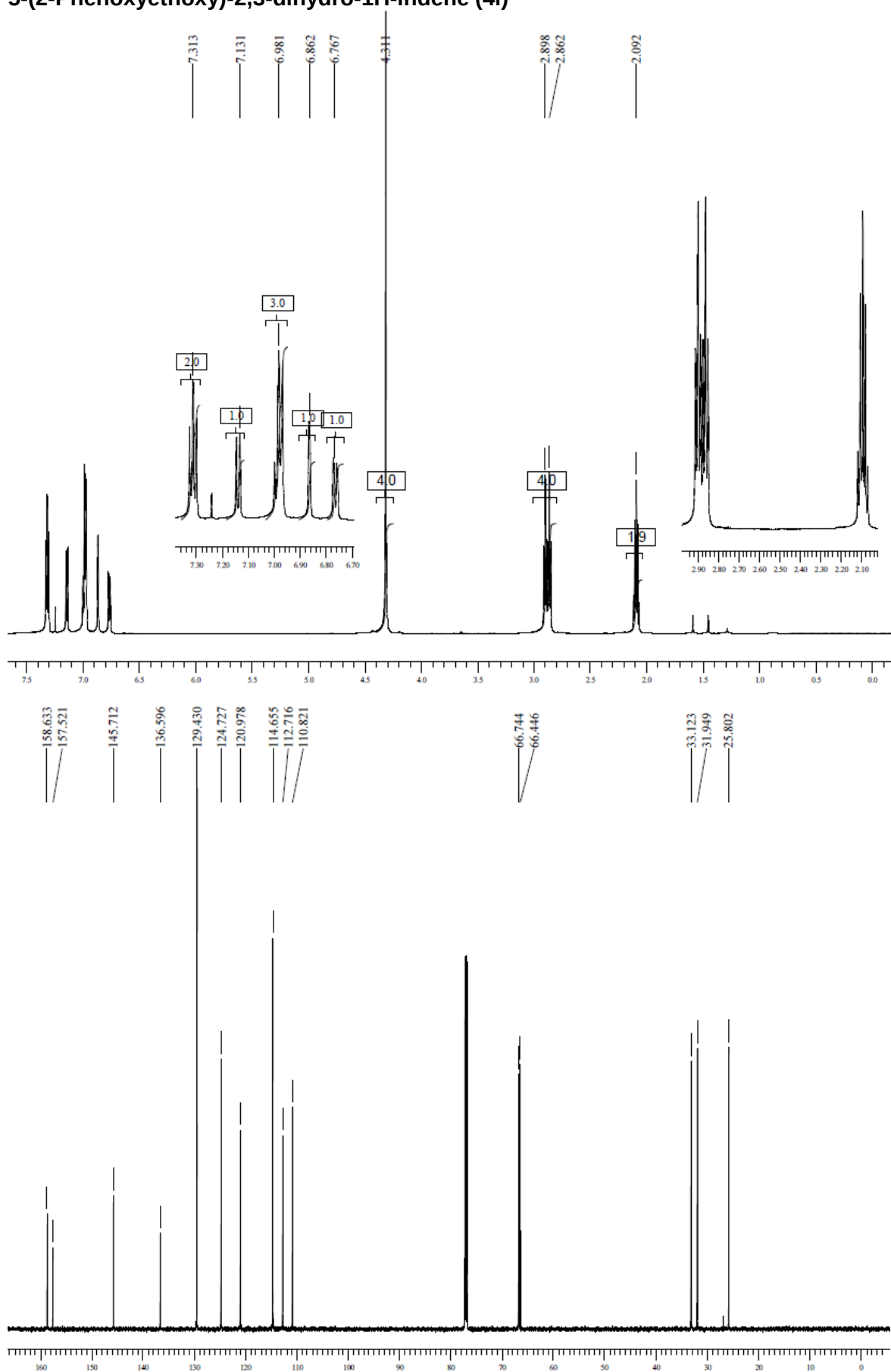
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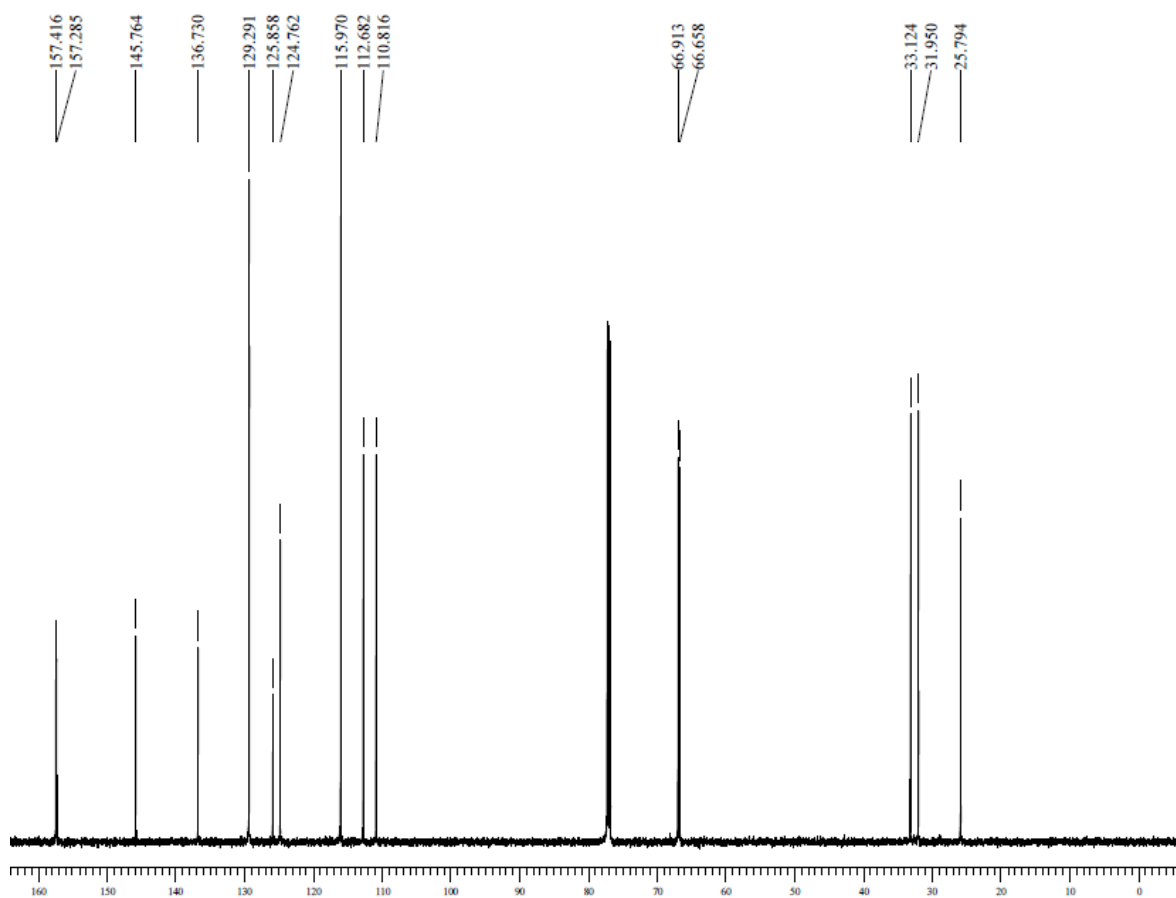
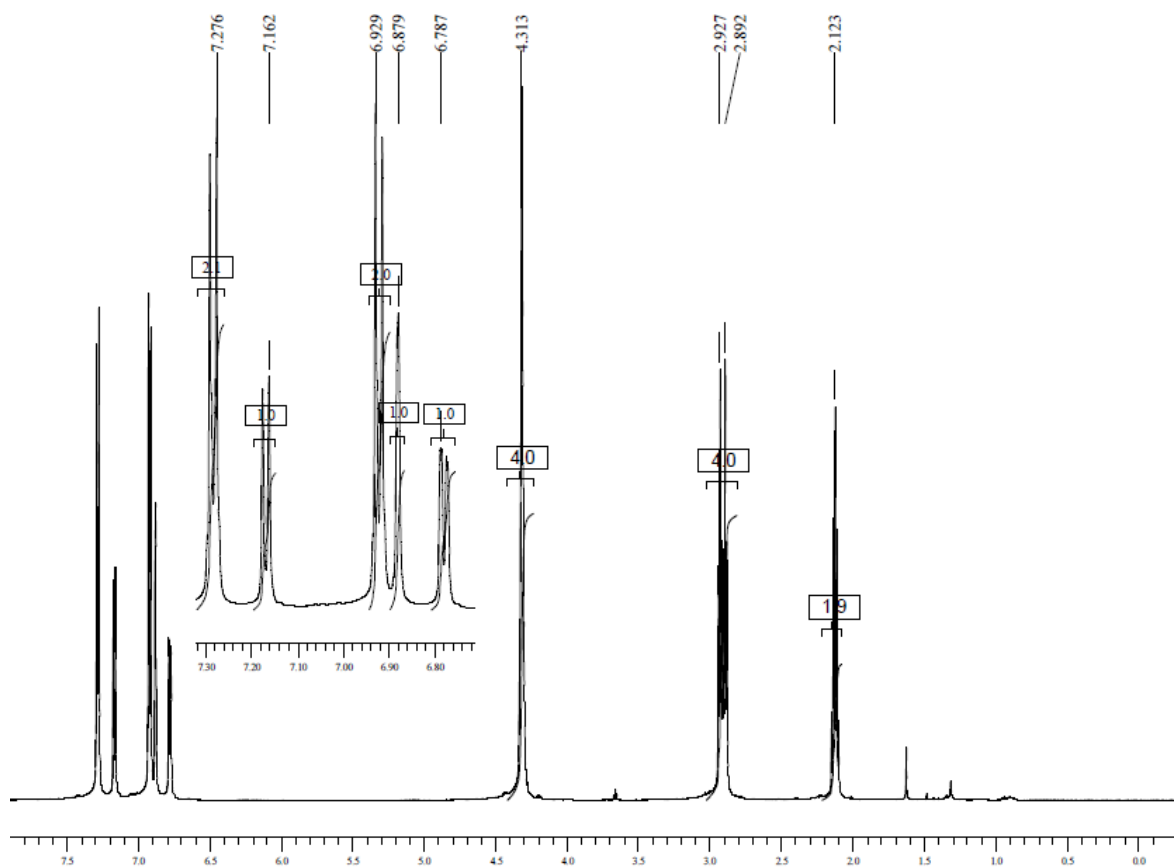
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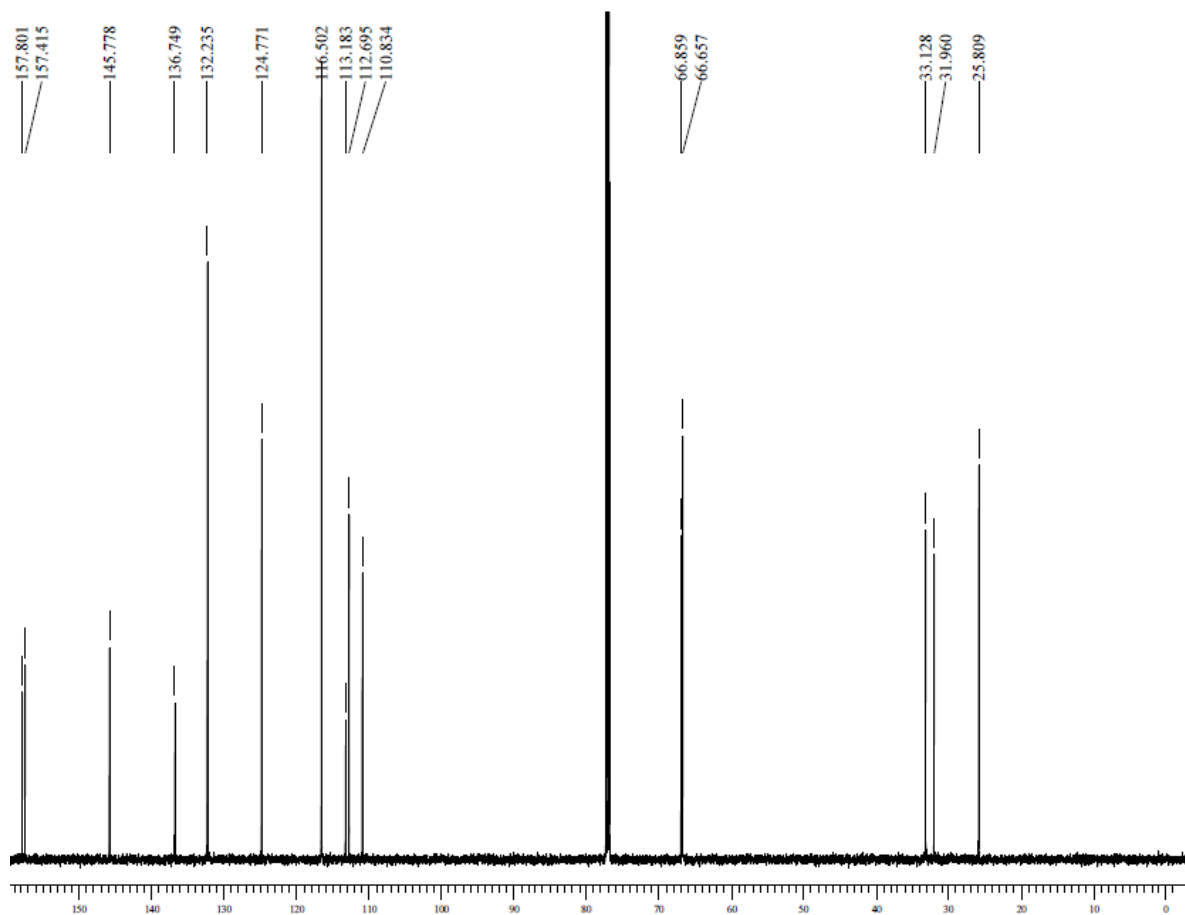
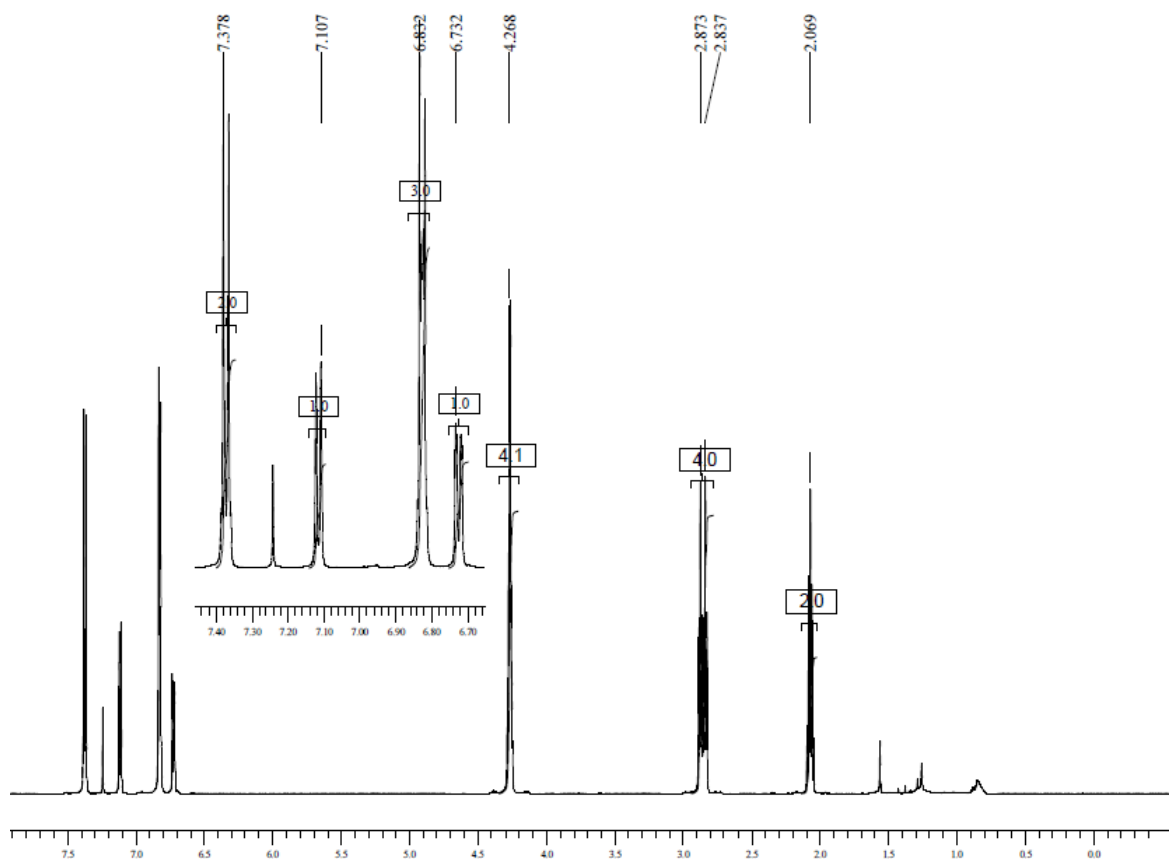
5-(2-Phenoxyethoxy)-2,3-dihydro-1H-indene (4i)



5-[2-(4-Chlorophenoxy)ethoxy]-2,3-dihydro-1H-indene (4j)



5-[2-(4-Bromophenoxy)ethoxy]-2,3-dihydro-1H-indene (4k)



Chapter 5

Manuscript B

Article awaiting submission to Chemical Biology and Drug Design entitled:

Inhibition of monoamine oxidase by benzoxathiolone analogues

No graphical abstract

The manuscript is written in a letter format and is presented according to the author guidelines as stipulated in addendum B.

Authors' contributions

- The experimental work, interpretation of results and documentation of this study was carried out by **S. Mostert**.
- This study was conceptualised and documented with the assistance of **J.P Petzer**.
- The enzymology section of this study was conducted under the supervision of **A. Petzer**.

All co-authors provided permission to use this manuscript as part of S Mostert's Ph.D thesis.

Inhibition of monoamine oxidase by benzoxathiolone analogues

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Running title: Benzoxathiolones as MAO inhibitors

Keywords: monoamine oxidase, MAO, inhibition, reversible, benzoxathiolone.

Abstract

Inhibitors of the monoamine oxidase (MAO) enzymes are considered useful therapeutic agents, and are used in the clinic for the treatment of depressive illness and Parkinson's disease. In addition, MAO inhibitors are also under investigation for the treatment of certain cardiovascular pathologies and as possible aids to smoking cessation. In an attempt to discover novel classes of compounds that inhibit the MAOs, the current study examines the human MAO inhibitory properties of a small series of 2*H*-1,3-benzoxathiol-2-one analogues. The results show that the benzoxathiolones are potent MAO-B inhibitors with IC₅₀ values ranging from 0.003 to 0.051 µM. Although the benzoxathiolones are selective for the MAO-B isoform, two compounds display good MAO-A inhibition with IC₅₀ values of 0.189 and 0.424 µM respectively. Dialysis studies show that a selected compound inhibits the MAOs reversibly. It may thus be concluded that the benzoxathiolone class is suitable for the design and development of MAO-B inhibitors, and that in some instances good MAO-A inhibition may also be achieved.

Letter

The monoamine oxidase (MAO) enzymes are responsible for the oxidation of a variety of amine substrates (1; 2). MAO consists of two isoforms, MAO-A and MAO-B, which share overall similar structures and possesses covalently attached flavin adenine dinucleotide (FAD) cofactors (3). Substrate oxidation occurs by two half reactions, the reductive half reaction where the FAD cofactor is reduced when accepting two electrons from the substrate amine, and an oxidative half reaction where the reduced FAD is reoxidised by molecular oxygen to yield hydrogen peroxide as by-product (4; 5; 6). In most instances, the product of amine oxidation, the corresponding imine, is hydrolysed to yield an aldehyde. Since the MAOs metabolise neurotransmitter amines, they have become targets for the treatment of neuropsychiatric and neurodegenerative disorders (3). Drugs that inhibit MAO-A are established antidepressants, and are thought to act by elevating central serotonin levels (7; 8). Examples of clinically used agents are phenelzine (**1**), isocarboxazid (**2**), tranylcypromine (**3**) and iproniazid (**4**) (Fig. 1). Drugs that inhibit the MAO-B isoform, in turn, are used in the treatment of Parkinson's disease, often in combination with L-Dopa (9). Since MAO-B metabolise dopamine in the brain, inhibitors are thought to enhance dopamine levels, particularly following therapy with L-Dopa (10; 11). Examples of clinically used agents are (R)-deprenyl (**5**) and rasagiline (**6**). MAO-B inhibitors are also thought to protect against neurodegeneration in Parkinson's disease. In this respect, central inhibition of MAO-B reduces the formation of hydrogen peroxide and aldehydes, species which may cause neuronal injury if not adequately cleared from the brain (1). This risk may be particularly important in the aged brain where MAO-B activity is significantly increased (12).

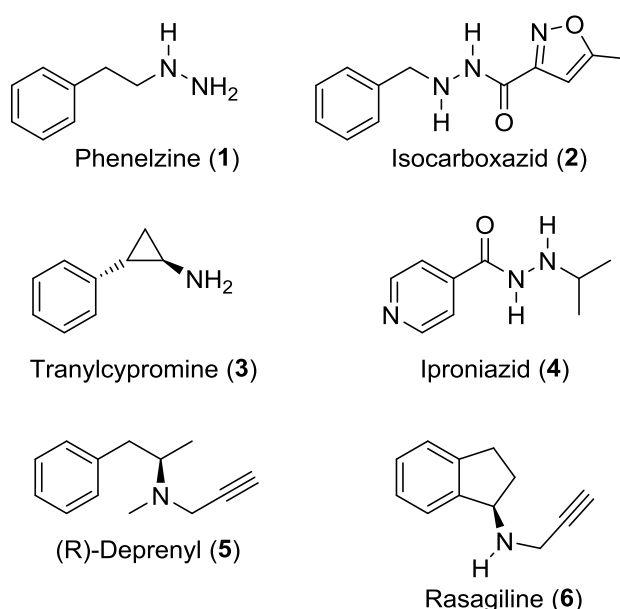


Figure 1: The structures of known MAO inhibitors.

The MAOs have also attracted attention as targets for the development of therapy for Alzheimer's disease. Laboratory evidence suggests that MAO inhibitors improve cognitive deficits and reverse amyloid β peptide (A β) pathology (13). In cardiovascular pathophysiology, MAO inhibitors may reduce the formation of hydrogen peroxide and thus improve cardiac function in patients with congestive heart failure (14). MAO-A appears to be the relevant isoform here since cardiac MAO-A activity and hydrogen peroxide generated by MAO-A show an age-dependent increase in the hearts of rats. MAO-A could be a major source of hydrogen peroxide in the aging heart, and thus a contributor to cardiac cellular degeneration (15). Another potential application of MAO inhibitors, particularly MAO-B inhibitors, is the possible treatment of smoking cessation. This is based on reports that MAO-B activity is reduced in smokers, and by increasing synaptic monoamines, MAO-B inhibitors may mimic the effects of smoking, at least in part (16). Interestingly, MAO-A levels are reported to be elevated in certain types of cancer tissue such as prostate cancer, and MAO-A inhibition may, in synergism with surviving suppressants, inhibit cancer cell growth, migration and invasion (17; 18).

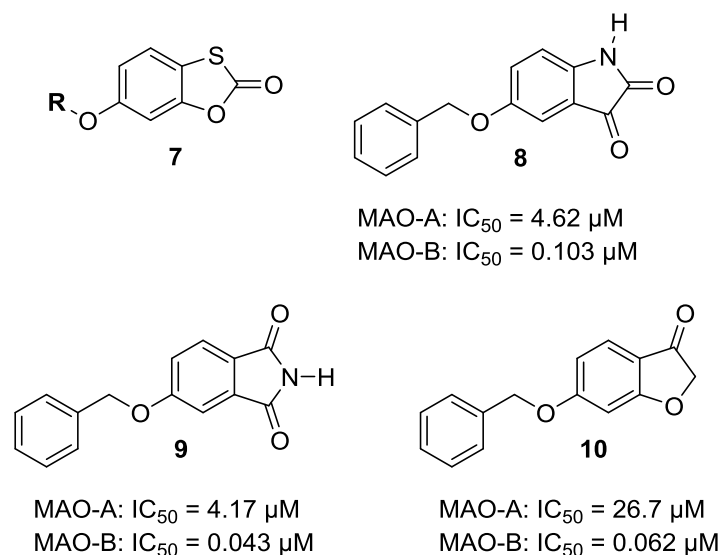
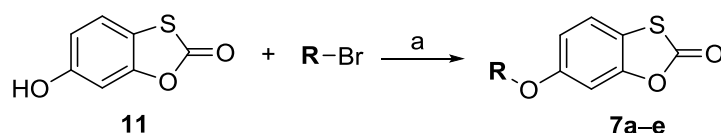


Figure 2: The structures of the 2H-1,3-benzoxathiol-2-one analogues (**7**) that will be investigated in this study as well as those of the lead compounds, 5-benzyloxyisatin (**8**), 5-benzyloxyphthalimide (**9**) and 3-coumaranone derivative **10** (19; 20; 21).

Based on the current therapeutic value and future potential of MAO inhibitors, the discovery of new classes of compounds that inhibit the MAOs are justified. In the present study we have investigated a small series of 2H-1,3-benzoxathiol-2-one analogues (**7**) as potential human MAO inhibitors (Fig. 2). These compounds are structurally related to a number of heterocycles that have been found in previous studies to be MAO inhibitors. For example, both 5-benzyloxyisatin (**8**) and 5-benzyloxyphthalimide (**9**) are potent and selective (over the MAO-A isoform) inhibitors of human MAO-B (19; 20). A recent study has reported that 3-coumaranone derivatives, such as compound **10**, also are potent MAO-B inhibitors (21). The benzyloxy side chains of these heterocycles appear

to be required for MAO inhibition since the removal thereof results in significant reduction or abolishment of MAO inhibition. The first benzoxathiolone analogue of the present study, **7a**, has thus been substituted on the C6 position with the benzyloxy moiety (Table 1). Since substitution on the benzyloxy ring frequently enhances MAO inhibition we have included the chlorine and methyl substituted homologues, **7b–d**. The effect of side chain elongation was investigated with phenylpropoxy substitution (**7e**). Although the series is limited, the objective was to determine if the benzoxathiolone class of compounds may possess MAO inhibition activity. This study will also investigate the reversibility of MAO inhibition by selected benzoxathiolones. For MAO-A inhibition, reversibility is an important consideration since irreversible acting MAO-A inhibitors are associated with a potentially fatal increase in blood pressure when taken with certain foods and can only be used in the clinic if dietary restrictions are followed (22). Reversible MAO-A inhibitors, on the other hand, appear to be safe in this regard (23; 24).



Scheme 1: Synthetic route to the 2H-1,3-benzoxathiol-2-one analogues, **7a–e**. Reagents and conditions: (a) Acetone, K₂CO₃, 60 °C, 5–24 h.

The benzoxathiolone analogues were synthesized in poor to fair yields (4–53%) by reacting commercially available 6-hydroxy-1,3-benzoxathiol-2-one (**11**) with an appropriate substituted arylalkyl bromide in acetone (Scheme 1). Potassium carbonate served as base. The reactions were carried out at 60 °C for 5–24 h and monitored with thin-layer chromatography (TLC). After completion, ethyl acetate was added to the reaction and the resulting mixture was washed with water and brine. The crude obtained after evaporation of the organic phase was purified by recrystallization. The structures and purities of the target compounds were verified by ¹H NMR, ¹³C NMR, mass spectrometry and HPLC analysis as cited in the supplementary data.

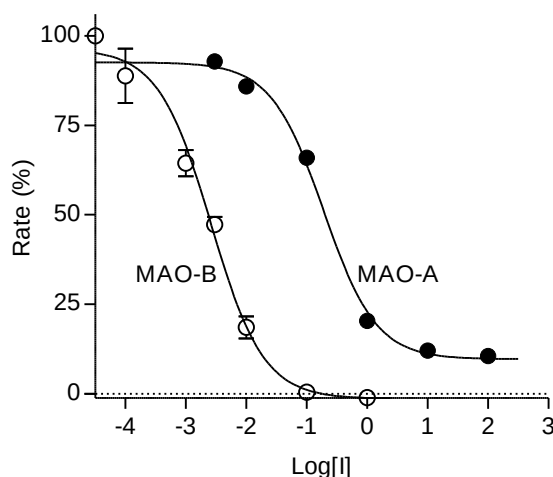
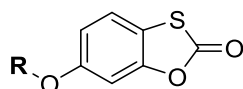


Figure 3: The sigmoidal plots for the inhibition of MAO-A (filled circles) and MAO-B (open circles) by **7c**.

The MAO inhibitory properties of the benzoxathiolone analogues **7a–e** were evaluated using the recombinant human MAO-A and MAO-B enzymes. Inhibition potencies are expressed as the IC_{50} values, which were determined from sigmoidal plots of residual enzyme activity versus the logarithm of inhibitor concentration. Residual enzyme activity, in turn, was measured by using kynuramine as substrate for both MAO isoforms. Kynuramine is oxidised by the MAOs to yield 4-hydroxyquinoline, which was quantified by fluorescence spectrophotometry (25; 26). Fig. 3 provides examples of sigmoidal plots obtained for the inhibition of the MAOs by compound **7c**.

The inhibition potencies of the benzoxathiolone analogues are given in Table 1. From the results it is evident that these compounds are selective for MAO-B over the MAO-A isoform. In this respect selectivity index (SI) values range from 63 to 645. All compounds may be viewed as potent MAO-B inhibitors with IC_{50} values $<0.051 \mu\text{M}$. For comparison, the known MAO-B inhibitor, lazabemide (**12**; $IC_{50} = 0.091 \mu\text{M}$), is a weaker inhibitor than the benzoxathiolones examined here, while the reversible MAO-B inhibitor safinamide (**13**; $IC_{50} = 0.048 \mu\text{M}$) is approximately equipotent to **7a** (Fig. 4) (27). Although weaker MAO-A inhibitors, two compounds display good MAO-A inhibition. These are **7b** and **7c** with IC_{50} values of $0.424 \mu\text{M}$ and $0.189 \mu\text{M}$, respectively. For comparison, the clinically used reversible MAO-A inhibitor, toloxatone (**14**), inhibits MAO-A with an IC_{50} value of $3.92 \mu\text{M}$ (27). These compounds as well as **7d** ($IC_{50} = 2.55 \mu\text{M}$) are thus more potent MAO-A inhibitors than toloxatone. This study also finds that 6-hydroxy-1,3-benzoxathiol-2-one (**11**) is a weak MAO-B inhibitor with an IC_{50} of $9.57 \mu\text{M}$. This shows that the C6 substituent is indeed a requirement for potent MAO-B inhibition by the benzoxathiolone analogues, and is in agreement with similar findings with other heterocycles, such as isatins (e.g. **8**) and phthalimides (e.g. **9**), for which an appropriate side chain also is a requirement for MAO-B inhibition (19; 20). Based on the inhibition potencies it may thus be concluded that, similar to the isatins and phthalimides reported previously, benzoxathiolones represent a class of highly potent MAO-B inhibitors.

Table 1: The IC₅₀ values for the inhibition of recombinant human MAO-A and MAO-B by 2H-1,3-benzoxathiol-2-one analogues, **7a–e**.



	R	IC ₅₀ (μM) ^a		SI ^b
		MAO-A	MAO-B	
7a	C ₆ H ₅ CH ₂ –	5.14 ± 0.928	0.051 ± 0.011	101
7b	3-ClC ₆ H ₄ CH ₂ –	0.424 ± 0.092	0.004 ± 0.0003	106
7c	4-ClC ₆ H ₄ CH ₂ –	0.189 ± 0.012	0.003 ± 0.001	63
7d	4-CH ₃ C ₆ H ₄ CH ₂ –	2.55 ± 0.258	0.005 ± 0.001	510
7e	C ₆ H ₅ (CH ₂) ₃ –	21.3 ± 0.826	0.033 ± 0.006	645
11	H–	9.77 ± 0.480	9.57 ± 1.07	1

^a All values are expressed as the mean ± standard deviation (SD) of triplicate determinations.

^b The selectivity index is the selectivity for the MAO-B isoform and is given as the ratio of IC₅₀(MAO-A)/IC₅₀(MAO-B).

When comparing **7a** with the corresponding isatin and phthalimide homologues, it is evident that **7a** is a more potent MAO-B inhibitor than 5-benzyloxyisatin (**8**; IC₅₀ = 0.103 μM) and equipotent to 5-benzyloxyphthalimide (**9**; IC₅₀ = 0.043 μM) (19; 20). The results also show that with the appropriate substitution good MAO-A inhibition may be obtained with the benzoxathiolones. Good MAO-A inhibitors also exist among the reported isatins and phthalimides as exemplified by **15** (IC₅₀ = 0.233 μM) and **16** (IC₅₀ = 0.22 μM) (Fig. 5) (19; 20).

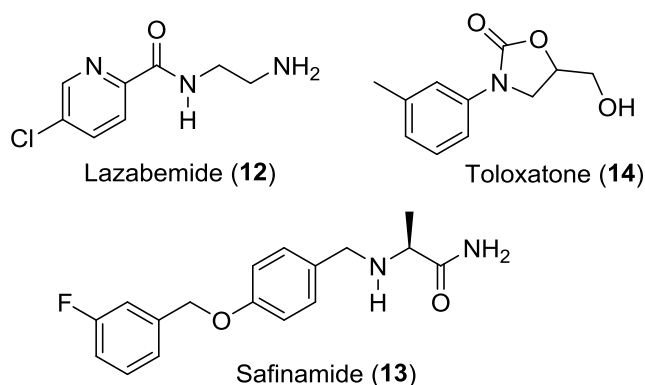


Figure 4: The structures of reference MAO inhibitors.

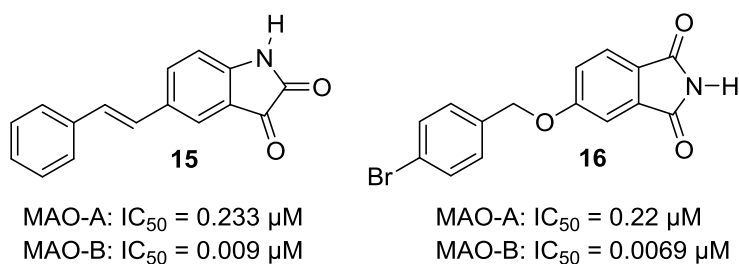


Figure 5: The structures of isatin (**15**) and phthalimide (**16**), both reported MAO inhibitors (19; 20).

As mentioned, reversibility of MAO inhibition is an important consideration in inhibitor design. This study thus investigates the reversibility of MAO-A and MAO-B inhibition by a selected benzoxathiolone analogue, compound **7c**. To examine the reversibility of inhibition, the MAO enzymes and test inhibitor (at a concentration of $4 \times IC_{50}$) were combined for 15 min and subsequently dialysed for 24 h. As negative control, similar dialysis of the MAOs in absence of inhibitor was carried out. As positive controls, the MAOs were combined with the irreversible inhibitors, pargyline (MAO-A) and (R)-deprenyl (MAO-B), and dialysed. For reversible inhibitors, enzyme activity is expected to recover to 100% of the negative control value following dialysis. For irreversible inhibition, enzyme activity is not recovered by dialysis. The results are given in Fig. 6, and show that, upon inhibition by **7c**, both MAO-A and MAO-B activities are recovered by dialysis to 93% and 78%, respectively. The MAO activities in undialysed mixtures of the MAOs and **7c**, in contrast are 73% and 39%, respectively. This result shows that **7c** is a reversible inhibitor of both MAO isoforms. As expected, after dialysis of mixtures of the MAOs and the irreversible inhibitors, pargyline and (R)-deprenyl, enzyme activity is not recovered with MAO-A and MAO-B at 0.9% and 4.8%, respectively, of the negative control.

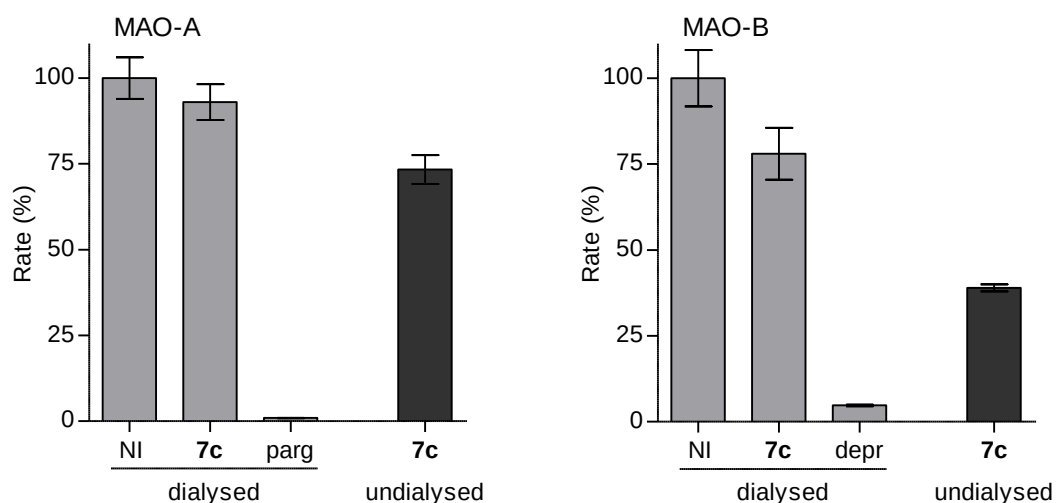


Figure 6: Dialysis reverses MAO-A and MAO-B inhibition by **7c**. The MAO enzymes and **7c** (at a concentration of $4 \times IC_{50}$) were combined for 15 min, dialysed for 24 h and the residual enzyme activity was measured (**7c** dialysed). Similar incubation and dialysis of the MAOs in the absence (NI dialysed) and presence of the irreversible inhibitors, pargyline (parg dialysed) and (R)-deprenyl (depr dialysed) were also carried out. The residual MAO activity of undialysed mixtures of the MAOs with **7c** was also recorded (**7c** undialysed).

To investigate the mode (e.g. competitive) of MAO-A and MAO-B inhibition by **7c**, Lineweaver-Burk plots were constructed. For each MAO isoform a set consisting of six Lineweaver-Burk plots was constructed by measuring the enzyme activities in the presence of the following inhibitor concentrations: $0 \mu M$, $\frac{1}{4} \times IC_{50}$, $\frac{1}{2} \times IC_{50}$, $\frac{3}{4} \times IC_{50}$, $1 \times IC_{50}$ and $1\frac{1}{4} \times IC_{50}$. For each plot, eight different substrate (kynuramine) concentrations were used, ranging from 15–250 μM . The results are given in Fig. 7 and show that, for both MAO-A and MAO-B inhibition, the lines are linear and intersect on the y-axis. This is in agreement with a competitive mode of inhibition of both MAO-A and MAO-B by **7c**. By plotting the slopes of the Lineweaver-Burk plots versus the inhibitor concentration, K_i values of 0.36 μM and 0.0035 μM for the inhibition of MAO-A and MAO-B, respectively, are estimated. The K_i values can also be determined by global (shared) fitting of the inhibition data directly to the Michaelis-Menten equation. This yielded similar results with K_i values of $0.56 \pm 0.07 \mu M$ ($R^2 = 0.99$) and $0.0039 \pm 0.0005 \mu M$ ($R^2 = 0.98$) μM for the inhibition of MAO-A and MAO-B, respectively.

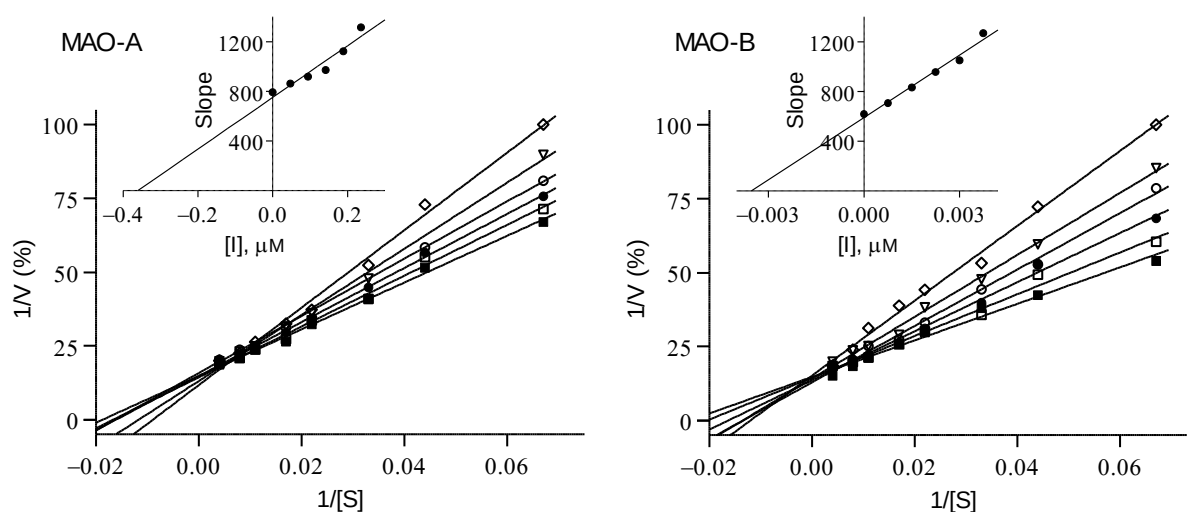


Figure 7: Lineweaver-Burk plots for the inhibition of human MAO-A and MAO-B by **7c**. The insets are plots of the slopes of the Lineweaver-Burk plots versus inhibitor concentration.

Since benzoxathiolone analogues show good promise as MAO inhibitors, selected properties were measured for the series as part of a preliminary investigation of the suitability of these compounds as drugs. For this purpose, the lipophilicity (logP) and aqueous solubility of the compounds were experimentally determined and are listed in Table 2. LogP was determined with the shake-flask method and represents the partitioning of the test compounds between n-octanol and water. The logP value, in general, provides an estimation of how readily a compound may diffuse passively across the lipid bilayer, an important process for absorption from the gastrointestinal tract and permeation across the blood-brain barrier. It is generally accepted that, for a compound to display good oral bioavailability and blood-brain barrier permeation, the logP value should be in a range of 0–3 (28). The logP values of the benzoxathiolone analogues are 3.80–5.61, indicating that a high degree of lipophilicity may be a concern, particularly from a solubility point of view. Indeed, the aqueous solubilities are $<0.081 \mu\text{M}$ and may be considered to be in the very weak solubility range. To improve the properties of the benzoxathiolone analogues, this study recommends that in future studies polar, and possibly ionisable groups should be incorporated in the C6 side chain to reduce lipophilicity and improve solubility. This would yield compounds with improved properties. The effect of polar groups on MAO inhibition, however, remains to be determined.

Table 2: The logP values and aqueous solubilities of 2*H*-1,3-benzoxathiol-2-one analogues, **7a–e**.

	LogP	Solubility (μM)
7a	3.80 ± 0.03	0.064 ± 0.066
7b	4.72 ± 0.12	0.081 ± 0.015
7c	5.61 ± 0.24	0.072 ± 0.013
7d	4.70 ± 0.17	0.013 ± 0.006
7e	4.54 ± 0.25	0.051 ± 0.005

All values are expressed as the mean ± SD of triplicate determinations.

In conclusion, the present study shows that a small series of 2*H*-1,3-benzoxathiol-2-one analogues are potent human MAO-B inhibitors, and may thus act as leads for the future design of therapies for disorders such as Parkinson's disease. The physicochemical properties of these compounds, however, are a concern and the high degree of lipophilicity and low aqueous solubilities require optimisation. Two of the 2*H*-1,3-benzoxathiol-2-one analogues also are relatively potent human MAO-A inhibitors, and may thus be suitable as leads for drugs aimed at the treatment of depression, certain cardiovascular pathologies and certain types of cancer. In this regard, the observation that a selected analogue is a reversible MAO-A inhibitor is of significance. Reversible MAO-A inhibitors are not likely to cause tyramine-induced changes in blood pressure and have better safety profiles than irreversible MAO-A inhibitors (23; 24). Compounds with dual MAO-A/B inhibition properties may be particularly relevant where depression is a comorbidity of Parkinson's disease.

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Conflict of Interest

The authors have no conflicts of interest to declare

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Supplementary material

Inhibition of monoamine oxidase by benzoxathiolone analogues

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1. Chemicals and instrumentation

Reagents: Unless otherwise stated, all reagents [including 6-hydroxy-1,3-benzoxathiol-2-one (**11**)] and solvents were obtained from Sigma-Aldrich and were used without further purification. Kynuramine.2HBr and insect cell microsomes containing recombinant human MAO-A and MAO-B (5 mg protein/mL) were obtained from Sigma-Aldrich.

NMR spectroscopy: NMR (¹H and ¹³C NMR) spectra were recorded with a Bruker Avance III 600 spectrometer in CDCl₃. The chemical shifts are reported in parts per million (δ) and were referenced to the residual solvent signal at 7.26 ppm (¹H NMR) and 77.0 (¹³C NMR) ppm. Spin multiplicities are abbreviated as follow: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), qn (quintet) or m (multiplet).

Mass spectrometry: High resolution mass spectra (HRMS) were recorded with a Bruker micrOTOF-Q II mass spectrometer functioning in atmospheric-pressure chemical ionisation (APCI) mode (positive mode).

Melting points (mp): Melting points were measured with a Buchi M-545 melting point apparatus and are uncorrected.

Thin-layer chromatography (TLC): TLC was conducted with silica gel 60 aluminium coated TLC sheets (Merck) and developed in a mobile phase consisting of ethyl acetate:petroleum ether (30:70). The developed TLC sheets were visualised under an UV-lamp at a wavelength of 254 nm.

Purity determination: To determine compound purity, HPLC analyses were carried out with an Agilent 1200 HPLC system, equipped with a quaternary pump and Agilent 1200 series diode array detector. HPLC grade acetonitrile (Merck) and Milli-Q water were used for the chromatography. A Venusil XBP C18 column (4.60 x 150 mm, 5 μm) was used with 30% acetonitrile and 70% Milli-Q water, as the initial mobile phase (flow rate of 1 mL/min). At the start of each HPLC run, a solvent gradient program was initiated by increasing the acetonitrile composition in the mobile phase linearly to 85% over a period of 5 min. Each HPLC run lasted 15 min with 5 min equilibration

between runs. The analytes were dissolved in acetonitrile (0.1 mM) and 20 μ L was injected into the HPLC system. The eluent was monitored at a wavelength of 254 nm.

Spectrophotometry: Fluorescence measurements were carried out with a Varian Cary Eclipse fluorescence spectrophotometer, while UV-Vis measurements were carried out with a Shimadzu MultiSpec-1501 UV-Vis photodiode array spectrophotometer.

2. Synthesis of 2*H*-1,3-benzoxathiol-2-one analogues, 7a–e

The 2*H*-1,3-benzoxathiol-2-one analogues were synthesised according to a literature procedure (1). Commercially available 6-hydroxy-1,3-benzoxathiol-2-one (**11**; 5 mmol) was dissolved in acetone (10 mL). To the reaction was added the appropriate substituted arylalkyl bromide (5 mmol) and anhydrous K_2CO_3 (12 mmol). The mixture was stirred at 60 °C for 5–24 h and monitored with thin-layer chromatography (TLC) until completion. The reaction was filtered to remove the K_2CO_3 and ethyl acetate (30 mL) was added to the reaction. The resulting solution was washed with deionised H_2O (30 mL) and brine (3 $\times$ 30 mL), and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the resulting residue was purified by recrystallisation from ethanol (40 mL).

6-Benzylloxy-1,3-benzoxathiol-2-one (7a):

The title compound was prepared in a yield of 53.4%: mp 90 °C (ethanol). ^1H NMR (600 MHz, $CDCl_3$) δ 5.06 (s, 2H), 6.89 (dd, J = 2.3, 8.7 Hz, 1H), 6.93 (d, J = 2.3 Hz, 1H), 7.24 (d, J = 8.7 Hz, 1H), 7.32–7.35 (m, 1H), 7.38–7.42 (m, 4H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 70.6, 99.6, 112.8, 113.8, 122.6, 127.4, 128.3, 128.7, 136.0, 148.8, 158.7, 169.5; APCI-HRMS m/z : calcd for $C_{14}H_{11}O_3S$ (MH^+), 259.0423, found 259.0437; purity (HPLC): 97%.

6-(3-Chlorobenzylloxy)-1,3-benzoxathiol-2-one (7b)

The title compound was prepared in a yield of 9.5%: mp 117.5 °C (ethanol). ^1H NMR (600 MHz, $CDCl_3$) δ 5.01 (s, 2H), 6.85 (dd, J = 2.3, 8.7 Hz, 1H), 6.88 (d, J = 2.6 Hz, 1H), 7.23 (d, J = 8.7 Hz, 1H), 7.24–7.26 (m, 1H), 7.27–7.29 (m, 2H), 7.39 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 69.7, 99.5, 112.7, 114.2, 122.7, 125.3, 127.4, 128.4, 130.0, 134.6, 138.0, 148.8, 158.4, 169.4; APCI-HRMS m/z : calcd for $C_{14}H_{10}ClO_3S$ (MH^+), 293.0034, found 293.0018; purity (HPLC): 87%.

6-(4-Chlorobenzylloxy)-1,3-benzoxathiol-2-one (7c)

The title compound was prepared in a yield of 3.5%: mp 114.1 °C (ethanol). ^1H NMR (600 MHz, $CDCl_3$) δ 5.02 (s, 2H), 6.86 (dd, J = 2.3, 8.7 Hz, 1H), 6.90 (d, J = 2.6 Hz, 1H), 7.25 (d, J = 8.3 Hz, 1H), 7.32–7.36 (m, 4H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 69.8, 99.5, 112.7, 114.1, 122.7, 128.8, 128.9, 134.1, 134.5, 148.8, 158.4, 169.4; APCI-HRMS m/z : calcd for $C_{14}H_{10}ClO_3S$ (MH^+), 293.0034, found 293.0026; purity (HPLC): 83%.

6-(4-Methylbenzyloxy)-1,3-benzoxathiol-2-one (7d)

The title compound was prepared in a yield of 3.7%: mp 77.3 °C (ethanol). ¹H NMR (600 MHz, CDCl₃) δ 2.35 (s, 3H), 5.01 (s, 2H), 6.88 (dd, J = 2.6, 8.7 Hz, 1H), 6.91 (d, J = 2.3 Hz, 1H), 7.19 (d, J = 7.9 Hz, 2H), 7.23 (d, J = 8.7 Hz, 1H), 7.29 (d, J = 7.9 Hz, 2H); ¹³C NMR (151 MHz, CDCl₃) δ 21.2, 70.5, 99.5, 112.9, 113.7, 122.6, 127.6, 129.4, 132.9, 138.1, 148.8, 158.8, 169.6; APCI-HRMS *m/z*: calcd for C₁₅H₁₃O₃S (MH⁺), 273.0580, found 273.0575; purity (HPLC): 96%.

6-(3-Phenylpropoxy)-1,3-benzoxathiol-2-one (7e)

The title compound was prepared in a yield of 13.4%: mp 136.6 °C (ethanol). ¹H NMR (600 MHz, CDCl₃) δ 2.11 (qn, J = 7.2 Hz, 2H), 2.80 (t, J = 7.5 Hz, 2H), 3.94 (t, J = 6.4 Hz, 2H), 6.80 (dd, J = 2.6, 8.7 Hz, 1H), 6.83 (d, J = 2.6 Hz, 1H), 7.19–7.21 (m, 3H), 7.23 (d, J = 8.7 Hz, 1H), 7.28 (t, J = 7.5 Hz, 2H); ¹³C NMR (151 MHz, CDCl₃) δ 30.5, 32.0, 67.5, 99.1, 112.5, 113.3, 122.5, 126.0, 128.5, 141.1, 148.9, 159.1, 169.6; APCI-HRMS *m/z*: calcd for C₁₆H₁₅O₃S (MH⁺), 287.0736, found 287.0713; purity (HPLC): 94%.

3. Protocol for the measurements of IC₅₀ values

The IC₅₀ values for the inhibition of the recombinant human MAO-A and MAO-B enzymes were measured as detailed in literature (2). The enzyme reactions were carried out in white 96-well microtiter plates (Eppendorf) and potassium phosphate buffer at pH 7.4 (K₂HPO₄/KH₂PO₄ 100 mM, made isotonic with KCl) served as reaction medium. The final volume of the reactions was 200 µL and contained the non-specific MAO substrate, kynuramine (50 µM), and the test inhibitors at concentrations of 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% DMSO. The enzyme reactions were initiated with the addition of MAO-A (0.0075 mg protein/mL) or MAO-B (0.015 mg protein/mL) and incubated for 20 min at 37 °C in a convection oven. A volume of 80 µL sodium hydroxide (2 N) was added to terminate the reactions and 4-hydroxyquinoline, the oxidation product of kynuramine, was analysed by fluorescence spectrophotometry (λ_{ex} = 310; λ_{em} = 400 nm) (3). To quantify 4-hydroxyquinoline, a linear calibration curve containing authentic 4-hydroxyquinoline (0.047–1.56 µM) was employed. These data were used to calculate enzyme catalytic rates, which were fitted to the one site competition model of the Prism 5 software package (GraphPad). The IC₅₀ values were determined in triplicate and expressed as mean ± standard deviation (SD).

4. Dialysis of enzyme reactions

The reversibility of MAO-A and MAO-B inhibition by **7c** was investigated by dialysis according to the reported protocol (2). Slide-A-Lyzer® dialysis cassettes (Thermo Scientific) with a molecular weight cut-off of 10 000 and a sample volume capacity of 0.5–3 mL were used for these studies. MAO-A or MAO-B (0.03 mg/mL) and compound **7c** were pre-incubated for 15 min at 37 °C to a final volume of 0.8 mL in a medium of potassium phosphate buffer (100 mM, pH 7.4, 5% sucrose).

Compound **7c** was dissolved in DMSO and added to the incubations to yield a final concentration of 4% DMSO and an inhibitor concentration of $4 \times \text{IC}_{50}$. These mixtures were subsequently dialysed at 4 °C in 80 mL of dialysis buffer (100 mM potassium phosphate, pH 7.4, 5% sucrose). The dialysis buffer was replaced with fresh buffer at 3 h and 7 h after the start of dialysis. After 24 h the reactions were diluted 2-fold with the addition of kynuramine (dissolved in potassium phosphate buffer, 100 mM, pH 7.4, made isotonic with KCl). The final concentrations of **7c** and kynuramine in these reactions were equal to $2 \times \text{IC}_{50}$ and 50 μM , respectively, and the final volume 500 μL . The reactions were incubated for 20 min at 37 °C in a water bath and terminated by adding NaOH (400 μL of 2 N) and 1000 μL water. The concentrations of the MAO-generated 4-hydroxyquinoline were measured by fluorescence spectrophotometry ($\lambda_{\text{ex}} = 310$; $\lambda_{\text{em}} = 400$ nm) employing a 3.5 mL quartz cuvette (pathlength 10 $\times$ 10 mm) (3). 4-Hydroxyquinoline was quantified by using a linear calibration curve constructed from authentic 4-hydroxyquinoline (0.047–1.56 μM).

As negative controls, MAO-A and MAO-B were similarly pre-incubated and dialysed in the absence of inhibitor while as positive controls MAO-A and MAO-B were pre-incubated and dialysed in the presence of the irreversible inhibitors, pargyline and (R)-deprenyl, respectively. The concentrations of pargyline and (R)-deprenyl employed were equal to $4 \times \text{IC}_{50}$ the reported IC_{50} values (13 μM and 0.079 μM , respectively) for the inhibition of the MAOs (4; 5). For comparison, the MAO-A and MAO-B activities of undialysed mixtures of these enzymes and **7c** were maintained at 4 °C for the same time period. All reactions were carried out in triplicate and the residual enzyme catalytic rates are expressed as mean $\pm$ SD.

5. The construction of Lineweaver-Burk plots and determination of K_i values

The modes of MAO-A and MAO-B inhibition by **7c** were examined by constructing sets of six Lineweaver-Burk plots for each MAO isoform. The first plot was constructed in the absence of inhibitor, while the remaining five plots were constructed in the presence of different concentrations of **7c**. These inhibitor concentrations were $\frac{1}{4} \times \text{IC}_{50}$, $\frac{1}{2} \times \text{IC}_{50}$, $\frac{3}{4} \times \text{IC}_{50}$, $1 \times \text{IC}_{50}$ and $1\frac{1}{4} \times \text{IC}_{50}$. Kynuramine, at concentrations of 15–250 μM , served as substrate and the concentrations of MAO-A and MAO-B was 0.015 mg protein/mL. All enzyme reactions and activity measurements were carried out as described above for the dialysis experiments. K_i values were estimated from plots of the slopes of the Lineweaver-Burke plots versus inhibitor concentration (x-axis intercept equals $-K_i$) as well as by global (shared) fitting of the inhibition data directly to the Michaelis-Menten equation using the Prism 5 software package.

6. Measurement of lipophilicity (logP)

n-Octanol and deionised water were mutually saturated and separated. The test compounds were dissolved in neat n-octanol to yield a concentration of 2 mM. In a microcentrifuge tube (2 mL) were placed, 640 μL saturated n-octanol, 800 μL saturated water, and 160 μL of the test compound. The

vessels were shaken by hand for 5 min and subsequently centrifuged at 4,000 g for 10 min. The n-octanol phase was diluted 30-fold into neat n-octanol and the absorbance of the test compound measured at a wavelength of 288 nm. To quantify the test compounds, molar extinction coefficients were recorded in n-octanol and were as follows: **7a** (4197 M^{-1}), **7b** (4398 M^{-1}), **7c** (4592 M^{-1}), **7d** (4930 M^{-1}), **7e** (3647 M^{-1}). The water phase was analysed by HPLC (Agilent 1200 HPLC system equipped with a quaternary pump and Agilent 1200 series diode array detector) without prior dilution. A Venusil XBP C18 column ($4.60 \times 150 \text{ mm}$, $5 \mu\text{m}$) was used with the mobile phase consisting of 75% acetonitrile and 25% MilliQ water at a flow rate of 1 mL/min . A volume of $20 \mu\text{L}$ of the water phases were injected into the HPLC system and the eluent was monitored at 210 nm. To quantify the test compounds, linear calibration curves were constructed using known concentrations ($0.05\text{--}10 \mu\text{M}$) prepared in acetonitrile. The ratio of the concentration of the test compound in the n-octanol phase to the water phase is the partition coefficient (P) from which the logP value is calculated. These studies were carried out in triplicate and the logP values are expressed as mean $\pm$ SD.

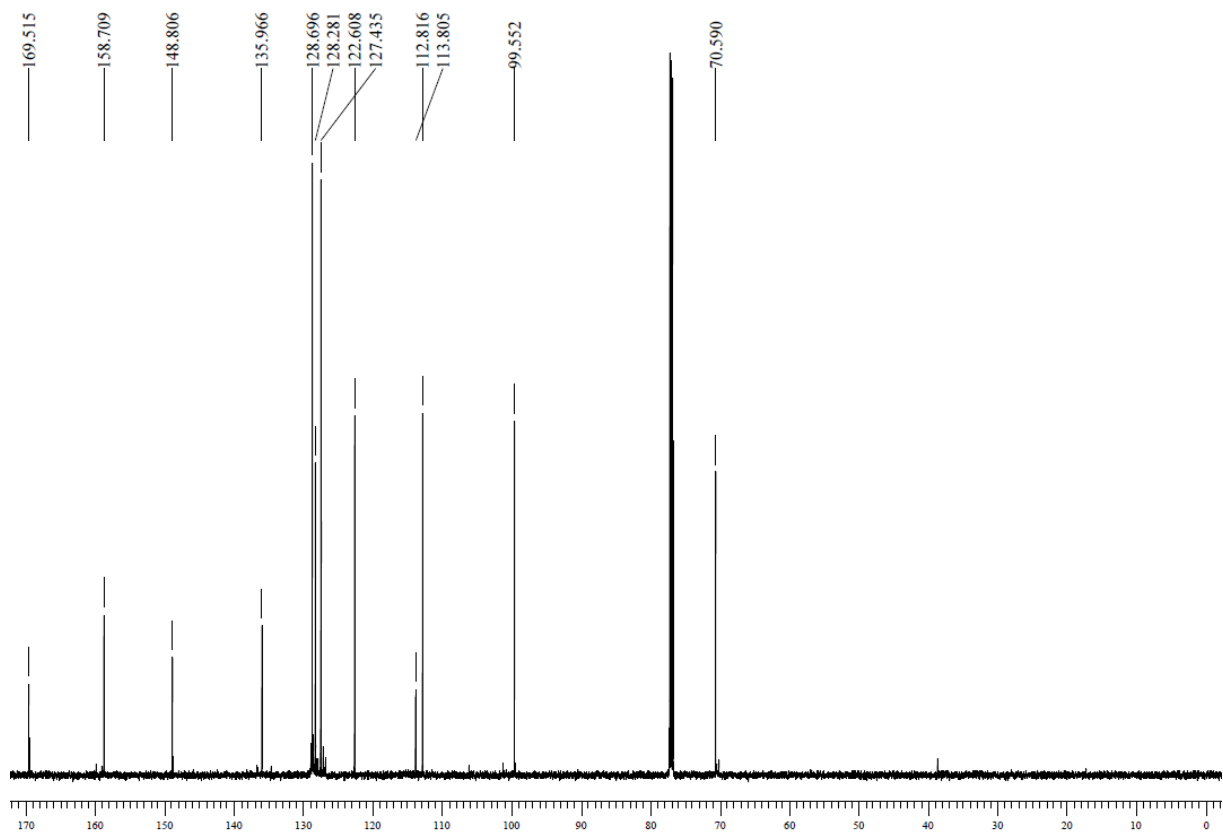
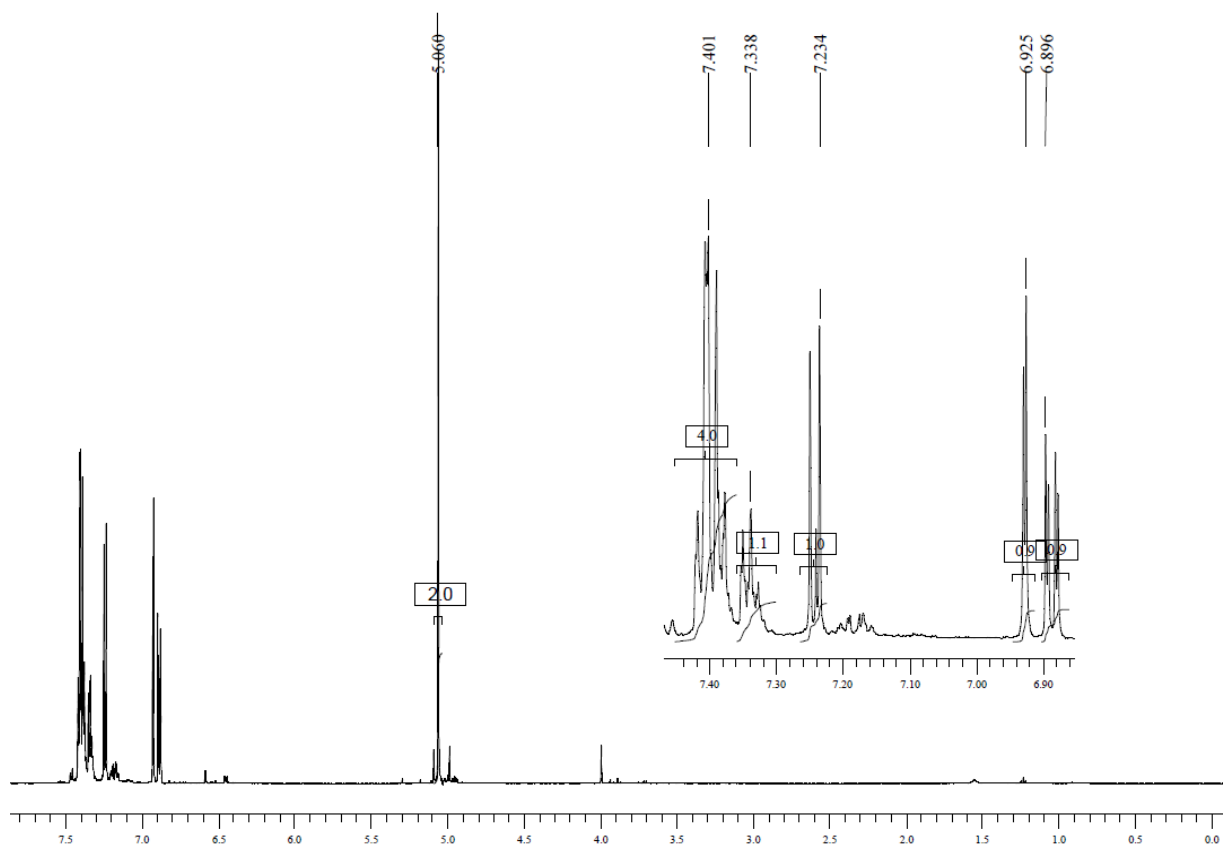
7. Measurement of aqueous solubility

To determine the aqueous solubilities of the test compounds, 3–5 mg was placed into a polypropylene tube followed by 3 mL deionised water. The tubes were agitated for 24 h in a water bath at 37°C and subsequently centrifuged at 16,000 g for 10 min. The supernatants were filtered with a $0.22 \mu\text{m}$ syringe filter and analysed by HPLC (without prior dilution) as described above for the measurement of lipophilicity. The solubility values were measured in triplicate and are given as mean $\pm$ SD.

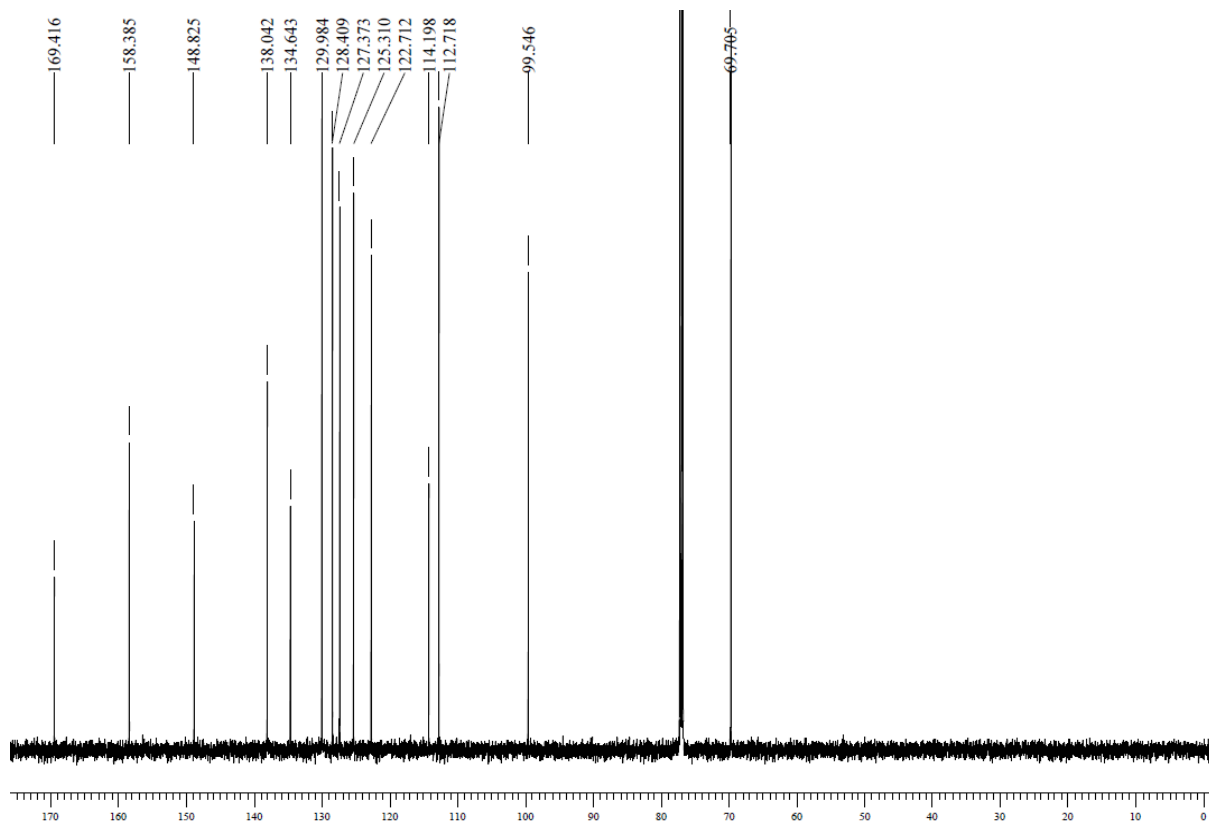
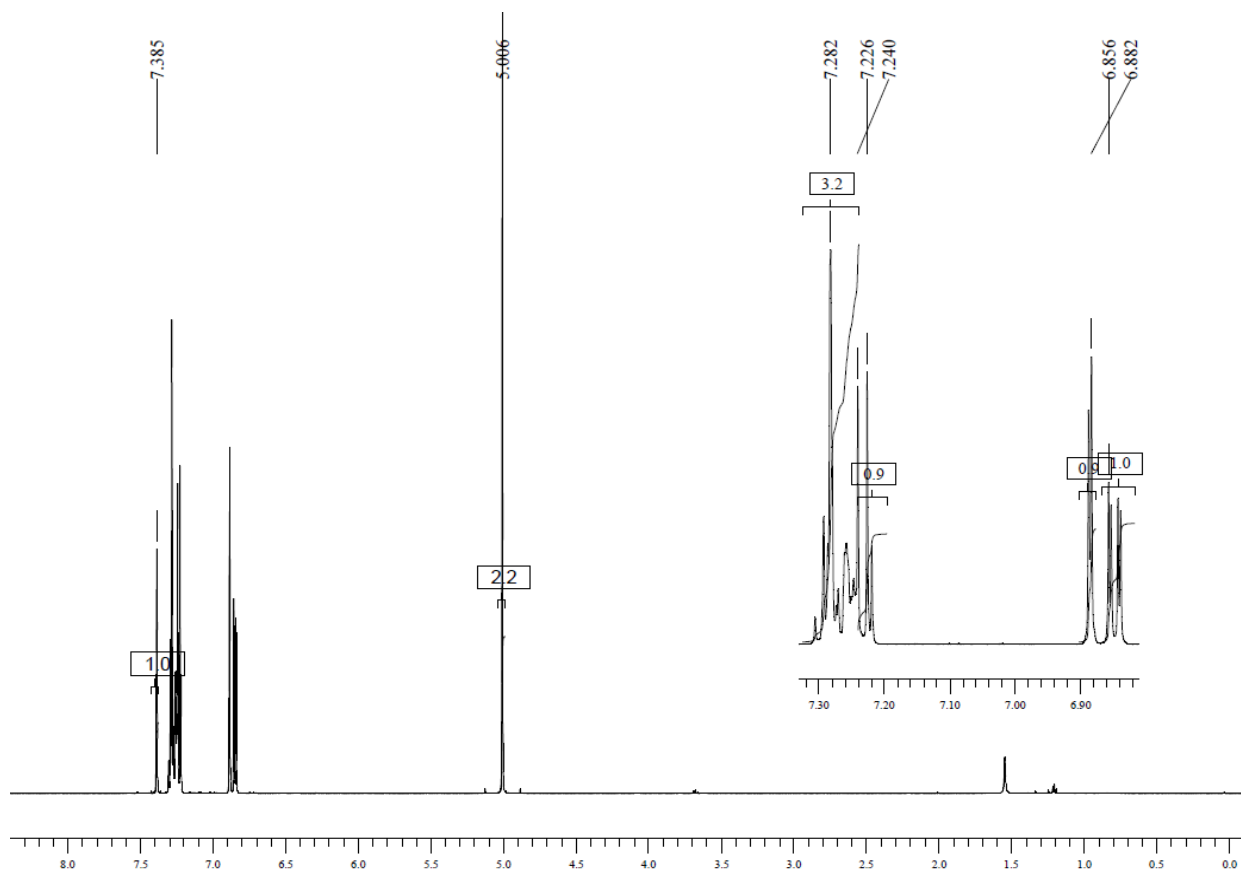
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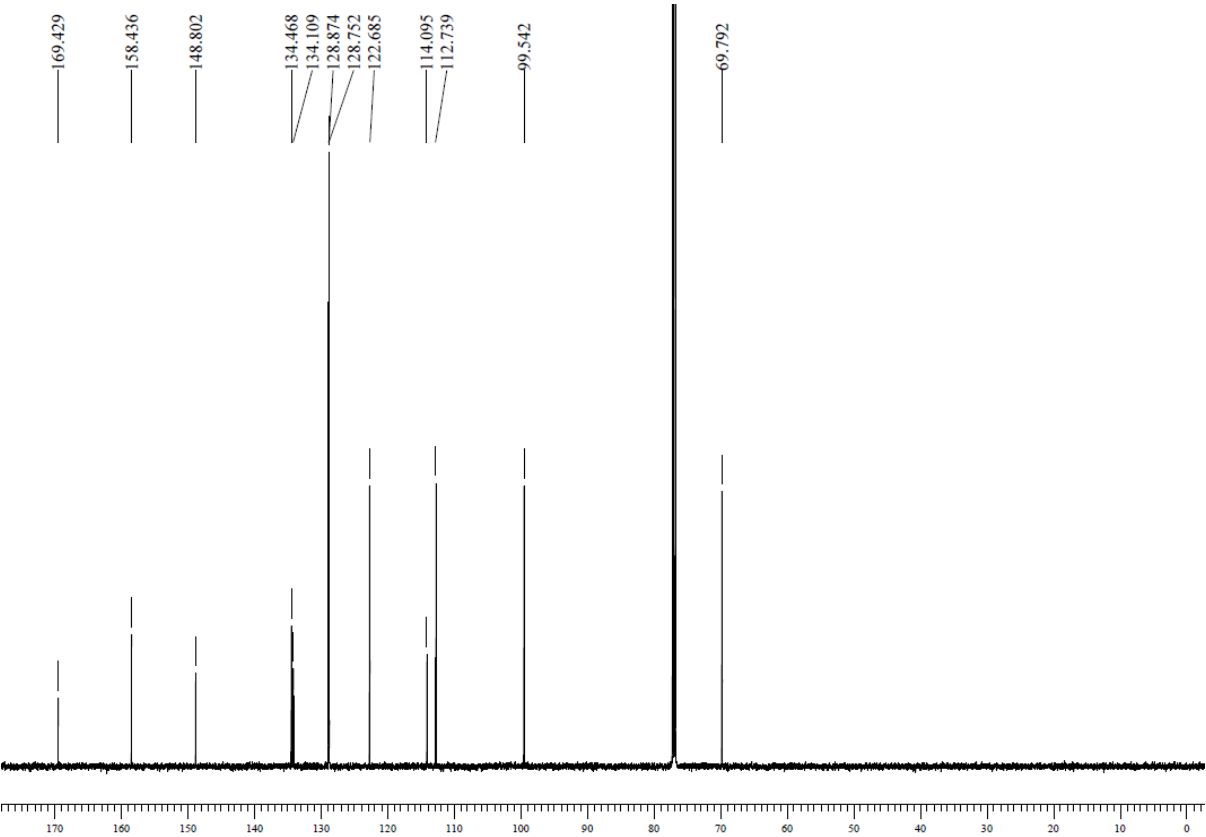
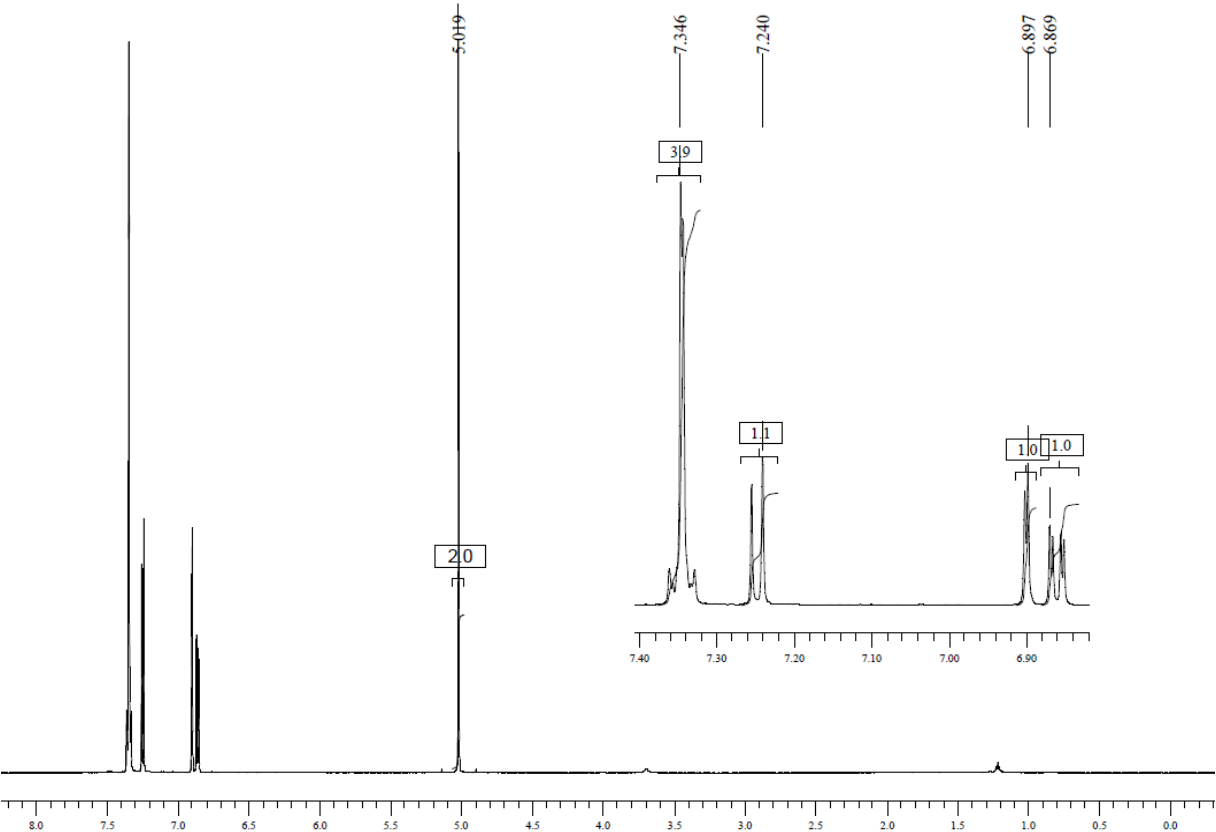
6-Benzoyloxy-1,3-benzoxathiol-2-one (7a)



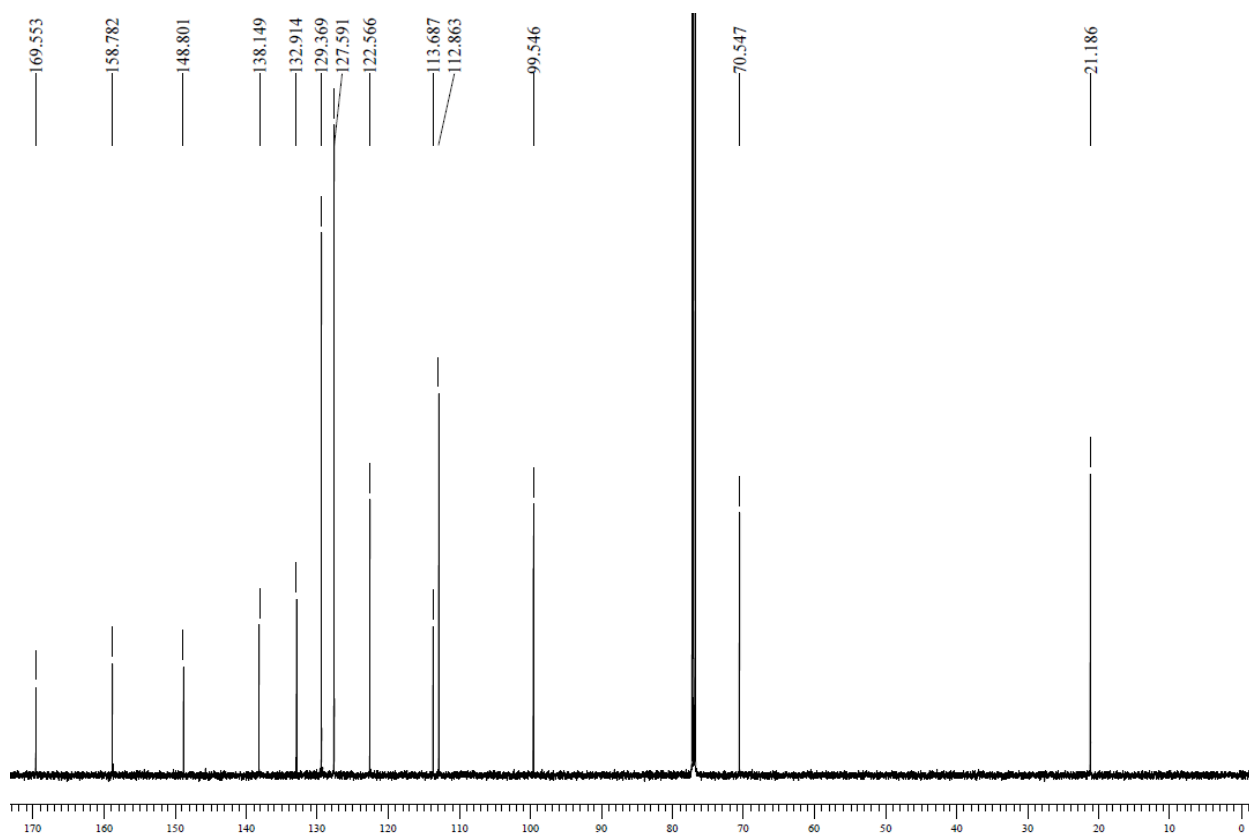
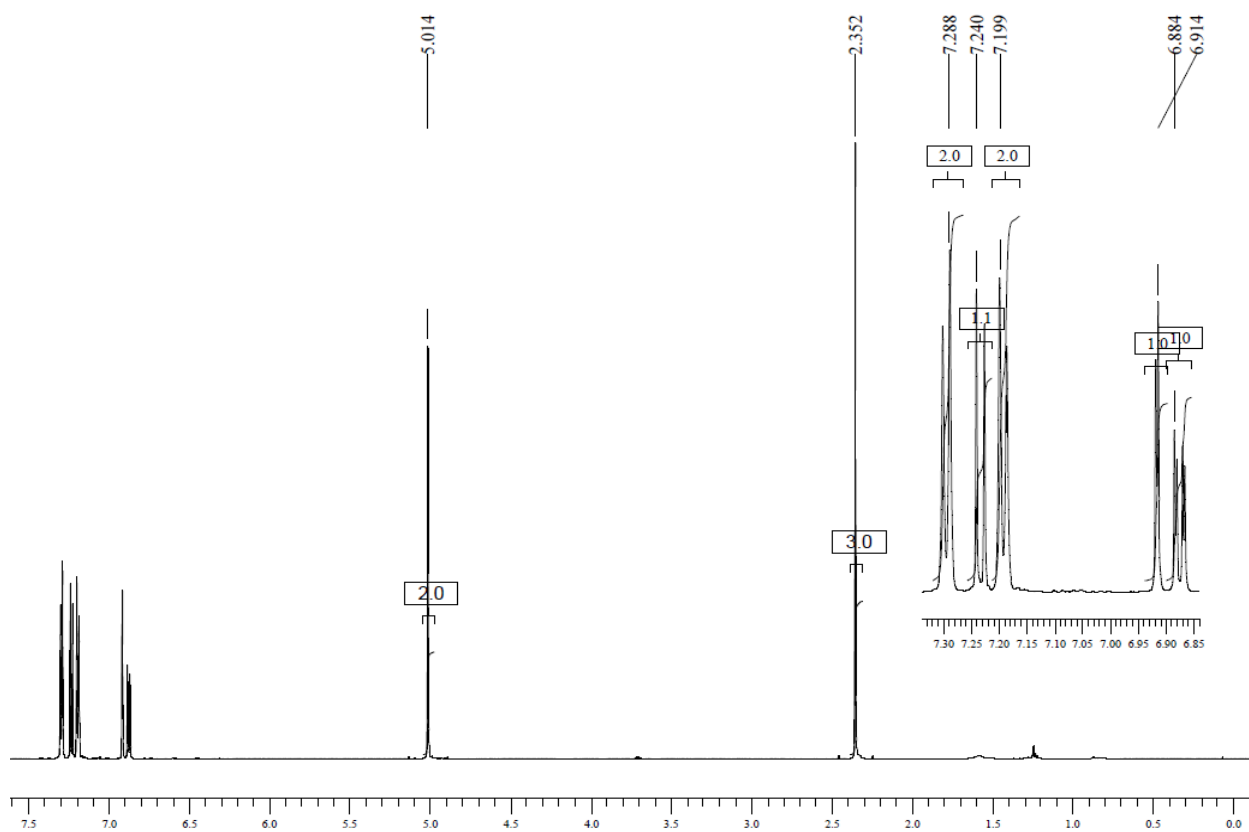
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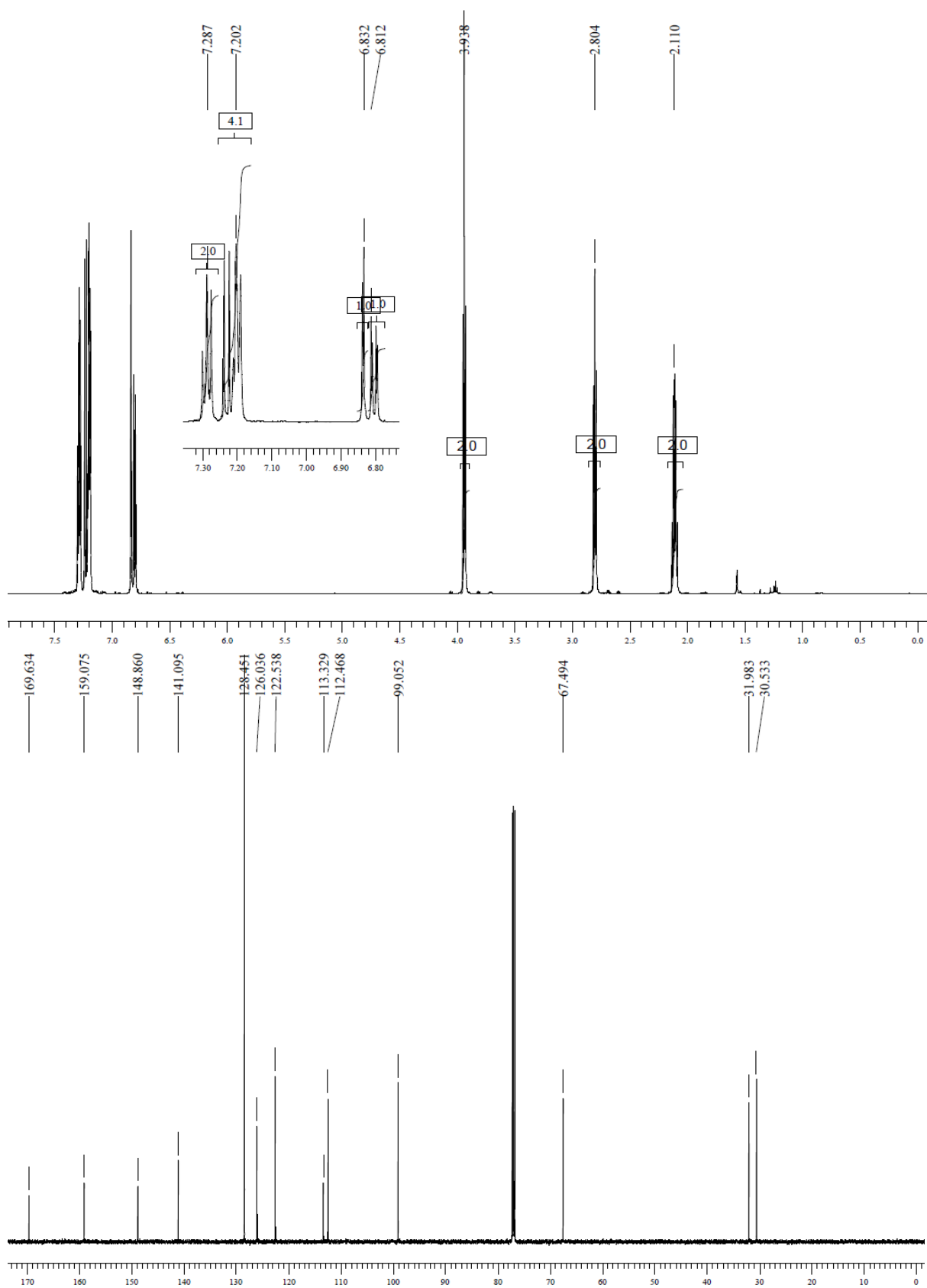
6-(4-Chlorobenzyloxy)-1,3-benzoxathiol-2-one (7c)



6-(4-Methylbenzyloxy)-1,3-benzoxathiol-2-one (7d)



6-(3-Phenylpropoxy)-1,3-benzoxathiol-2-one (7e)



6-Benzoyloxy-1,3-benzoxathiol-2-one (7a)

Mass Spectrum SmartFormula Report

Analysis Info

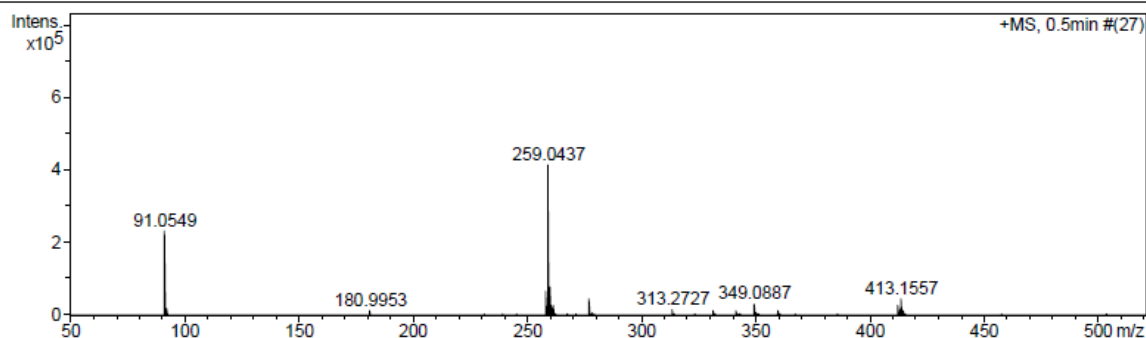
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Comment

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Operator JHL Jordaan
Instrument / Ser# micrOTOF-Q II 10390

Acquisition Parameter

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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	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e ⁻ Conf
91.0549	1	C 7 H 7	100.00	91.0542	-0.6	-6.9	2.0	4.5	ok	even
	2	C 4 H 11 S	12.52	91.0576	2.7	30.1	30.8	-0.5	ok	even
259.0437	1	C 14 H 11 O 3 S	100.00	259.0423	-1.3	-5.1	16.2	9.5	ok	even
413.1557	1	C 27 H 25 O 2 S	100.00	413.1570	1.3	3.2	5.3	15.5	ok	even
	2	C 30 H 21 O 2	46.69	413.1536	-2.0	-5.0	18.6	20.5	ok	even
	3	C 23 H 25 O 7	6.08	413.1595	3.8	9.3	33.1	11.5	ok	even

6-(3-Chlorobenzoyloxy)-1,3-benzoxathiol-2-one (7b)



Mass Spectrum SmartFormula Report



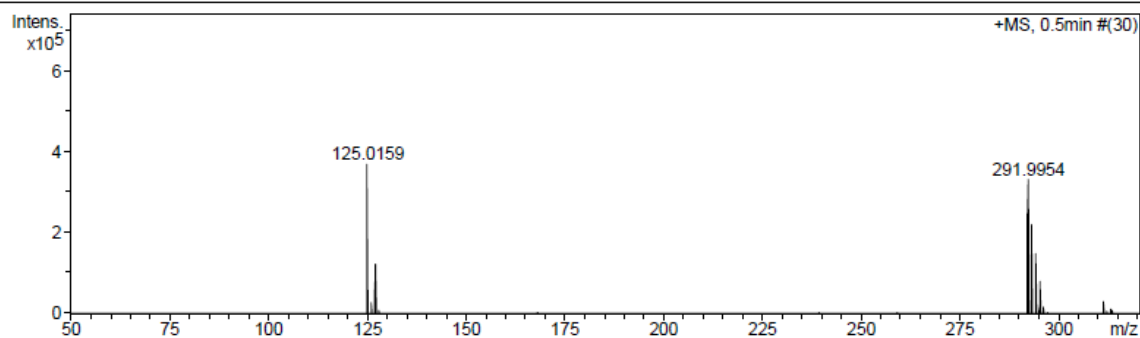
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Operator JHL Jordaan
Instrument / Ser# micrOTOF-Q II 10390

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Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e ⁻ Conf
125.0159	1	C 7 H 6 Cl	100.00	125.0153	-0.7	-5.4	6.4	4.5	ok	even
291.9954	1	C 14 H 9 Cl O 3 S	100.00	291.9955	0.1	0.5	242.8	10.0	ok	odd
	2	C 23 O	0.00	291.9944	-1.0	-3.6	342.1	24.0	ok	odd
293.0018	1	C 17 H 6 Cl O 3	100.00	293.0000	-1.8	-6.3	222.0	14.5	ok	even
	2	C 14 H 10 Cl O 3 S	44.77	293.0034	1.5	5.2	232.7	9.5	ok	even
	3	C 23 H O	0.01	293.0022	0.4	1.2	315.5	23.5	ok	even

6-(4-Chlorobenzoyloxy)-1,3-benzoxathiol-2-one (7c)



Mass Spectrum SmartFormula Report



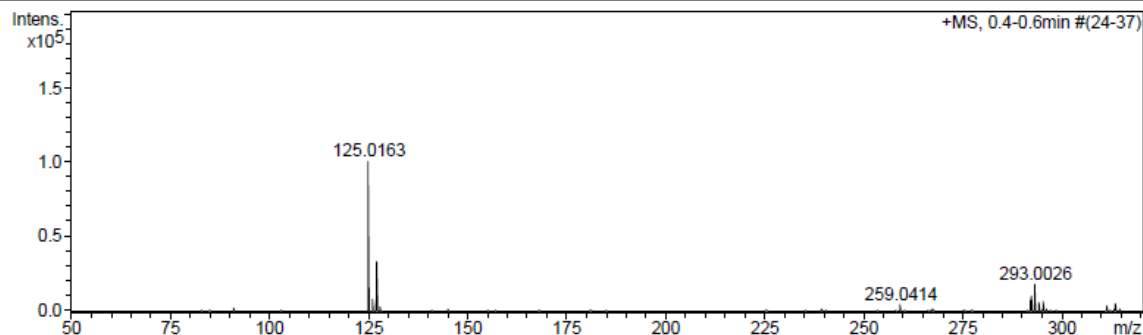
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Scan End	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e ⁻ Conf
125.0163	1	C 7 H 6 Cl	100.00	125.0153	-1.0	-8.0	3.7	4.5	ok	even
291.9954	1	C 14 H 9 Cl O 3 S	100.00	291.9955	0.2	0.6	446.1	10.0	ok	odd
	2	C 23 O	0.00	291.9944	-1.0	-3.4	530.5	24.0	ok	odd
293.0026	1	C 14 H 10 Cl O 3 S	100.00	293.0034	0.7	2.5	79.1	9.5	ok	even
	2	C 23 H O	0.04	293.0022	-0.4	-1.5	204.3	23.5	ok	even
293.9980	1	C 16 H 6 O 4 S	100.00	293.9981	0.1	0.4	416.4	14.0	ok	odd
	2	C 6 H 11 Cl O 11	17.97	293.9984	0.4	1.5	425.6	1.0	ok	odd
294.9997	1	C 13 H 8 Cl O 6	100.00	295.0004	0.7	2.3	130.0	9.5	ok	even
	2	C 17 H 8 Cl O S	14.71	294.9979	-1.8	-6.2	149.6	13.5	ok	even

6-(4-Methylbenzyloxy)-1,3-benzoxathiol-2-one (7d)



Mass Spectrum SmartFormula Report



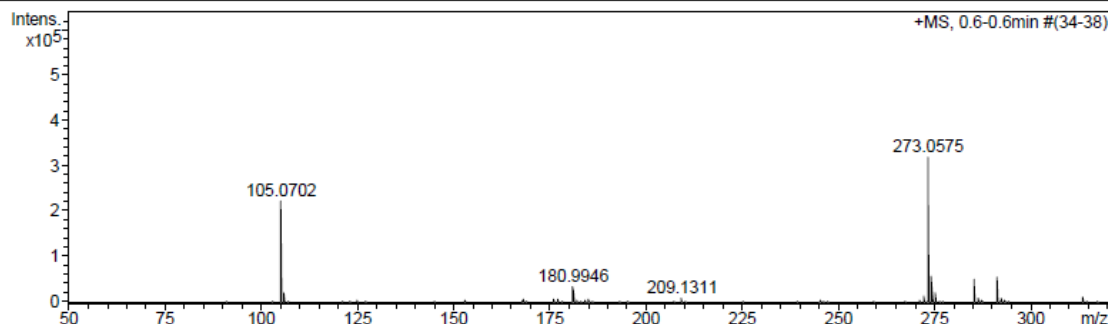
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105.0702	1	C 8 H 9	100.00	105.0699	-0.4	-3.5	5.6	4.5	ok	even
273.0575	1	C 15 H 13 O 3 S	100.00	273.0580	0.5	2.0	3.5	9.5	ok	even

6-(3-Phenylpropoxy)-1,3-benzoxathiol-2-one (7e)



Mass Spectrum SmartFormula Report



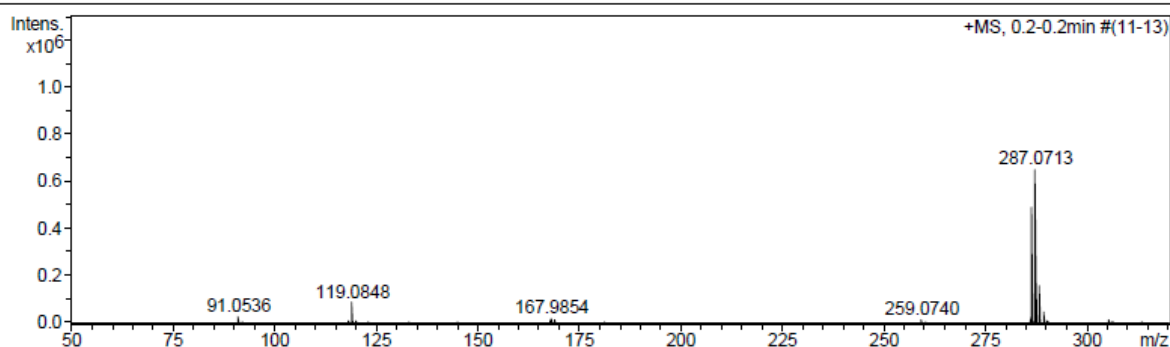
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Scan End	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e ⁻ Conf
287.0713	1	C ₂₂ H ₉ N	86.89	287.0730	1.6	5.6	26.4	19.0	ok	odd
	2	C ₁₆ H ₁₅ O ₃ S	45.95	287.0736	2.3	8.0	31.2	9.5	ok	even
	3	C ₁₉ H ₁₁ O ₃	100.00	287.0703	-1.1	-3.7	34.9	14.5	ok	even
	4	C ₁₇ H ₉ N ₃ O ₂	32.98	287.0689	-2.4	-8.4	40.7	15.0	ok	odd

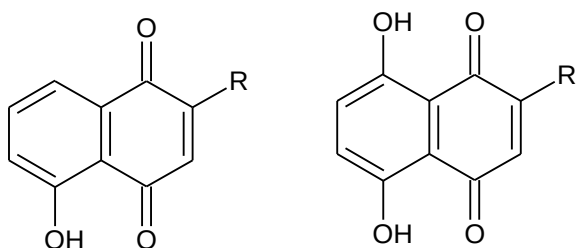
Chapter 6

Manuscript C

Article submitted to Chemical Biology and Drug Design entitled:

The evaluation of natural and synthetic 1,4-naphthoquinones as inhibitors of monoamine oxidase

Graphical abstract



$IC_{50} = 1.50\text{-}4.91 \mu\text{M}$ (MAO-A)
 $0.86\text{-}4.36 \mu\text{M}$ (MAO-B)

The current study expands on the structure-activity relationships (SARs) of MAO inhibition by the 1,4-naphthoquinone class of compounds. Noteworthy inhibitors included juglone, plumbagin, 5,8-dihydroxy-1,4-naphthoquinone and shikonin. Interestingly, most 2-hydroxy-containing compounds evaluated do not inhibit the MAOs.

The manuscript is written as a research article and is presented according to the author guidelines as stipulated in addendum B.

Authors' contributions

- The experimental work, interpretation of results and documentation of this study was carried out by **S. Mostert**.
- This study was conceptualised and documented with the assistance of **J.P Petzer**.
- The enzymology section of this study was conducted under the supervision of **A. Petzer**.

All co-authors provided permission to use this manuscript as part of **S Mostert's Ph.D thesis**.

The evaluation of natural and synthetic 1,4-naphthoquinones as inhibitors of monoamine oxidase

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Running title: 1,4-Naphthoquinones as MAO inhibitors

Keywords: monoamine oxidase, MAO, inhibition, reversible, competitive, dialysis, 1,4-naphthoquinone.

Abstract

Previous reports have documented that 1,4-naphthoquinones act as inhibitors of the monoamine oxidase (MAO) enzymes. In particular, fractionation of extracts of cured tobacco leaves have led to the characterisation of 2,3,6-trimethyl-1,4-naphthoquinone, a non-selective MAO inhibitor. To derive structure-activity relationships for MAO inhibition by the 1,4-naphthoquinone class of compounds, the present study investigates the human MAO inhibitory activities of fourteen structurally diverse 1,4-naphthoquinones of natural and synthetic origin. Among these, 5,8-dihydroxy-1,4-naphthoquinone was found to be the most potent inhibitor with an IC₅₀ value of 0.860 µM for the inhibition of MAO-B. A related compound, shikonin, inhibits both the MAO-A and MAO-B isoforms with IC₅₀ values of 1.50 and 1.01 µM, respectively. It is further shown that MAO-A and MAO-B inhibition by these compounds are reversible since inhibition is completely reversed by dialysis. In this respect, kinetic analysis suggests that the modes of MAO inhibition are competitive. This study contributes to the discovery of novel MAO inhibitors, which may be useful in disorders such as Parkinson's disease, depressive illness, congestive heart failure and cancer.

6.1 Introduction

The monoamine oxidase (MAO) enzymes are represented by two isoforms, MAO-A and MAO-B (1). These isoforms are encoded by separate genes and share approximately 70% sequence identity at the amino acid level (2; 3). The function of the MAOs is the oxidation of amine substrates to yield the corresponding iminium species, which for most known substrates hydrolyse to form the aldehydes. With the reoxidation of the flavin adenine dinucleotide (FAD) redox cofactor of MAO, molecular oxygen is reduced to yield an equivalent of hydrogen peroxide for each equivalent substrate metabolised (1). The substrate specificities of MAO-A and MAO-B often overlap with neurotransmitter amines such as dopamine, epinephrine and norepinephrine serving as substrates for both MAO isoforms (4). Other substrates such as serotonin (MAO-A specific) and the false neurotransmitter, β -phenylethylamine (MAO-B specific), are isoform specific.

Since the MAOs metabolise amine neurotransmitters in the brain and peripheral tissues, inhibitors of these enzymes are often useful in the treatment of disease states that result from deficient levels of neurotransmitters (1; 5). For example, major depression has been linked to reduced levels of serotonin in the central nervous system, and MAO-A inhibitors are thus an established class of antidepressant drugs (6; 7). In Parkinson's disease, where dopamine depletion in the basal ganglia is responsible for the characteristic motor symptoms, MAO-B inhibitors are employed (8). In this respect, MAO-B inhibitors prolong the physiological action of dopamine and are often combined with L-dopa, the metabolic precursor of dopamine, to further enhance dopamine levels (9; 10). By reducing the MAO-catalysed production of hydrogen peroxide and aldehyde intermediates, MAO inhibitors may be of further pharmacological importance. These species may injure neuronal cells if not effectively cleared, and it has thus been suggested that MAO inhibitors may protect against neurodegeneration (1; 11). This is of particular relevance in Parkinson's disease since MAO-B activity increases as the human brain ages, thereby enhancing the production of injurious metabolic by-products (12). Hydrogen peroxide and aldehyde intermediates of the MAO catalytic cycle may also have roles in cardiovascular pathophysiology. By blocking the formation of these species in the myocardium, MAO inhibitors may improve cardiac function in patients with congestive heart failure (13). In this instance, MAO-A inhibition may be more relevant since MAO-A levels and MAO-A-generated hydrogen peroxide are significantly increased in the hearts of aged rats, an observation that may be associated with cardiac cellular degeneration (14). Interestingly, MAO-B inhibitors have been investigated as a possible treatment for smoking cessation. This is based on the observation that MAO-B activity is reduced in smokers and MAO-B inhibitors may thus mimic the effects of smoking, particularly with respect to increasing synaptic monoamines (15).

Based on the pharmacological importance of MAO, the discovery of novel chemical classes that may serve as MAO inhibitors is of value. In this respect, 1,4-naphthoquinones may represent such a chemical class. In a previous study the fractionation of extracts of cured tobacco leaves have led

to the characterisation of 2,3,6-trimethyl-1,4-naphthoquinone (TMN; **1**), a non-selective MAO inhibitor (Fig. 1) (16). TMN inhibits human MAO-A and MAO-B with K_i values of 3 and 6 μM , respectively. A subsequent study has shown that the structurally related menadione (**2**), also a 1,4-naphthoquinone compound, is a MAO inhibitor with selectivity for the MAO-B isoform ($K_i = 0.4 \mu\text{M}$) over MAO-A ($K_i = 26 \mu\text{M}$) (17). Lapachol (**3**), a 1,4-naphthoquinone from natural origin, and norlapachol (**4**), semi-synthetic derivative of lapachol, also proved to inhibit the MAOs, albeit with lower potencies compared to TMN (17). By acting as topoisomerase inhibitors via DNA intercalation, the 1,4-naphthoquinone class of compounds is also of interest as potential antitumor agents (18). Since MAO inhibition is considered to be a potential mechanism for the treatment for malignant tumors, MAO inhibitors of the 1,4-naphthoquinone class may be of particular importance in future cancer therapy (17).

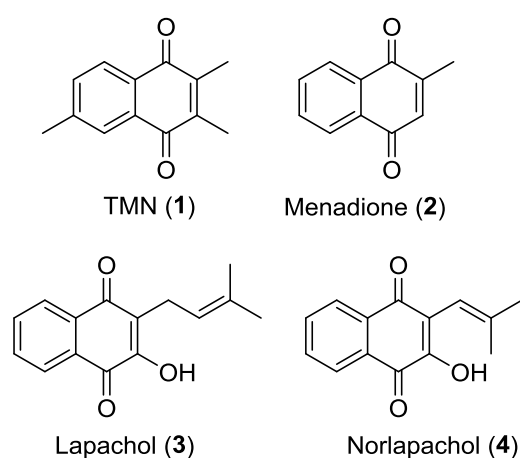


Figure 1: The structures of compounds discussed in the text.

The focus of the current study is thus to explore the MAO inhibitory properties of natural and synthetic 1,4-naphthoquinones in the hope of discovering compounds with potent activities. As outlined above, such compounds may represent interesting therapeutic agents and leads for future MAO inhibitor design. Fourteen commercially available 1,4-naphthoquinone compounds from three different structural classes were selected for this study. These are 1,4-naphthoquinone derivatives **5a–h**, 5-hydroxy-1,4-naphthoquinone derivatives **6a–b** and 5,8-dihydroxy-1,4-naphthoquinone derivatives **7a–b** (Table. 1). These series include menadione (**5d**), lapachol (**3**) and norlapachol (**4**) as reference compounds. Also included are plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone; **6b**) (19), a compound originally isolated from the plant genus *Plumbago*, lawsone (2-hydroxy-1,4-naphthoquinone; **5b**) present in the leaves of the henna plant (20), juglone (5-hydroxy-1,4-naphthoquinone; **6a**) a natural product of walnut trees (21) and shikonin [(±)-5,8-dihydroxy-2-(1-hydroxy-4-methyl-3-pentenyl)-1,4-naphthoquinone; **7b**], a component of Chinese herbal medicine (22).

6.2 Methods and materials

6.2.1 Chemicals and instrumentation

A Varian Cary Eclipse fluorescence spectrophotometer was used for fluorescence spectrophotometry. Microsomes from insect cells containing recombinant human MAO-A and MAO-B (5 mg/mL), and kynuramine.2HBr were obtained from Sigma-Aldrich. Slide-A-Lyzer dialysis cassettes with a molecular weight cut-off of 10 000 and a sample volume capacity of 0.5–3 mL were from Thermo Scientific. The 1,4-naphthoquinone compounds were obtained from Sigma-Aldrich.

6.2.2 IC_{50} value determination

The protocol for measuring IC_{50} values for the inhibition of MAO-A and MAO-B has been reported in detail in a recent publication (23). The recombinant human MAO enzymes were used and kynuramine served as non-selective MAO substrate. The MAOs oxidise kynuramine to ultimately yield a fluorescent metabolite, 4-hydroxyquinoline, which was measured by fluorescence spectrophotometry ($\lambda_{ex} = 310$; $\lambda_{em} = 400$ nm). Inhibitor concentrations spanning at least 3 orders of magnitude were used to construct sigmoidal dose-response curves from which the IC_{50} values were determined. Each experiment was conducted in triplicate and IC_{50} values are expressed as mean $\pm$ standard deviation (SD).

6.2.3 Recovery of enzyme activity after dialysis

The protocol for examining the reversibility of MAO-A and MAO-B inhibition has been reported in detail in a recent publication (23). The test inhibitors, **7b** and **7a**, were combined with MAO-A and MAO-B at concentrations of $4 \times IC_{50}$ for 15 min, and the resulting mixtures were subsequently dialysed for 24 h at 4 °C. After diluting the mixtures twofold with the addition of kynuramine, the residual MAO activities were measured. As positive controls, similar experiments were carried out with the irreversible inhibitors, pargyline and (R)-deprenyl. Dialysis experiments in the absence of inhibitor served as negative controls. For comparison, undialysed mixtures of the MAOs and the test inhibitors were maintained over the same time period at 4 °C. All reactions were carried out in triplicate and the residual enzyme catalytic rates were expressed as mean $\pm$ SD.

6.2.4 The construction of Lineweaver-Burk plots

The protocol for constructing Lineweaver-Burk plots has been reported in detail in a recent publication (23). For the inhibition of MAO-A and MAO-B by **7b** and **7a**, respectively, sets of six Lineweaver-Burk plots were constructed. The first plot was constructed in the absence of inhibitor and remaining plots were constructed in the presence of five different concentrations of the test inhibitors ($\frac{1}{4} \times IC_{50}$, $\frac{1}{2} \times IC_{50}$, $\frac{3}{4} \times IC_{50}$, $1 \times IC_{50}$ and $1\frac{1}{4} \times IC_{50}$). For each plot, kynuramine was used at concentrations of 15–250 μ M. K_i values were determined from graphs of the slopes of the

Lineweaver-Burk plots versus inhibitor concentration as well as by global (shared) fitting of the inhibition data directly to the Michaelis-Menten equation using the Prism 5 software package (GraphPad).

6.2.5 Molecular docking experiments

The protocol for docking ligands into the active site models of MAO-A and MAO-B has been reported in detail (23). All calculations were carried out with the Windows based Discovery Studio 3.1 software (24). The X-ray crystal structures of human MAO-A (PDB code, 2Z5X) (25) and human MAO-B (PDB code, 2V5Z) (26) were employed for these studies and the CDOCKER algorithm was used to dock the test ligands. For both MAO-A and MAO-B three active site waters were retained since these are considered to be conserved: HOH 710, 718 and 739 in MAO-A, and HOH 1155, 1170 and 1351 in the A-chain of MAO-B. The FAD cofactor was set to the oxidised state and the illustrations were generated with PyMOL (27).

6.3 Results

6.3.1 MAO inhibition potencies

To determine the inhibition potencies of the 1,4-naphthoquinones, the recombinant human MAO-A and MAO-B enzymes were used (28). The non-selective MAO-A/B substrate, kynuramine, served as enzyme substrate. The MAOs oxidise kynuramine to yield 4-hydroxyquinoline as final metabolite. Since 4-hydroxyquinoline fluoresces in alkaline media, its MAO-catalysed formation may be conveniently measured by fluorescence spectrophotometry. By recording MAO catalytic rates in the absence and presence of various concentrations of the 1,4-naphthoquinone inhibitors, sigmoidal plots of rate versus logarithm of inhibitor concentration may be constructed. From these plots the corresponding IC_{50} values were calculated as a measure of the inhibition potencies. IC_{50} values were measured in triplicate and inhibitor concentrations spanning at least three orders of magnitude (0.003–100 μ M) were used. Fig. 2 provides, as examples, the sigmoidal plots for the inhibition of MAO-A and MAO-B by selected compounds.

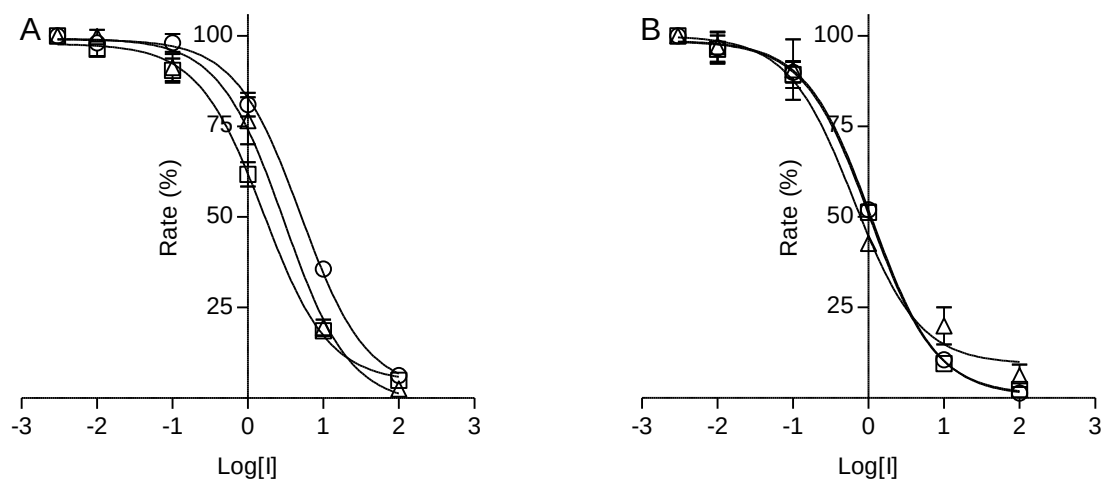
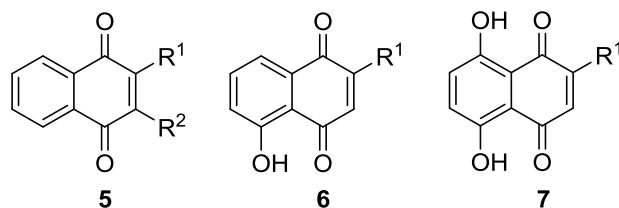


Figure 2: The sigmoidal plots for the inhibition of MAO-A and MAO-B by compounds **6b** (circles), **7a** (triangles) and **7b** (rectangles).

The IC_{50} values for the inhibition of human MAO-A and MAO-B by the 1,4-naphthoquinones are given in table 1. With the exception of **5f** and **5g**, which inhibit MAO-B but not MAO-A, the 1,4-naphthoquinones exhibit little isoform selectivity. This finding is similar to that observed for TMN, a non-selective MAO inhibitor (16). Among the compounds that inhibited MAO, the MAO-A inhibition potencies ranged from 1.50 to 20.4 μ M. The MAO-B potencies were in a similar range, 0.860 to 24.8 μ M. Compounds **7a** and **7b** may be emphasized as the most potent MAO-B and MAO-A inhibitors, respectively. A number of 1,4-naphthoquinones, however, possess similar inhibition potencies with 9 (of fourteen) homologues exhibiting $IC_{50} < 5$ μ M for at least one of the MAO isoforms. Interestingly, the parent 1,4-naphthoquinone (**5a**) is a moderately potent MAO inhibitor with IC_{50} values for the inhibition of MAO-A and MAO-B of 3.43 and 8.40 μ M, respectively. These potencies are similar to the reported K_i values of 7.7 and 1.5 μ M for the inhibition of MAO-A and MAO-B, respectively (17). The current study, however, finds menadione (**5d**; $IC_{50} = 3.02$ μ M) a significantly weaker MAO-B inhibitor than the previous report ($K_i = 0.4$ μ M), while the MAO-A inhibition potencies are similar, with values of 10.2 μ M in the current study and 26 μ M (K_i) reported (29). The MAO-A and MAO-B inhibition potencies of lapachol and norlapachol ($IC_{50} > 100$ μ M in the current study) are also much weaker than those reported. The reported K_i values for the inhibition of MAO-A are >100 μ M and 35 μ M for lapachol and norlapachol, respectively, and for the inhibition of MAO-B 59 μ M and 18 μ M for lapachol and norlapachol, respectively (17). While reasons for these differences are not apparent, from both the reported and current studies it may be concluded that lapachol and norlapachol are relatively weak MAO inhibitors.

Table 1. The IC₅₀ values for the inhibition of recombinant human MAO-A and MAO-B by 1,4-naphthoquinone derivatives.



	R ¹	R ²	IC ₅₀ (μM) ^a		SI ^b
			MAO-A	MAO-B	
3 (lapachol)	OH	CH ₂ CH=C(CH ₃) ₂	NI ^c	NI ^c	—
4 (norlapachol)	OH	CHC(CH ₃) ₂	NI ^c	NI ^c	—
5a	H	H	3.43 ± 0.630	8.40 ± 1.20	0.4
5b (lawsone)	OH	H	NI ^c	NI ^c	—
5c	OCH ₃	H	20.4 ± 0.878	24.8 ± 0.579	0.8
5d (menadione)	CH ₃	H	10.2 ± 3.29	3.02 ± 0.638	3.4
5e	Cl	Cl	2.00 ± 0.448	2.61 ± 0.616	0.8
5f	CH ₃	CH ₂ Cl	NI ^c	10.8 ± 1.26	—
5g	OH	Cl	NI ^c	3.45 ± 0.041	—
5h	O(CH ₂) ₄ OH	Cl	5.06 ± 0.156	3.06 ± 0.926	1.7
6a (juglone)	H	—	1.71 ± 0.172	4.36 ± 0.180	0.4
6b (plumbagin)	CH ₃	—	4.91 ± 0.781	1.09 ± 0.063	4.5
7a	H	—	3.19 ± 0.531	0.860 ± 0.241	3.7
7b (shikonin)	CH(OH)CH ₂ CH=C(CH ₃) ₂	—	1.50 ± 0.302	1.01 ± 0.052	1.5

^a All values are expressed as the mean ± SD of triplicate determinations.

^b The selectivity index is the selectivity for the MAO-B isoform and is given as: IC₅₀(MAO-A)/IC₅₀(MAO-B).

^c No inhibition at a maximal tested concentration of 100 μM.

6.3.2 Structure-activity relationships

Considering the inhibition data, some structure-activity relationships may be derived. Among the series of 1,4-naphthoquinones, five compounds did not inhibit MAO-A while three compounds did not inhibit MAO-B. With the exception of **5f**, all of the compounds that did not inhibit MAO-A, are substituted with the hydroxy group on position C2 of the 1,4-naphthoquinone moiety. None of the four homologues with C2-hydroxy substituents are thus MAO-A inhibitors while only one 2-hydroxy-containing compound exhibit MAO-B inhibition, compound **5g**. This trend is particularly obvious when comparing **5a**, which is a MAO-A/B inhibitor, with its 2-hydroxy analogue **5b**, which does not inhibit either isoform. A further point of interest is that a 1,4-naphthoquinone with a relatively larger substituent such as **7b** has comparable MAO inhibition potency to the homologue lacking a substituent (compound **7a**). Also noteworthy is that all compounds containing hydroxyl groups on the phenyl ring (**6** and **7**) are relatively potent MAO inhibitors ($IC_{50} < 4.91 \mu M$). To gain further insight, an analysis of the interactions of these hydroxyl groups with the MAO enzymes' active sites will be carried out below. Also of interest is that methyl substitution on C2 of **5a** and **6a** to yield menadione (**5d**) and plumbagin (**6b**), respectively, is accompanied with a reversal of isoform selectivity. In this respect, the methyl substituted homologues exhibit selective inhibition of MAO-B while the desmethyl homologues are MAO-A selective. Lastly, methoxy substitution on C2 of **5a** to yield **5c** significantly reduces the inhibition potencies towards both MAO isoforms with the MAO-A inhibition, in particular, decreasing sixfold.

6.3.3 Reversibility of MAO inhibition

1,4-Napthoquinones such as TMN have previously been reported to interact reversibly and competitively with the MAOs (16; 17). Reversibility of MAO inhibition is an important consideration, particularly for MAO-A inhibition. Inhibitors of MAO-A are used with care in the clinic because of a potentially fatal hypertensive reaction that may occur when MAO-A inhibitors are taken with dietary tyramine (30). For reversible MAO-A inhibitors, this is of lesser concern and reversible inhibitors in general do not cause tyramine-induced hypertension (31; 32). Irreversible MAO-A inhibitors on the other hand, are well-known to increase blood pressure when combined with tyramine. Although tyramine also serves as a substrate for MAO-B, in the intestine, tyramine is metabolised predominantly by MAO-A (4). MAO-B inhibitors are therefore not associated with tyramine-induced hypertension.

The present study therefore investigates the reversibility of MAO inhibition by two selected 1,4-napthoquinones, compounds **7a** and **7b**. These compounds are respectively the most potent MAO-B and MAO-A inhibitors of the present series. The reversibility of MAO inhibition was examined by measuring the degree by which enzyme activity recovers after dialysis of enzyme-inhibitor mixtures (33). MAO-A and MAO-B were thus incubated for 15 min with **7b** and **7a**, respectively, and subsequently dialysed for 24 h. For this purpose, the concentrations of the inhibitors were

equal to $4 \times IC_{50}$. The results given in Fig. 3 show that dialysis reverses MAO-A and MAO-B inhibition by **7b** and **7a**, with the activities recovering to 124% and 110% of the negative control value (activity after dialysis in the absence of inhibitor). With undialysed mixtures of the MAOs and **7b** and **7a**, inhibition is still observed with the MAO-A and MAO-B activities at 53% and 66%, respectively, of the control value. These data suggest that **7b** and **7a** are reversible inhibitors of MAO-A and MAO-B, respectively. As expected, enzyme activity cannot recover after the enzymes have been incubated with the irreversible inhibitors, pargyline (MAO-A) and (R)-deprenyl (MAO-B), and after dialysis the residual enzyme activities are only 1.6% and 4.8%, respectively.

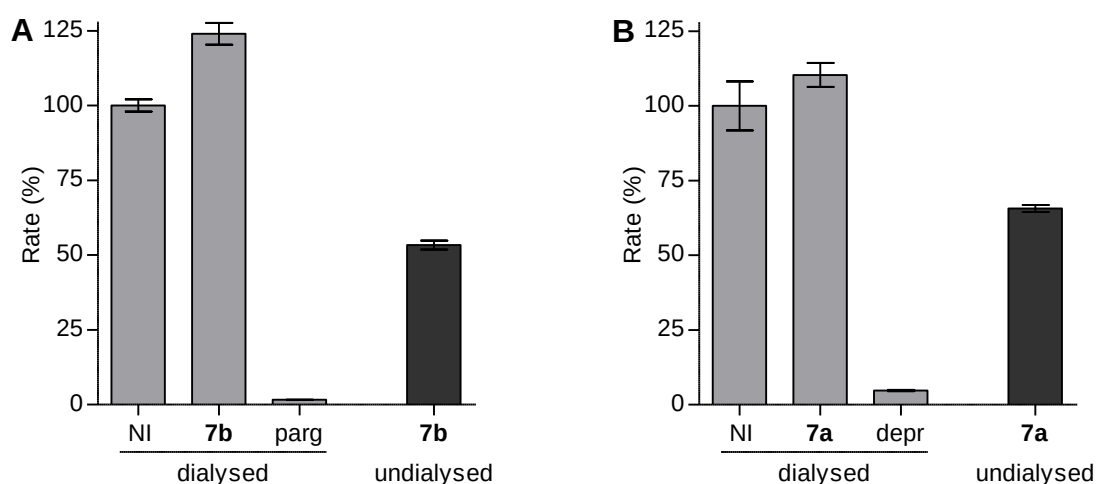


Figure 3: Dialysis of mixtures containing MAO-A and **7b** (panel A), and mixtures containing MAO-B and **7a** (panel B) suggests reversibility. The enzymes were preincubated with the inhibitors at a concentration of $4 \times IC_{50}$ for 15 min and dialysed for 24 h. The residual enzyme activities were subsequently measured. As controls, similar experiments in the presence of the irreversible inhibitors, pargyline and (R)-deprenyl, and in the absence of inhibitor (NI) were performed. MAO activities of undialysed enzyme–inhibitor mixtures were also measured.

6.3.4 MAO inhibition is competitive

Since the inhibition of MAO-A and MAO-B by **7b** and **7a** is reversible, a set of Lineweaver-Burk plots were constructed for each inhibitor to determine whether these compounds act as competitive inhibitors. Each plot was constructed by measuring the MAO activities with eight different concentrations (15–250 μM) of kynuramine as substrate. Each set consisted of six plots constructed at the following inhibitor concentrations: 0 μM , $\frac{1}{4} \times IC_{50}$, $\frac{1}{2} \times IC_{50}$, $\frac{3}{4} \times IC_{50}$, $1 \times IC_{50}$ and $1\frac{1}{4} \times IC_{50}$. The results are given in Fig. 4, and show that, for both compounds, the linear lines intersect on the y-axis. This suggests that these are competitive inhibitors of human MAO-A and MAO-B, and supports the observation that these compounds are reversible inhibitors. From graphs of the slopes of the Lineweaver-Burk plots versus the concentration of inhibitor, the K_i values may

be determined. For the inhibition of MAO-A by **7b**, $K_i = 1.63 \mu\text{M}$, while for the inhibition of MAO-B by **7a**, $K_i = 1.88 \mu\text{M}$. K_i values of $0.81 \pm 0.11 \mu\text{M}$ ($r^2 = 0.96$) and $1.91 \pm 0.14 \mu\text{M}$ ($r^2 = 0.98$) for **7b** and **7a** were obtained by global (shared) fitting of the inhibition data directly to the Michaelis-Menten equation.

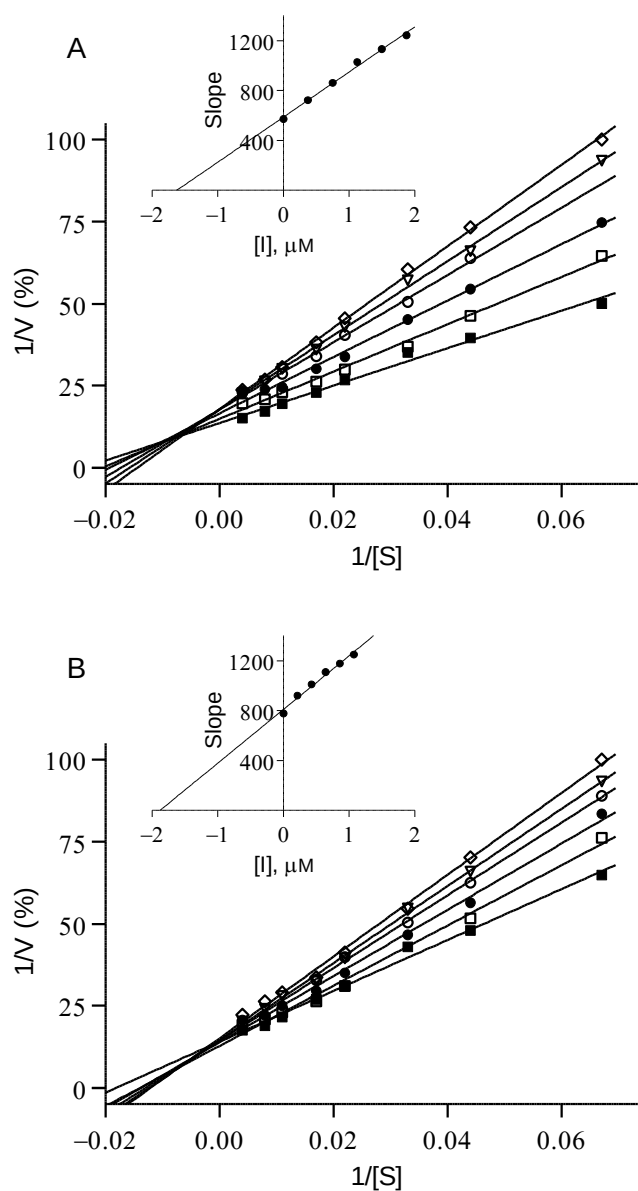


Figure 4: Lineweaver-Burk plots for the inhibition of human MAO-A and MAO-B by **7b** (panel A) and **7a** (panel B).

6.3.5 Docking of selected inhibitors

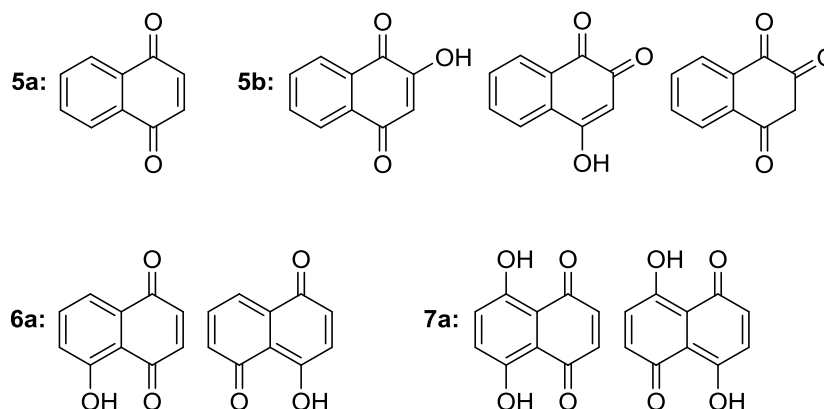


Figure 5: The structures of the compounds and their respective tautomers that were considered for the docking study.

As mentioned above, compounds containing hydroxyl groups on the phenyl ring (**6** and **7**) are relatively potent MAO inhibitors. To gain further insight, **6a** and **7a** were docked into the active sites of MAO-A and MAO-B, and compared to the orientation of **5a**, the parent 1,4-naphthoquinone. Furthermore, compound **5b**, which differs from **5a** in substitution with a hydroxyl group on C2 of the naphthoquinone moiety is, in contrast to **5a**, not a MAO inhibitor. Compound **5b** was thus also docked into the active sites of the MAO enzymes, and compared to **5a**. The structures of the compounds and their respective tautomers considered for the docking study are shown in Fig. 5. The X-ray crystal structures of human MAO-A (PDB code: 2Z5X) (25) and human MAO-B (PDB code: 2V5Z) (26) were used as protein models. All calculations were carried out with the Windows based Discovery Studio 3.1 modelling software (24) according to the literature procedure (23; 34). The CDocker protocol was used for the docking calculations as this protocol has been shown to accurately predict the binding orientations of the cocrystallised ligands in the active sites of these MAO models (23; 34).

The docked orientations in MAO-A are given in Fig. 6 and show that 1,4-naphthoquinone binds in the MAO-A active site with the quinone moiety orientated towards the FAD cofactor. The space in front of the FAD is considered to be the polar region of the MAO-A and MAO-B active sites and the amine groups of substrates bind here, with the amine placed between the phenolic side chains of Tyr-407/Tyr-444 in MAO-A and Tyr-398/Tyr-435 in MAO-B (35). As expected, the 1,4-naphthoquinones are hydrogen bonded with polar functional groups in MAO-A, most notably Tyr-444, Asn-181 and water molecules. All inhibitors (**5a**, **5b**, **6a**, **7a**) form π - π interactions between the quinone moieties and Tyr-407. With the exception of the triketo-tautomer of **5b**, this π - π interaction as well as extensive hydrogen bonding is present for all tautomers of the docked inhibitors.

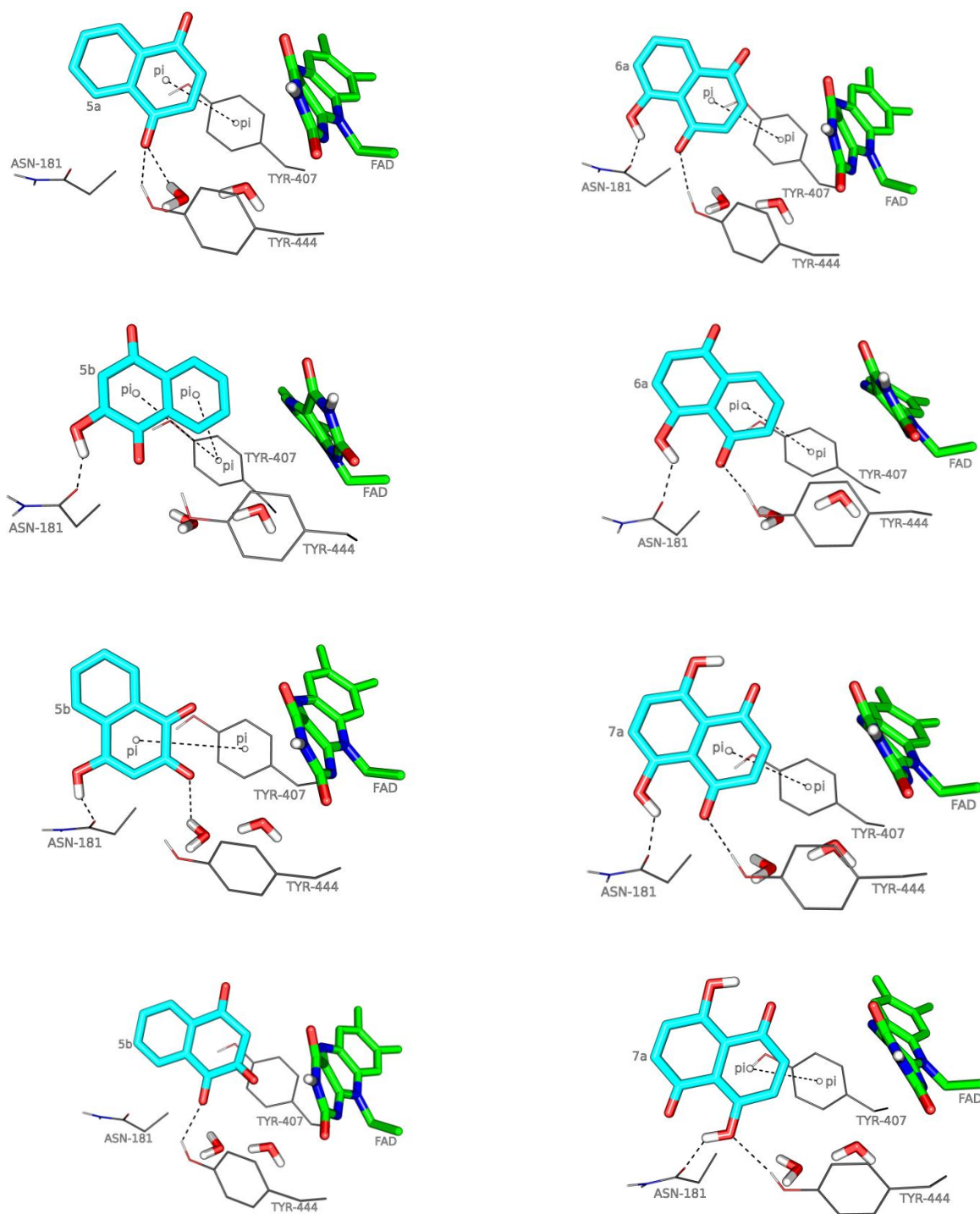


Figure 6: The docked binding orientations of **5a**, **5b**, **6a** and **7a** in an active site model of human MAO-A.

The docked orientations of the 1,4-naphthoquinones in MAO-B are given in Fig. 7. Similarly to MAO-A, the 1,4-naphthoquinones bind in the substrate cavity of MAO-B, in proximity to the FAD cofactor. The 1,4-naphthoquinones undergoes extensive hydrogen bonding with water molecules, and in most orientations, with the phenolic group of Tyr-435. All tautomers of the docked compounds (**5a**, **5b**, **6a**, **7a**) form π - π interactions with Tyr-398.

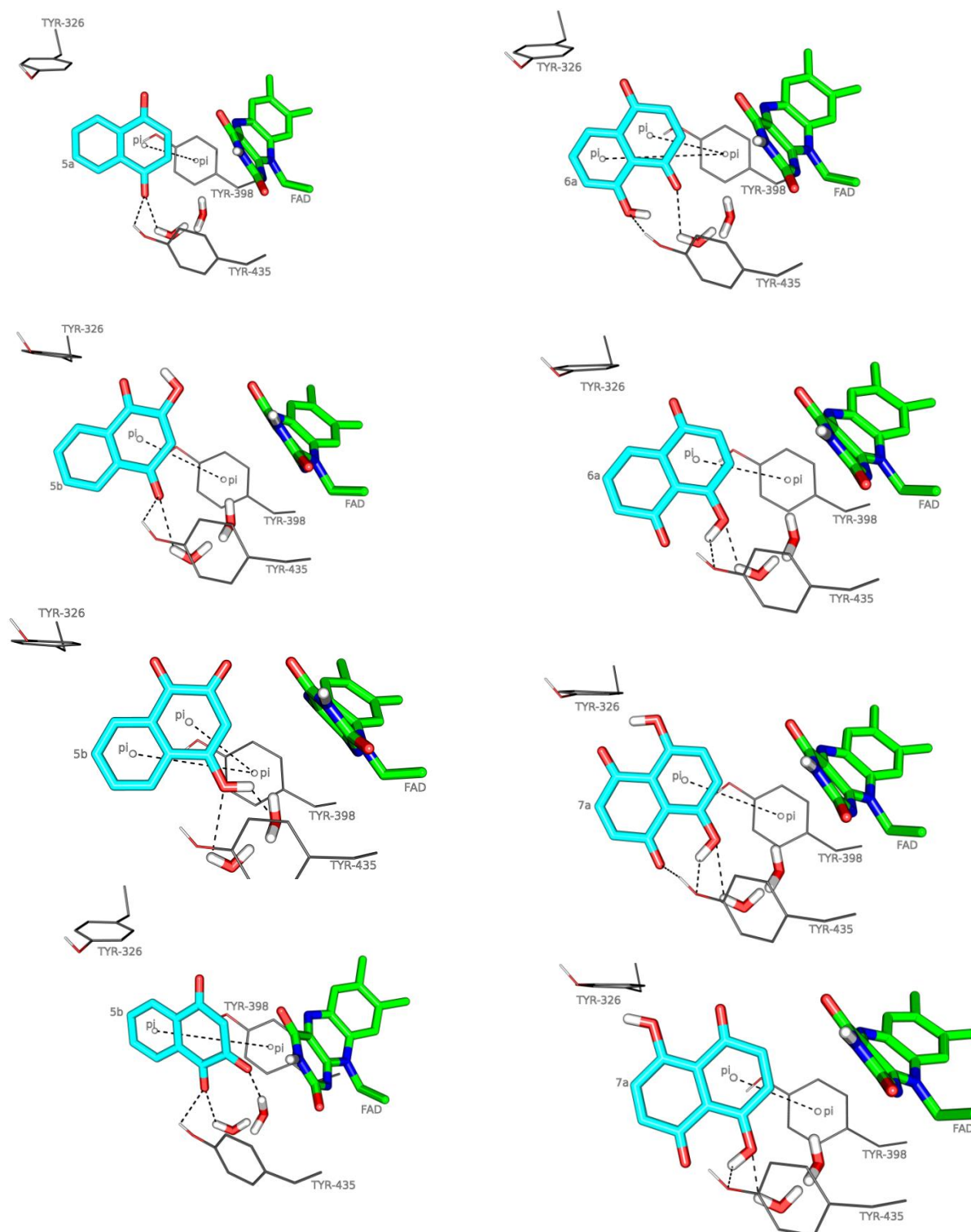


Figure 7: The docked binding orientations of **5a**, **5b**, **6a** and **7a** in an active site model of human MAO-B.

The binding orientations of the 1,4-naphthoquinones are similar to that reported for isatin in complex with MAO-B (Fig. 8) (36). The 2-oxo and pyrrole NH of isatin forms hydrogen bonds with ordered water molecules in the MAO-B active site while a π - π interaction occurs with Tyr-398. For small molecules such as isatin and the 1,4-naphthoquinones, hydrogen bonding and π - π interactions are probably key interactions responsible for inhibitor stabilisation. In support of this,

isatin and the active 1,4-naphthoquinone inhibitors, which form similar interactions with the MAO enzymes, display similar inhibition potencies towards MAO-A and MAO-B. Isatin is reported to inhibit MAO-A and MAO-B with K_i values of 15 μM and 3 μM , respectively, while the active 1,4-naphthoquinones inhibit these isoforms with IC_{50} values of 1.50–20.4 μM and 0.86–24.8 μM , respectively (37). Although **5b** and its tautomers also undergo extensive hydrogen bonding and form π – π interactions, this compound is inactive as a MAO inhibitor. A possible explanation for this observation is not readily apparent. Differing π – π interactions with the tyrosine phenolic rings of MAO probably do not explain the weak MAO inhibition of **5b**, particularly since the binding orientations and interactions of the 1,4-naphthoquinone inhibitors are similar. Differing electronic properties of the 1,4-naphthoquinone inhibitors may, however, affect the formation of π – π interactions, but since the bioactive tautomeric forms of the compounds are not known this proposal requires further investigation (38; 39). A further consideration is the acidity of 1,4-naphthoquinones. C2-Hydroxy-substituted 1,4-naphthoquinones are acidic and are expected to be mostly negatively charged (e.g. **5b**, $\text{pK}_a = 3.98$) at physiological pH where the inhibition studies were carried out (40). Since other hydroxy-substituted 1,4-naphthoquinones, such as **6a** ($\text{pK}_a = 3.65$), act as MAO inhibitors although being of similar acidity, differences in the acidities of the 1,4-naphthoquinones probably also do not explain the differing MAO inhibition activities (41).

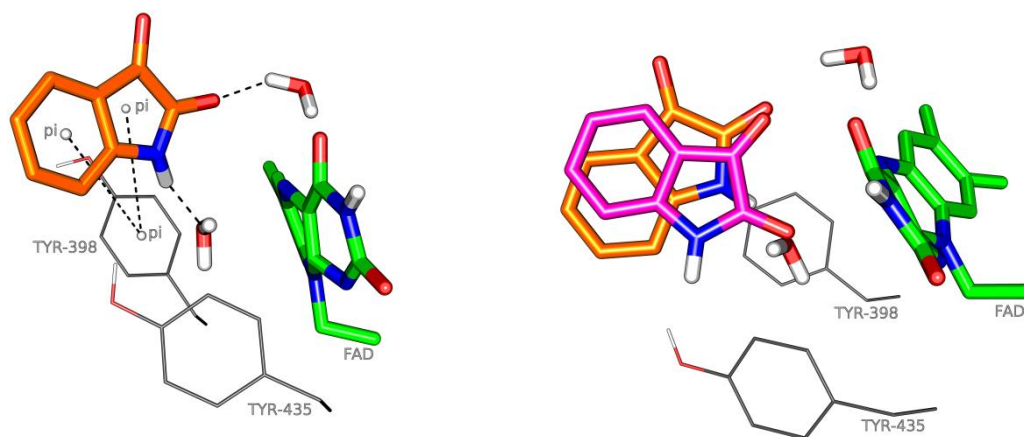


Figure 8: The binding orientation of isatin in a co-crystal (orange) with human MAO-B (left) and a comparison of the docked orientation (magenta) of isatin to the orientation in the co-crystal (right) ($\text{RMSD} = 2.30 \text{ \AA}$) (36).

6.4 Discussion

This study explored the MAO inhibitory properties of a series of fourteen natural and synthetic 1,4-naphthoquinones. The MAO-A inhibition potencies of the active compounds ranged from 1.50 to 20.4 μM , while for MAO-B the potencies ranged from 0.860 to 24.8 μM . Nine (of fourteen) homologues exhibit $\text{IC}_{50} < 5 \mu\text{M}$ for at least one of the MAO isoforms. The inhibition potencies of these inhibitors are within the same range as that of TMN, which is reported to inhibit MAO-A and

MAO-B with K_i values of 3 and 6 μM , respectively (16). In general, the 1,4-naphthoquinones exhibited little isoform selectivity. The only compounds with appreciable isoform selectivity are **5f** and **5g**, which are MAO-B inhibitors, but not MAO-A inhibitors. Compound **5g** may be emphasised as a particularly interesting MAO-B selective inhibitor with an $\text{IC}_{50} < 5 \mu\text{M}$. An interesting finding of the present study was that, with the exception of **5g**, 2-hydroxy-substituted 1,4-naphthoquinones are not MAO inhibitors. By employing molecular docking experiments further insight into the inhibition of the MAOs by 1,4-naphthoquinones were obtained. The modelling study shows that 1,4-naphthoquinones have similar binding orientations in the active sites of the MAO enzymes and form similar interactions, particularly with respect to hydrogen bonding and π – π interactions. Of importance is the observation that in MAO-B, the 1,4-naphthoquinones bind in the substrate cavity, leaving the entrance cavity unoccupied. As was shown with isatin, appropriate substitution with the styryl or benzyloxy side chains could yield compounds that bind to both the substrate and entrance cavities (42; 43). This, in turn, greatly enhances MAO-B inhibition potency (37). For the 1,4-naphthoquinones the appropriate substitution on C6 and C7 of the 1,4-naphthoquinone moiety would lead to such cavity-spanning compounds. This study shows that the 1,4-naphthoquinone class of compounds are active MAO inhibitors, which may be useful in disorders such as Parkinson's disease, depressive illness, congestive heart failure and cancer. Further structure modification should lead to more active inhibitors, particularly of MAO-B.

6.5 Acknowledgements

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Conflict of Interest

The authors have no conflicts of interest to declare

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Chapter 7

Manuscript D

Article awaiting submission to Chemical Biology and Drug Design entitled:

The evaluation of 1,4-benzoquinones as inhibitors of human monoamine oxidase

No graphical abstract

The manuscript is written in a letter format and is presented according to the author guidelines as stipulated in addendum B.

Authors' contributions

- The experimental work, interpretation of results and documentation of this study was carried out by **S. Mostert**.
- This study was conceptualised and documented with the assistance of **J.P. Petzer**.
- The enzymology section of this study was conducted under the supervision of **A. Petzer**.

All co-authors provided permission to use this manuscript as part of S Mostert's Ph.D thesis.

The evaluation of 1,4-benzoquinones as inhibitors of human monoamine oxidase

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Keywords: monoamine oxidase, MAO, inhibition, irreversible, dialysis, 1,4-benzoquinone.

Running title: 1,4-Benzoquinones as MAO inhibitors

Abstract

The monoamine oxidase (MAO) enzymes are of considerable pharmacological interest and inhibitors are used in the clinic for the treatment of major depressive disorder and Parkinson's disease. A limited number of studies have shown that the quinone class of compounds possesses MAO inhibition properties. Most notable among these is a report that 2,3,6-trimethyl-1,4-naphthoquinone (TMN), present in extracts of cured tobacco leaves, is a non-selective inhibitor of both MAO isoforms. An older study reports that 1,4-benzoquinone inhibits MAO-A and MAO-B from human synaptosomes. Both 1,4-naphthoquinones and 1,4-benzoquinone are reported to inhibit the MAOs with a reversible mode of action. Since the MAO inhibition properties of additional members of the 1,4-benzoquinone class of compounds have not yet been explored, the present study investigates a small series of four 1,4-benzoquinones which incorporate phenyl, benzyl, benzyloxy and cyclopentyl monosubstitution on C2. The 1,4-benzoquinones were found to be moderately potent MAO inhibitors with IC₅₀ values of 5.03–13.2 µM (MAO-A) and 3.69–23.2 µM (MAO-B). These values are comparable to those recorded for 1,4-benzoquinone of 4.82 µM (MAO-A) and 10.2 µM (MAO-B). Of interest however, is the finding that the 1,4-benzoquinones are irreversible inhibitors of MAO-A since prolonged incubation results in near complete inhibition, and enzyme activity is not recovered by dialysis. MAO-B is much less sensitive to inactivation by the 1,4-benzoquinones. These findings are discussed with reference to a possible mechanism by which irreversible inhibition occurs. It may be concluded that irreversible 1,4-benzoquinone-derived inhibitors may act as probes for investigating quinone reactive sites in the MAOs.

Letter

The monoamine oxidase (MAO) enzymes are involved in the metabolism of neurotransmitter amines and amine-containing compounds from the diet (1). Mammals have two forms of the enzyme, namely MAO-A and MAO-B. Both isoforms are found in the brain (2). In the periphery, MAO-A is predominantly present in the gastrointestinal mucosa and placenta, while MAO-B is the main isoform in platelets. Hepatic tissue expresses both MAO-A and MAO-B (3; 4). The MAO enzymes are encoded by distinct genes and exhibit a high degree of similarity (approximately 70%) at the amino acid level (5; 6). The active sites of MAO-A and MAO-B are also similar in amino acid residues and their respective orientations, and only six of the sixteen active site residues differ between the two isozymes (7; 8). It is therefore not surprising that significant overlap in substrate specificities occur between the two MAO isoforms. For example, epinephrine, norepinephrine, dopamine and tyramine are good substrates for both MAO-A and MAO-B. Serotonin, on the other hand, may be considered as a MAO-A-specific substrate, while the dietary amines, benzylamine and 2-phenylethylamine are MAO-B-specific (9). Since the MAOs exhibit different tissue distributions, in certain instances they have tissue-specific functions. For example, MAO-B present in the microvasculature of the brain may act as a metabolic barrier for false neurotransmitters such as benzylamine and 2-phenylethylamine (10). Similarly, in gastrointestinal mucosa, MAO-A is a barrier for the uptake of dietary tyramine into the systemic circulation. Tyramine is a sympathomimetic amine which may induce the release of norepinephrine from peripheral neurons if absorbed in excessive amounts from the gastrointestinal tract (1). This leads to an acute increase in blood pressure, known as tyramine-induced hypertensive crisis or the “cheese-effect” (11; 12). Tyramine-induced hypertensive crisis often occurs when irreversible MAO-A inhibitors are taken with food such as cheese, which is rich in tyramine.

Based on their roles in the metabolism of neurotransmitters, the MAOs are of considerable pharmacological importance (9). MAO-A inhibitors are used in the clinic for the treatment of major depressive disorder and act by inhibiting the metabolism of serotonin, norepinephrine and dopamine, thereby enhancing neurotransmission mediated by these neurotransmitters (9). MAO-A inhibitors that have been used in the clinic for depression include phenelzine, isocarboxazid, tranylcypromine and iproniazid (Fig. 1) (9; 13). These are irreversible MAO-A inhibitors, which are used restrictively in the clinic due to the occurrence of tyramine-induced hypertensive crisis. Reversible inhibitors such as moclobemide and toloxatone, on the other hand, represent safer and better-tolerated MAO-A inhibitors (14; 15). A transdermal delivery system of the MAO-B selective inhibitor, (R)-deprenyl (selegiline), has also been shown to be effective in the treatment of major depressive disorder in clinical trials (16). Since this formulation does not lead to the inhibition of MAO-A in the gastrointestinal and hepatic systems, the risk of tyramine-induced hypertension is low. MAO-B inhibitors are, however, most frequently used as adjuncts to L-Dopa in the treatment of Parkinson's disease (9; 13). In Parkinson's disease MAO-B inhibitors elevate central dopamine

levels, especially when combined with L-Dopa, the metabolic precursor of dopamine. MAO inhibitors may also act as potential neuroprotective agents in Parkinson's disease. Hydrogen peroxide and aldehydes produced by the MAOs in the brain, are thought to contribute to neurodegeneration (1; 17). MAO inhibitors reduce the formation of these metabolic by-products and thus may slow the degenerative process. MAO-B inhibitors, in particular, may be of relevance in age-related degenerative disorders such as Parkinson's disease since MAO-B activity, and not MAO-A activity, increases in the brain with age (18). Two irreversible MAO-B inhibitors, (R)-deprenyl and rasagiline, are registered for this purpose, while a reversible MAO-B inhibitor, safinamide, has completed phase III development for the management of Parkinson's disease (19; 20).

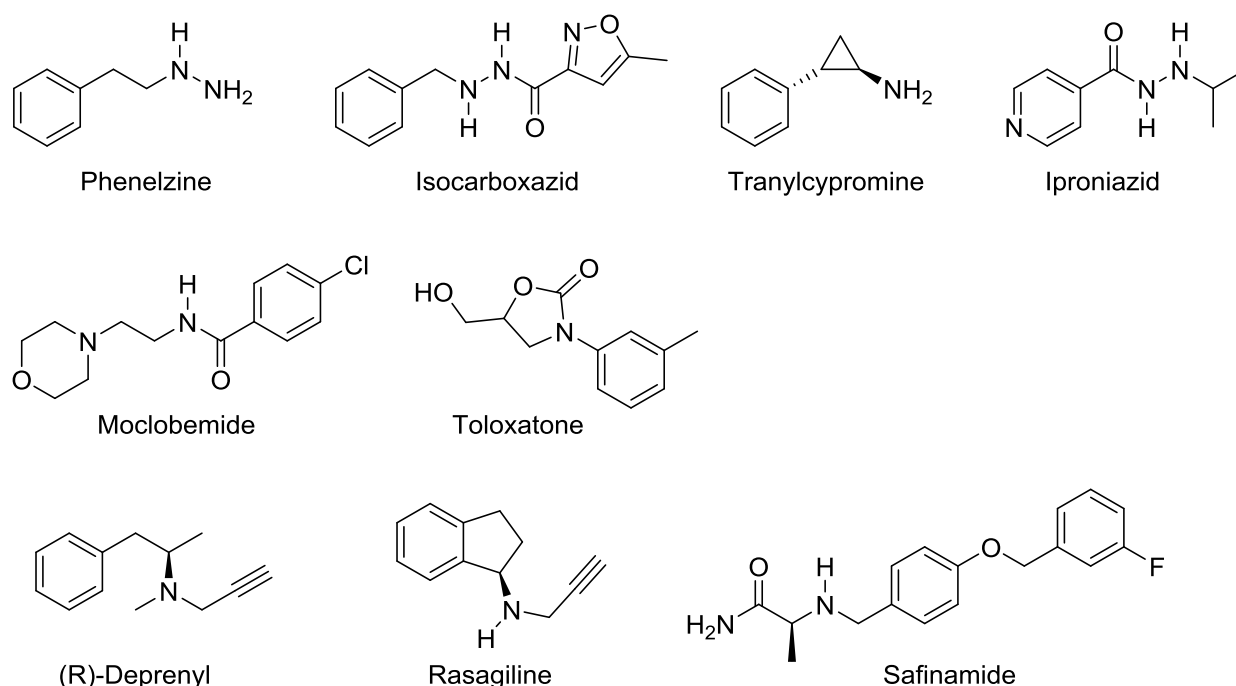


Figure 1: The structures of selected MAO inhibitors discussed in the text.

MAO inhibitors may also be relevant to other conditions, and are thus under investigation as therapies for Alzheimer's disease, certain types of cancer and age-related impairment of cardiac function. In Alzheimer's disease, MAO inhibitors may reverse amyloid β peptide ($A\beta$) pathology and improve cognitive deficits (21), while MAO-A inhibitors acting synergistically with surviving suppressants, may inhibit prostate cancer cell growth, migration and invasion (22; 23). Based on the observation that hydrogen peroxide produced by MAO-A in the heart increases with age in rats, it has been suggested that MAO-A inhibitors may slow age-related impairment of cardiac function by reducing hydrogen peroxide-mediated cardiac cellular degeneration (24).

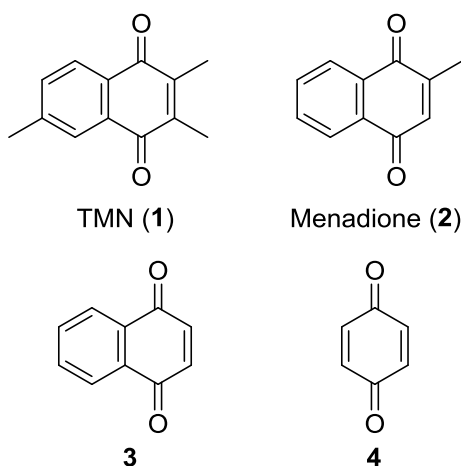


Figure 2: The structures of selected 1,4-naphthoquinones and 1,4-benzoquinone.

Motivated by a financial interest in MAO inhibitors, but also the academic challenge of designing high potency and isoform-specific inhibitors, the discovery of new MAO inhibitors is pursued by a number of research groups. A limited number of studies have reported that the quinone class of compounds, specifically 1,4-naphthoquinones and 1,4-benzoquinone, possess MAO inhibition properties. For example, 2,3,6-trimethyl-1,4-naphthoquinone (TMN, **1**), a compound isolated from cured tobacco leaves, was shown to inhibit human MAO-A and MAO-B with K_i values of 3 μM and 6 μM , respectively (Fig. 2) (25). Another study reported that other 1,4-naphthoquinones such as menadione (**2**) and the parent 1,4-naphthoquinone (**3**), also inhibits the MAO isoforms (26). An older study reports that 1,4-benzoquinone (**4**) inhibits MAO-A and MAO-B from human brain synaptosomes with K_i values of 9.62 μM (MAO-A) and 20.3 μM (MAO-B) (27). Both 1,4-naphthoquinones and 1,4-benzoquinone inhibit the MAOs with a reversible mode of action. Since the MAO inhibition properties of additional members of the 1,4-benzoquinone class of compounds have not yet been explored, the present study investigates a small series of four 1,4-benzoquinones (**5a–d**) which incorporate phenyl, benzyl, benzyloxy and cyclopentyl monosubstitution on C2 (Table 1). As shown by many literature examples, substitution of a small molecule MAO inhibitor with a variety of ring systems often has a large effect on potency as well as isoform-specificity (28; 29). For example, substitution (on C5) of the small molecule, isatin (**6**), with the phenyl ring (to yield **7**) enhances MAO-A inhibition by 56-fold, while substitution with the benzyloxy moiety to yield **8**, enhances MAO-B inhibition by 120-fold (Fig. 3) (29).

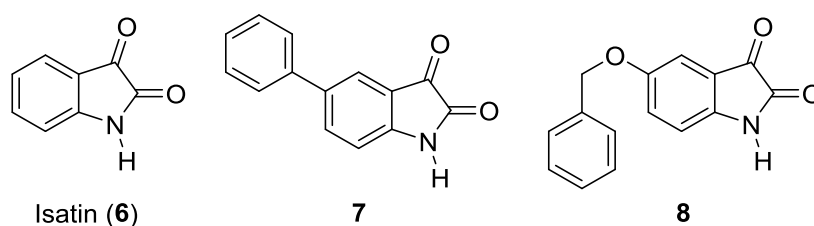
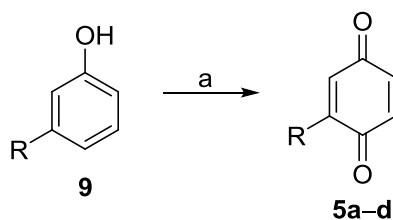


Figure 3: The structures of isatin and synthetic analogues thereof.

A number of synthetic approaches have been reported for the preparation of 1,4-benzoquinones (30). In the present study the 1,4-benzoquinone analogues **5a–d** were synthesised by oxidation of the corresponding commercially available phenols (**9**) according to a previously reported procedure (Scheme 1) (31). The phenols (4.5 equiv.) were thus combined with hydrated salcomine [N,N'-bis(salicylidene)ethylenediaminocobalt(II) hydrate; 1 equiv.] in acetonitrile and the resulting mixtures were stirred at room temperature under an atmosphere of oxygen. The reaction progress was monitored with neutral aluminium oxide thin-layer chromatography (n-hexane/ethyl acetate 9:1; visualised by staining with iodine vapour), and after approximately 24 h the reaction solvent was removed in vacuo and petroleum ether (boiling range 60–80° C) was added to the crude. After heating the resulting mixture to boiling, the salcomine was removed by hot filtration and the filtrate was cooled to room temperature. The crystals which formed were collected and, for compounds **5a** and **5c**, were found to be analytically pure. For compounds **5b** and **5d**, the target 1,4-benzoquinone was purified by distillation under reduced pressure using a quick-distillation setup (Aldrich®). These compounds were finally recrystallised from n-hexane/ethyl acetate to remove any residual phenol reagent. The yields obtained ranged from 6.4 to 24%. The structures and purities of the target compounds were verified by ¹H NMR, ¹³C NMR, mass spectrometry and HPLC analysis as cited in the supplementary data.



Scheme 1: Synthetic route to the 1,4-benzoquinone derivatives **5a–d**. Reagents and conditions: (a) acetonitrile, hydrated salcomine, O₂, 24 h.

The MAO inhibition properties of the 1,4-benzoquinone analogues (**5a–d**) were evaluated using the recombinant human MAO-A and MAO-B enzymes (32). For comparison, 1,4-benzoquinone (**4**) was included in this study. The potencies by which the 1,4-benzoquinones inhibit the MAOs are expressed as the IC₅₀ values, which were determined from sigmoidal plots of residual enzyme activity versus inhibitor concentration. To measure MAO activities in the absence and presence of the test inhibitors, the non-specific MAO substrate, kynuramine, was used. Kynuramine is metabolised by the MAOs to yield 4-hydroxyquinoline, a fluorescent metabolite which can be readily quantified by fluorescence spectrophotometry (33; 34). Examples of sigmoidal plots for the inhibition of MAO-A and MAO-B by **5d** and **4**, respectively, are shown in Fig. 4.

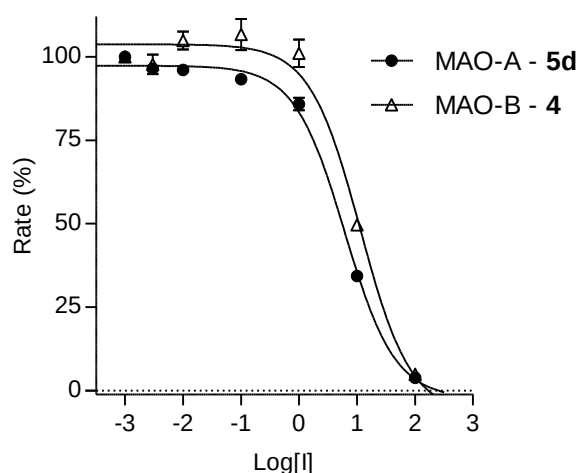
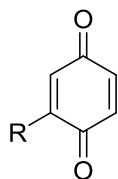


Figure 4: Examples of sigmoidal plots for the inhibition of MAO-A and MAO-B by compound **5d** and **4**, respectively.

The IC_{50} values for the inhibition of the MAOs by the 1,4-benzoquinone analogues (**5a–d**) are given in Table 1. The results show that 1,4-benzoquinones **5a–d** inhibit the MAOs with IC_{50} values in the ranges of 5.03–13.2 μ M (MAO-A) and 3.69–23.2 μ M (MAO-B). The parent 1,4-benzoquinone (**4**) exhibits IC_{50} values of 4.82 μ M (MAO-A) and 10.2 μ M (MAO-B). The IC_{50} values recorded for **4** are comparable to the K_i values reported previously (27). As mentioned, compound **4** is reported to inhibit MAO-A and MAO-B from human brain synaptosomes with K_i values of 9.62 μ M and 20.3 μ M, respectively (27). From the inhibition data it is evident that the 1,4-benzoquinones are moderately potent MAO inhibitors. In general, high potency MAO inhibitors possess inhibition potencies (IC_{50} or K_i values) in the lower nanoMolar range. Examples of these are the MAO-A inhibitor, methylene blue, which exhibits an IC_{50} value of 0.07 μ M (35) and the MAO-B inhibitors, lazabemide and safinamide, with IC_{50} values of 0.091 μ M and 0.048 μ M, respectively (36). The 1,4-benzoquinones also display little isoform selectivity with selectivity index values ranging from 0.3 to 4.3. It is, however, notable that with the exception of **5c**, all 1,4-benzoquinones display slightly lower IC_{50} values for MAO-A compared to the MAO-B isoform. As shown below, this may, at least in part, be due to differences in reversibility of inhibition between the two isoforms and not necessarily due to higher affinities of the 1,4-benzoquinones for the MAO-A enzyme. Another point of interest is that, although the 1,4-benzoquinones are only moderate MAO inhibitors, their inhibition potencies are in a similar range as those reported for the 1,4-naphthoquinone, TMN [IC_{50} (MAO-A) = 3 μ M; IC_{50} (MAO-B) = 6 μ M] (25). Similarly, the clinically used MAO-A inhibitor, toloxatone, inhibits MAO-A with an IC_{50} value of 3.92 μ M, which is similar in potency to those of the 1,4-benzoquinones examined here (36).

Table 1: The IC₅₀ values for the inhibition of recombinant human MAO-A and MAO-B by 1,4-benzoquinone derivatives **5a–d** and 1,4-benzoquinone (**4**).



	R	IC ₅₀ (μM) ^a		SI ^b
		MAO-A	MAO-B	
5a	C ₆ H ₅ –	5.03 ± 0.928	7.87 ± 0.647	1.6
5b	C ₆ H ₅ CH ₂ –	5.37 ± 0.092	23.2 ± 1.48	4.3
5c	C ₆ H ₅ CH ₂ O–	13.2 ± 2.40	3.69 ± 0.457	0.3
5d	C ₅ H ₉ –	5.84 ± 0.190	17.8 ± 1.34	3.0
4	H	4.82 ± 0.120	10.2 ± 0.421	2.1

^a All values are expressed as the mean ± standard deviation (SD) of triplicate determinations.

^b The selectivity index is the selectivity for the MAO-A isoform and is given as the ratio of IC₅₀(MAO-B)/IC₅₀(MAO-A).

When investigating the MAO inhibitory properties of new compounds, the reversibility of inhibition is an important factor to consider. This is particularly relevant to MAO-A inhibition since, as mentioned, irreversible MAO-A inhibitors may induce a severe elevation in blood pressure when taken with food rich in tyramine (11; 12). Reversible MAO-A inhibitors such as moclobemide and toloxatone are safer in this regard and do not potentiate the sympathomimetic effects of tyramine when administered in clinically relevant doses and taken with amounts of tyramine consistent with normal dietary intake (14; 15). Both reversible and irreversible inhibitors of MAO-B, on the other hand, do not cause tyramine-induced changes in blood pressure and these agents have good safety profiles (16). To investigate the reversibility of MAO inhibition by the 1,4-benzoquinones, mixtures containing the MAO enzymes and test inhibitors were incubated for 15 min and subsequently dialysed for 24 h (34). The concentrations of the test inhibitor in these mixtures were equal to 4 × IC₅₀ for the inhibition of a particular MAO isoform. After dialysis, the mixtures were diluted twofold (to yield 2 × IC₅₀ of the test inhibitor) with the addition of the enzyme substrate, kynuramine, and the residual enzyme activities were measured. Dialysis studies were conducted with compounds **5a**, **5b** and **5d** as well as 1,4-benzoquinone (**4**). As positive controls, dialysis

studies with the irreversible MAO-A and MAO-B inhibitors, pargyline and (R)-deprenyl, were included. The negative control experiments constituted dialysis of the MAO enzymes in the absence of inhibitor and signified 100% residual enzyme activity. For comparison, the residual MAO activities of undialysed mixtures of the enzymes and test inhibitors (at $4 \times \text{IC}_{50}$) were also recorded after twofold dilution as above.

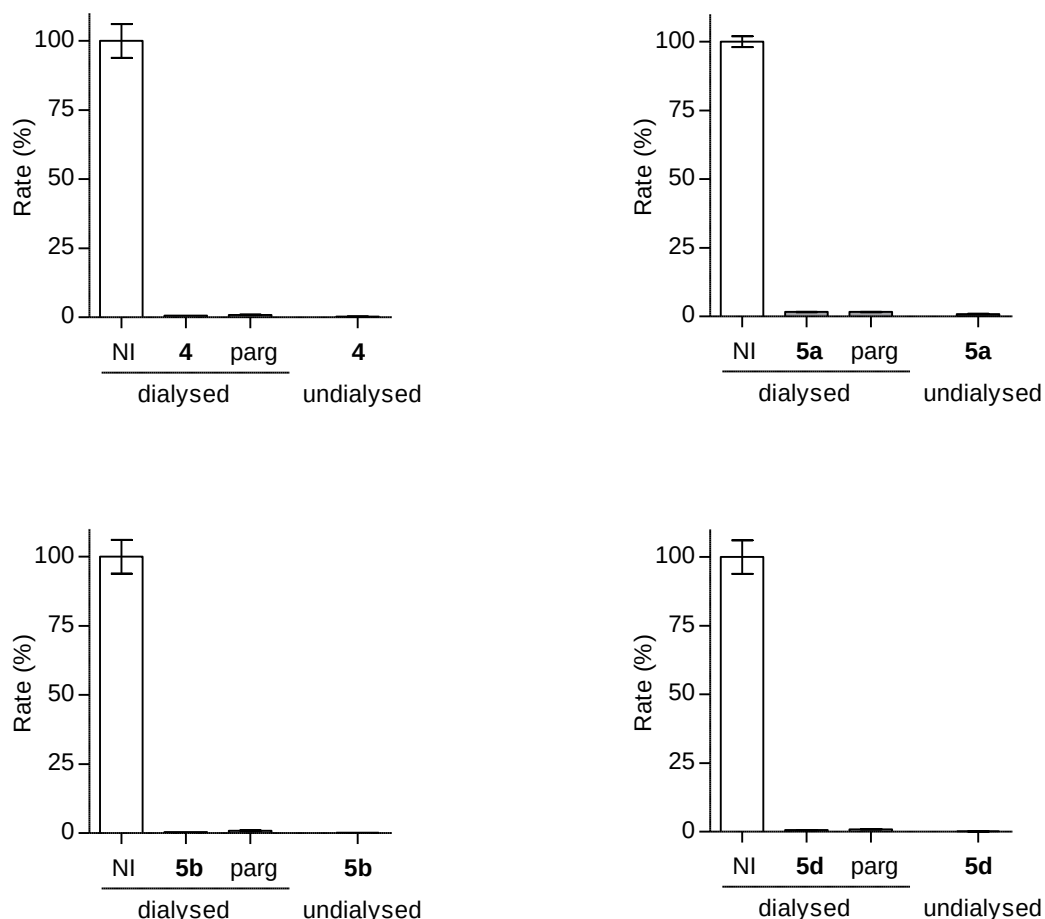


Figure 5: The reversibility of MAO-A inhibition by 1,4-benzoquinone (**4**) and compounds **5a**, **5b** and **5d**. MAO-A was incubated for 15 min in the presence of the test inhibitors (at $4 \times \text{IC}_{50}$), dialysed for 24 h and the residual enzyme activities were measured. Dialysis of MAO-A in the absence of inhibitor (NI dialysed) and presence of the irreversible inhibitor, pargyline (parg dialysed), were also conducted. The residual activities of undialysed mixtures of MAO-A and the test inhibitors are also shown (undialysed).

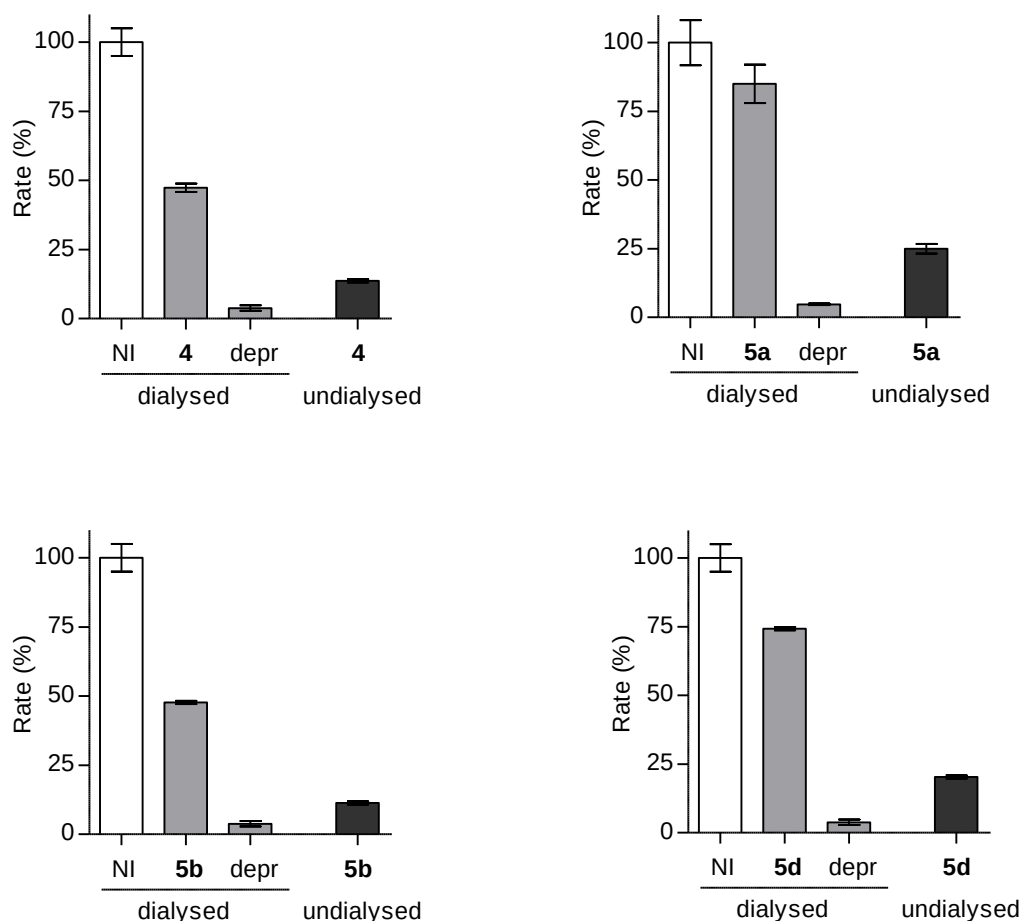


Figure 6: The reversibility of MAO-B inhibition by 1,4-benzoquinone (**4**) and compounds **5a**, **5b** and **5d**. MAO-B was incubated for 15 min in the presence of the test inhibitors (at $4 \times \text{IC}_{50}$), dialysed for 24 h and the residual enzyme activities were measured. Dialysis of MAO-B in the absence of inhibitor (NI dialysed) and presence of the irreversible inhibitor, (R)-deprenyl (depr dialysed), were also conducted. The residual activities of undialysed mixtures of MAO-B and the test inhibitors are also shown (undialysed).

The results of the dialysis studies are given in Figs. 5 and 6. As shown, dialysis restores MAO-B activity at least partially. After dialysis of mixtures containing MAO-B and the test inhibitors (**4**, **5a**, **5b** and **5d**) MAO-B activity is recovered to 47–85% of the negative control (Fig. 6). The enzyme activities of undialysed mixtures are comparatively lower at 11–25%. For reversible inhibition, dialysis should restore enzyme activity to 100%. As expected MAO-B activity is not recovered after incubation and dialysis in the presence of the irreversible inhibitor (R)-deprenyl, with the residual activities at 3.9–4.8%. Unexpected results were obtained with the MAO-A dialysis experiments. As shown in Fig 5, dialysis of mixtures containing MAO-A and the test inhibitors failed to restore enzyme activity with the residual activity at only 0.4–1.7% of the negative control. The enzyme activities of undialysed mixtures of MAO-A and the 1,4-benzoquinones also are well below the

expected 25%, with values of 0.9%. In undialysed mixtures, a residual activity of 25% is expected for a reversible inhibitor at a concentration of $2 \times \text{IC}_{50}$. Dialysis of mixtures of MAO-A and the irreversible inhibitor, pargyline, also failed to restore enzyme activity (residual activity at 0.1–1.6%). The dialysis experiments with the test 1,4-benzoquinones thus yielded similar results as those with pargyline and suggests that **4**, **5a**, **5b** and **5d** are irreversible inhibitors of MAO-A. The irreversible nature of MAO-A inhibition by the 1,4-benzoquinones are best illustrated by (1) the failure of dialysis to restore enzyme activity and (2) the almost complete inhibition at a concentration of $2 \times \text{IC}_{50}$, behaviour consistent with time-dependent irreversible inhibition and not reversible inhibition. This irreversibility of inhibition is in contrast to literature, which reports that 1,4-benzoquinone is a reversible inhibitor of human MAO-A and MAO-B (27). Reasons for the different findings are not apparent.

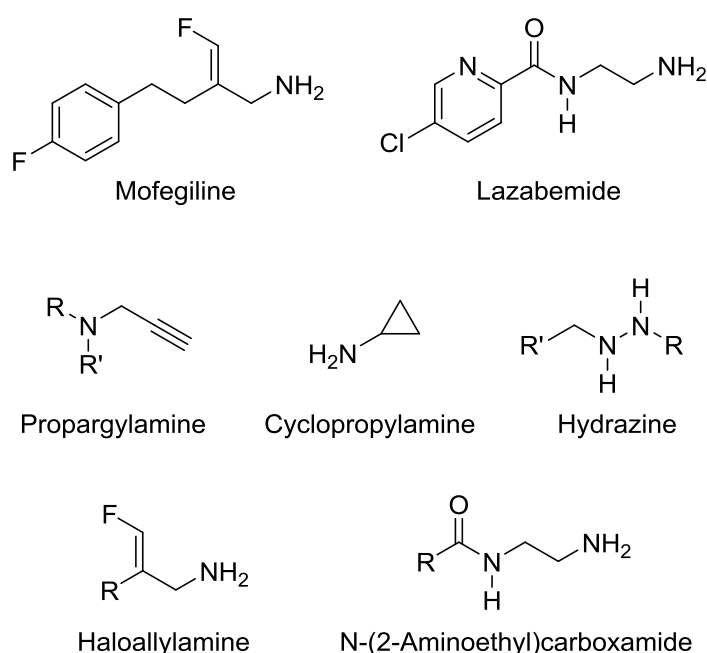


Figure 7: The structures of mofegiline and lazabemide, and the general structures of classes of mechanism-based MAO inhibitors.

Irreversible inhibitors of the MAOs are well known. Irreversible MAO inhibitors are usually mechanism-based inhibitors and contain functionalities such as the propargylamine [e.g. (R)-deprenyl, rasagiline], cyclopropylamine (e.g. tranlylcypromine), hydrazine (e.g. phenelzine, isocarboxazid), haloallylamine (mofegiline) and N-(2-aminoethyl)carboxamide (e.g. lazabemide) moieties (Fig. 7). These inhibitors are metabolised by the MAOs to reactive intermediates which form covalent adducts with the flavin cofactor at the N(5) and C(4a) positions of the flavin (37; 38; 39). The 1,4-benzoquinone inhibitors, however, are not expected to act as mechanism-based inhibitors but since the 1,4-benzoquinone moiety is an electrophile, these compounds may covalently modify cysteine and lysine residues in proteins (40; 41; 42; 43; 44; 45). Covalent modification of residues in the MAO-A active site at a location which prevents or hinders substrate

binding would explain the irreversibility of inhibition of this class of compounds. While the active site of MAO-A does not contain cysteine or lysine residues (which are accessible to an inhibitor) (7), the reduced FAD co-factor may represent an appropriate solvent accessible nucleophile for reacting with 1,4-benzoquinones. Although structural studies will provide conclusive evidence, the covalent modification of the FAD seems most likely since this is also the site of interaction of the various classes of mechanism-based inhibitors. The MAO-B active site, however, contains a cysteine residue (Cys172) (8), which may, in addition to the reduced FAD co-factor, represent a site for modification by 1,4-benzoquinones. As shown by the dialysis experiments, MAO-B is, however, much less sensitive to inactivation by 1,4-benzoquinones since dialysis restores activity at least partially. An explanation for the differential sensitivities of MAO-A and MAO-B towards inactivation by 1,4-benzoquinones would require further investigation, particularly with respect to the site and mechanism of covalent modification. A potential mechanism by which 1,4-benzoquinones reacts with nucleophiles is proposed in Fig. 8. The first step involves 1,4-addition of the nucleophile to the quinone. Subsequent protonation yields the covalent adduct. Although not shown, instead of the protonation step, reoxidation to yield the functionalised quinone also is a possibility.

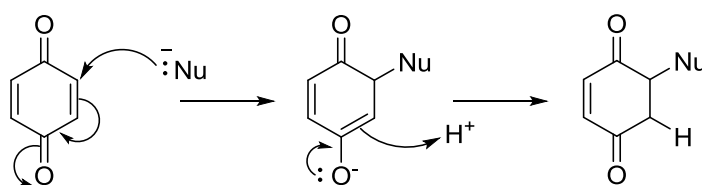


Figure 8: A potential mechanism by which nucleophiles react with 1,4-benzoquinones to form covalent adducts.

In conclusion, this study provides the first evidence that 1,4-benzoquinones inactivate human MAO-A. Human MAO-B is much less sensitive to this inactivation. Structural studies are required to determine the site and mechanism of covalent modification by 1,4-benzoquinones. 1,4-Benzoquinone-derived inactivators may thus act as probes for investigating quinone reactive sites in the MAOs. The implications of irreversible inhibition of MAO-A by endogenous as well as 1,4-benzoquinones present in the environment should also be considered in future studies, particularly with respect to the possibility of tyramine-induced changes in blood pressure following inactivation of MAO-A.

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Conflict of Interest

The authors confirm that this article content has no conflict of interest.

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Supplementary material

The evaluation of 1,4-benzoquinones as inhibitors of human monoamine oxidase

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1. Chemicals and instrumentation

Reagents: All reagents were obtained from Sigma-Aldrich and were used without further purification.

NMR spectroscopy: Proton (¹H) and carbon (¹³C) NMR spectra were recorded on a Bruker Avance III 600 spectrometer in CDCl₃. The chemical shifts are reported in parts per million (δ) and were referenced to the residual solvent signal at 7.26 and 77.0 ppm for ¹H and ¹³C NMR spectra, respectively. Spin multiplicities are abbreviated as follow: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), qn (quintet) or m (multiplet).

Mass spectrometry (MS): High resolution mass spectra (HRMS) were recorded with a Bruker micrOTOF-Q II mass spectrometer in atmospheric-pressure chemical ionization (APCI), positive ion mode.

Melting point (mp): Melting points were measured with a Buchi M-545 melting point apparatus and are uncorrected.

High performance liquid chromatography (HPLC): To determine the purity, HPLC analyses were carried out with an Agilent 1200 HPLC system, equipped with a quaternary pump and Agilent 1200 series diode array detector. HPLC grade acetonitrile (Merck) and Milli-Q water were used for the chromatography. A Venusil XBP C18 column (4.60 × 150 mm, 5 μm) was used with 30% acetonitrile and 70% Milli-Q water as the initial mobile phase (flow rate of 1 mL/min). At the start of each HPLC run, a solvent gradient program was initiated by increasing the acetonitrile composition in the mobile phase linearly to 85% over a period of 5 min. Each HPLC run lasted 15 min with 5 min equilibration between runs. The analytes were dissolved in acetonitrile (1 mM) and 20 μL was injected into the HPLC system. The eluent was monitored at a wavelength of 254 nm.

Fluorescence spectrophotometry: Fluorescence spectrophotometry was carried out with a Varian Cary Eclipse fluorescence spectrophotometer.

Biological reagents: Kynuramine.2HBr and insect cell microsomes containing recombinant human MAO-A and MAO-B (5 mg/mL) were obtained from Sigma-Aldrich.

2. Synthesis of 1,4-benzoquinone analogues 5a–d

The appropriately substituted phenols were commercially available from Sigma Aldrich and were converted to the respective 1,4-benzoquinone analogues according to a previously reported protocol (1). The phenols (4.5 mmol) were dissolved in acetonitrile (50 mL). Hydrated salcomine [N,N'-bis(salicylidene)ethylenediaminocobalt(II) hydrate; 1 mmol] was added to the reaction. The mixture was stirred in an atmosphere of oxygen for 24 h. During this time the reaction progress was monitored with neutral aluminium oxide thin-layer chromatography using n-hexane/ethyl acetate (9:1) as mobile phase. The developed sheets were visualised by staining with iodine vapour. Upon completion of the reaction, the acetonitrile was removed *in vacuo* and petroleum ether (boiling range 60–80 °C) was added to the residue. After heating the resulting mixture to boiling, the salcomine was removed by hot filtration and the filtrate was cooled to room temperature. The crystals which formed were collected and, for compounds **5a** and **5c**, were found to be analytical pure. For compounds **5b** and **5d**, the target 1,4-benzoquinone was purified by distillation under reduced pressure using a quick-distillation setup (Aldrich®). Compounds **5b** and **5d** were finally recrystallised from n-hexane:ethyl acetate (n-hexane in large excess) to remove any residual phenol reagent.

2-Phenyl-1,4-benzoquinone (5a)

The title compound was prepared in a yield of 9.3%: mp 113 °C. ¹H NMR (600 MHz, CDCl₃) δ 6.80 (dd, J = 2.3, 9.7 Hz, 1H), 6.84–6.86 (m, 2H), 7.40–7.47 (m, 5H); ¹³C NMR (151 MHz, CDCl₃) δ 128.5, 129.2, 130.1, 132.6, 132.6, 136.2, 137.0, 145.9, 186.6, 187.6; APCI-HRMS *m/z*: calcd for C₁₂H₉O₂ (MH⁺), 185.0597, found 185.0599; purity (HPLC): 95.7%.

2-Benzyl-1,4-benzoquinone (5b)

The title compound was prepared in a yield of 14%: mp 43 °C. ¹H NMR (600 MHz, CDCl₃) δ 3.72 (s, 2H), 6.35 (d, J = 2.3 Hz, 1H), 6.69 (dd, J = 2.6, 10.1 Hz, 1H), 6.75 (d, J = 9.8 Hz, 1H), 7.17 (d, J = 7.2 Hz, 2H), 7.22–7.25 (m, 1H), 7.31 (t, J = 7.2 Hz, 2H); ¹³C NMR (151 MHz, CDCl₃) δ 35.2, 127.0, 128.8, 129.3, 133.2, 136.3, 136.6, 148.7, 187.2, 187.7; APCI-HRMS *m/z*: calcd for C₁₃H₁₀O₂ (M⁺), 198.0681, found 198.0688; purity (HPLC): 99.0%.

2-Benzyloxy-1,4-benzoquinone (5c)

The title compound was prepared in a yield of 6.4%: mp 114.2 °C. ¹H NMR (600 MHz, CDCl₃) δ 5.02 (s, 2H), 5.97 (d, J = 1.9 Hz, 1H), 6.67 (dd, J = 2.3, 10.2 Hz, 1H), 6.69 (d, J = 10.2 Hz, 1H), 7.32–7.35 (m, 1H), 7.36–7.39 (m, 4H); ¹³C NMR (151 MHz, CDCl₃) δ 71.0, 108.9, 127.6, 128.8, 128.8, 133.9, 134.5, 137.0, 157.4, 181.6, 187.5; APCI-HRMS *m/z*: calcd for C₁₃H₁₀O₃ (M⁺), 214.0630, found 214.0617; purity (HPLC): 93.7%.

2-Cyclopentyl-1,4-benzoquinone (5d)

The title compound was prepared in a yield of 23.9%: mp 47 °C. ^1H NMR (600 MHz, CDCl_3) δ 1.36–1.42 (m, 2H), 1.61–1.68 (m, 2H), 1.68–1.76 (m, 2H), 1.91–1.96 (m, 2H), 3.02 (qn, J = 7.9 Hz, 1H), 6.52–6.53 (m, 1H), 6.67 (dd, J = 2.6, 10.2 Hz, 1H), 6.72 (d, J = 10.2 Hz, 1H); ^{13}C NMR (151 MHz, CDCl_3) δ 25.1, 32.0, 38.7, 130.1, 136.0, 137.0, 153.2, 187.6, 188.1; APCI-HRMS m/z : calcd for $\text{C}_{11}\text{H}_{13}\text{O}_2$ (MH^+), 177.0910, found 177.0901; purity (HPLC): 98.0%.

3. Measurement of IC_{50} values

The IC_{50} values for the inhibition of recombinant human MAO-A and MAO-B by the 1,4-benzoquinones (**4** and **5a–d**) were determined by the literature protocol (2). The enzymatic reactions were performed in white 96-well microtiter plates (Eppendorf) at pH 7.4 ($\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ 100 mM, made isotonic with KCl). The final volume of the reactions was 200 μL and contained the non-selective MAO substrate, kynuramine (50 μM) and the test inhibitors at concentrations of 0.003–100 μM . Each reaction also contained DMSO as co-solvent (4%). The reactions were initiated with the addition of MAO-A (0.0075 mg protein/mL) or MAO-B (0.015 mg protein/mL) and incubated for 20 min at 37 °C in a convection oven. The reactions were terminated by adding NaOH (80 μL of 2 N) and the concentrations of the MAO-generated 4-hydroxyquinoline were measured by fluorescence spectrophotometry (λ_{ex} = 310; λ_{em} = 400 nm) (3). For this purpose, a linear calibration curve containing authentic 4-hydroxyquinoline (0.047–1.56 μM) was employed. The enzyme catalytic rates were calculated and fitted to the one site competition model incorporated into the Prism 5 software package (GraphPad). The IC_{50} values were determined from the resulting sigmoidal plots and are expressed as mean $\pm$ standard deviation (SD) of triplicate measurements.

4. Recovery of enzyme activity after dialysis

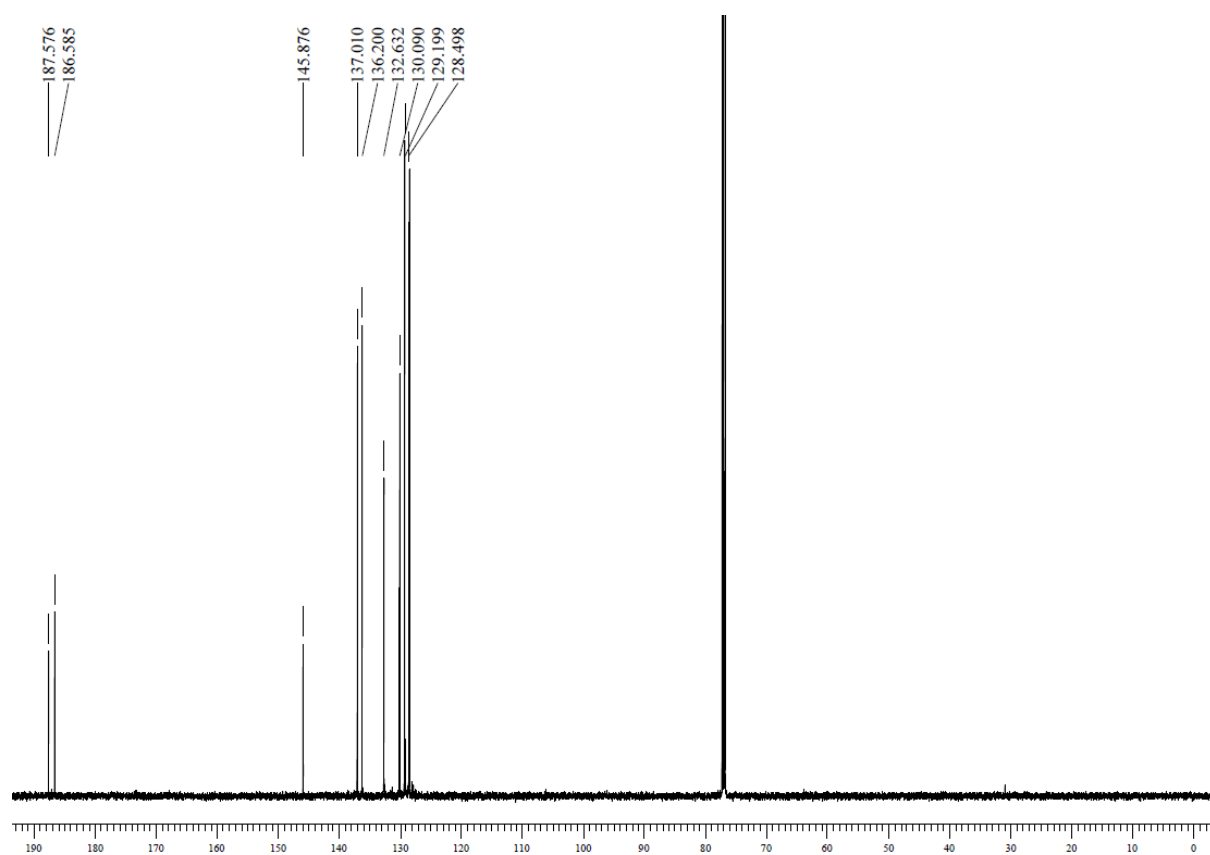
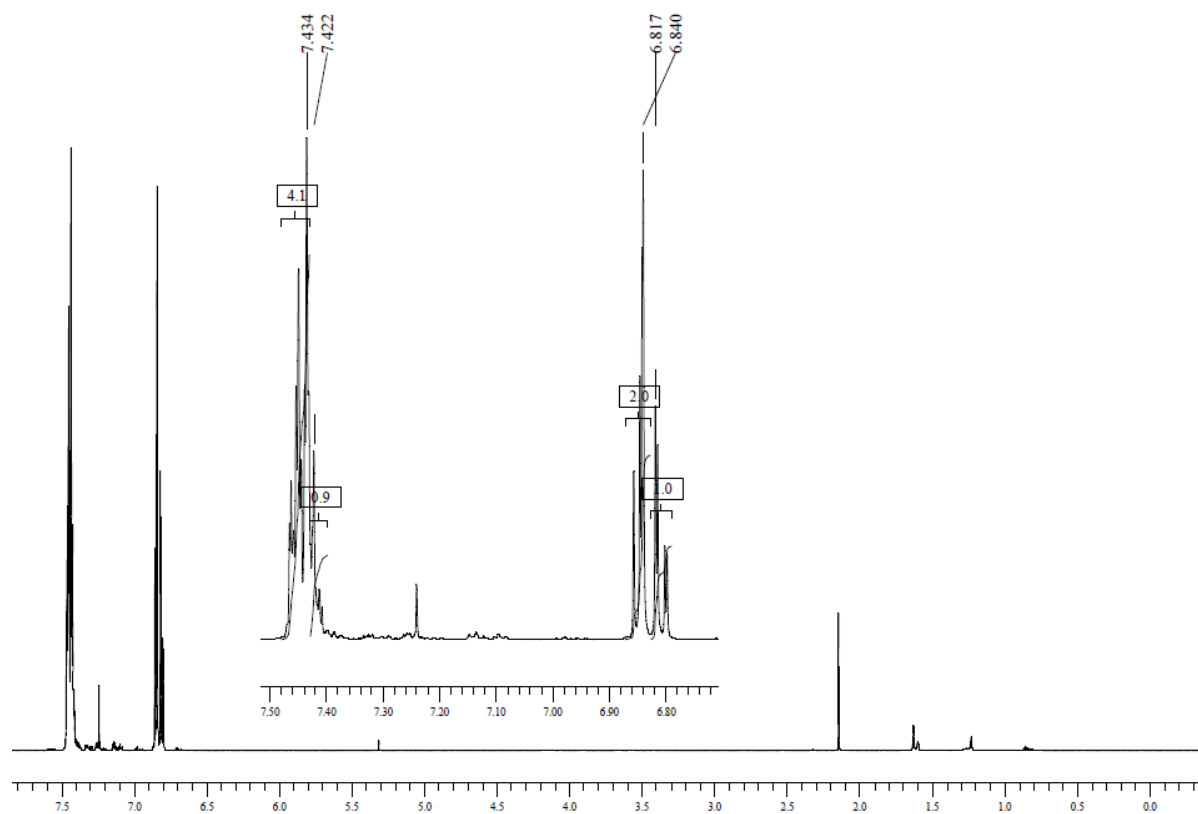
The reversibility of MAO inhibition by selected 1,4-benzoquinones (**4**, **5a**, **5b**, and **5d**) were examined by dialysis according to the literature protocol (2). Slide-A-Lyzer® dialysis cassettes (Thermo Scientific) with a molecular weight cut-off of 10 000 and a sample volume capacity of 0.5–3 mL were used for these studies. The MAO enzymes (0.03 mg protein/mL) were combined with the test inhibitors (for 15 min at 37 °C) at an inhibitor concentration equal to $4 \times \text{IC}_{50}$ for the inhibition of the MAOs. The volume of these mixtures was 0.8 mL and potassium phosphate buffer (100 mM, pH 7.4, 5% sucrose) served as solvent. The mixtures also contained 4% DMSO as co-solvent. Similar mixtures containing the MAO enzymes without inhibitor present (negative control) and with the irreversible inhibitors (positive controls), pargyline and (R)-deprenyl, respectively, present were also prepared. The concentrations of pargyline [$\text{IC}_{50}(\text{MAO-A})$ = 13 μM] and (R)-deprenyl [$\text{IC}_{50}(\text{MAO-B})$ = 0.079 μM] employed were equal to $4 \times \text{IC}_{50}$ their reported IC_{50} values for the inhibition of the respective enzymes (4; 5). The mixtures were dialysed at 4 °C in 80 mL of dialysis buffer (100 mM potassium phosphate, pH 7.4, 5% sucrose). The dialysis buffer was

replaced with fresh buffer at 3 h and 7 h after the start of dialysis. After 24 h the reactions were diluted 2-fold with the addition of kynuramine (dissolved in potassium phosphate buffer, 100 mM, pH 7.4, made isotonic with KCl). The final inhibitor concentrations in these reactions were thus equal to $2 \times \text{IC}_{50}$ their IC_{50} values and the final concentration of kynuramine was 50 μM . These enzymatic reactions (500 mL final volume) were incubated for 20 min at 37 °C in a water bath and terminated by adding NaOH (400 μL of 2 N) and 1000 μL water. The concentrations of the MAO-generated 4-hydroxyquinoline were measured by fluorescence spectrophotometry ($\lambda_{\text{ex}} = 310$; $\lambda_{\text{em}} = 400$ nm) employing a 3.5 mL quartz cuvette (10 $\times$ 10 mm pathlength) (3). For the purpose of comparison, undialysed mixtures of the MAOs and the test inhibitors were maintained at 4 °C for the same time period (24 h) as that used for dialysis. All reactions were carried out in triplicate and the residual enzyme catalytic rates were expressed as mean $\pm$ SD.

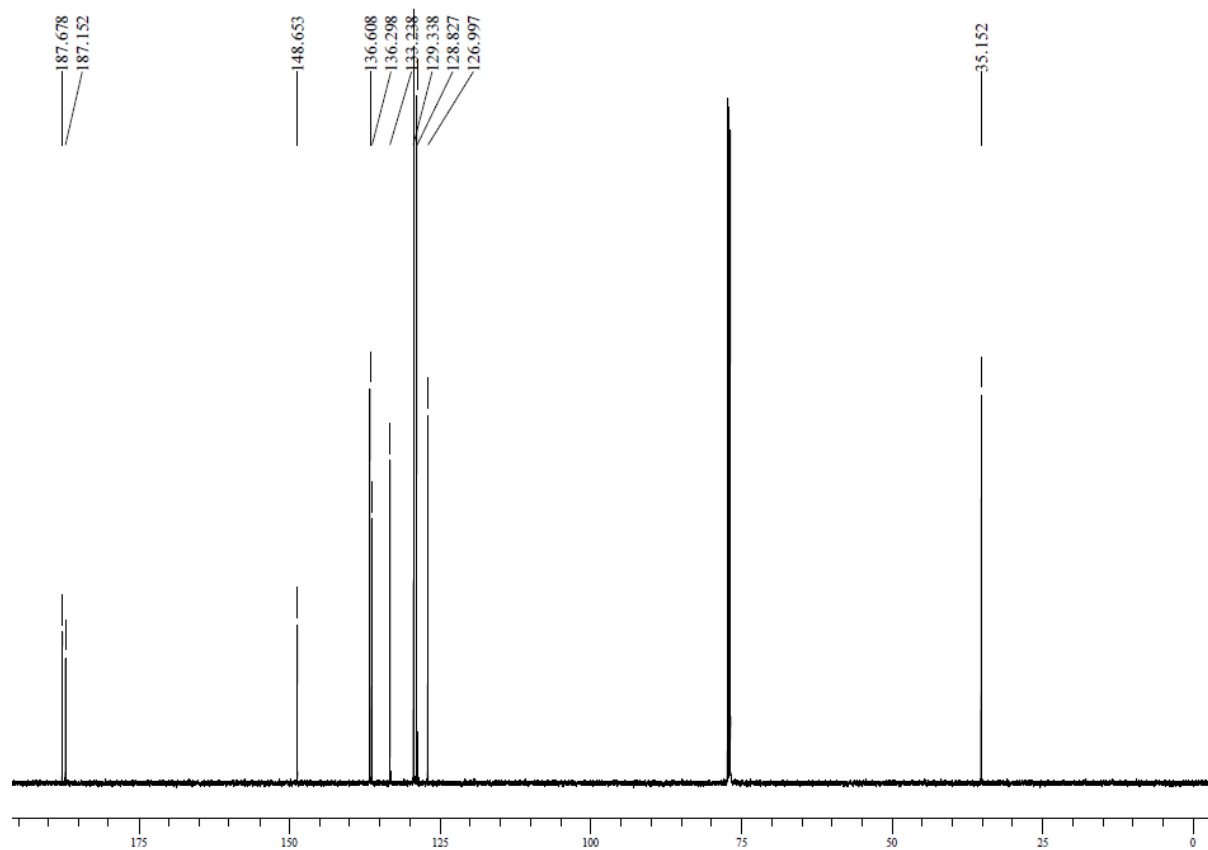
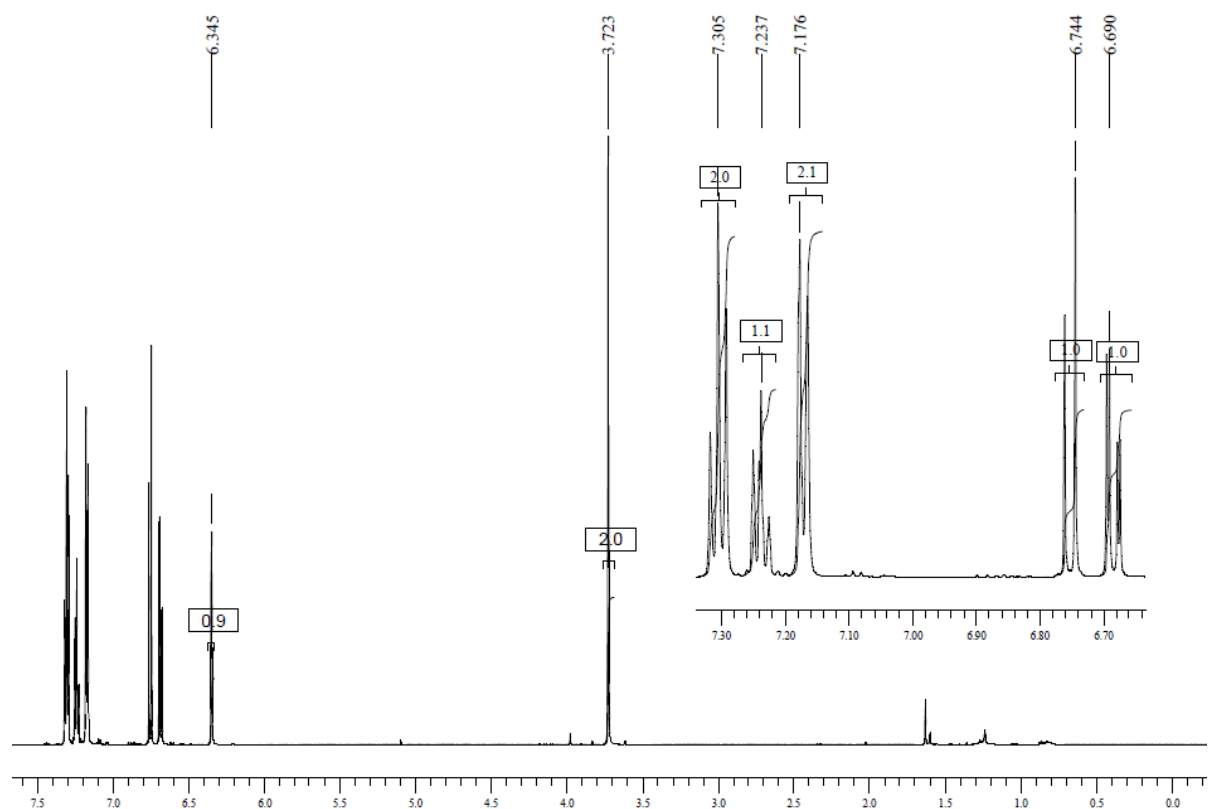
5. References

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2. Mostert, S., Petzer, A., Petzer, J.P. (2015) Indanones as high-potency reversible inhibitors of monoamine oxidase. *ChemMedChem* 10:862-873.
3. 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. *Bioorg Med Chem* 13:6212-6217.
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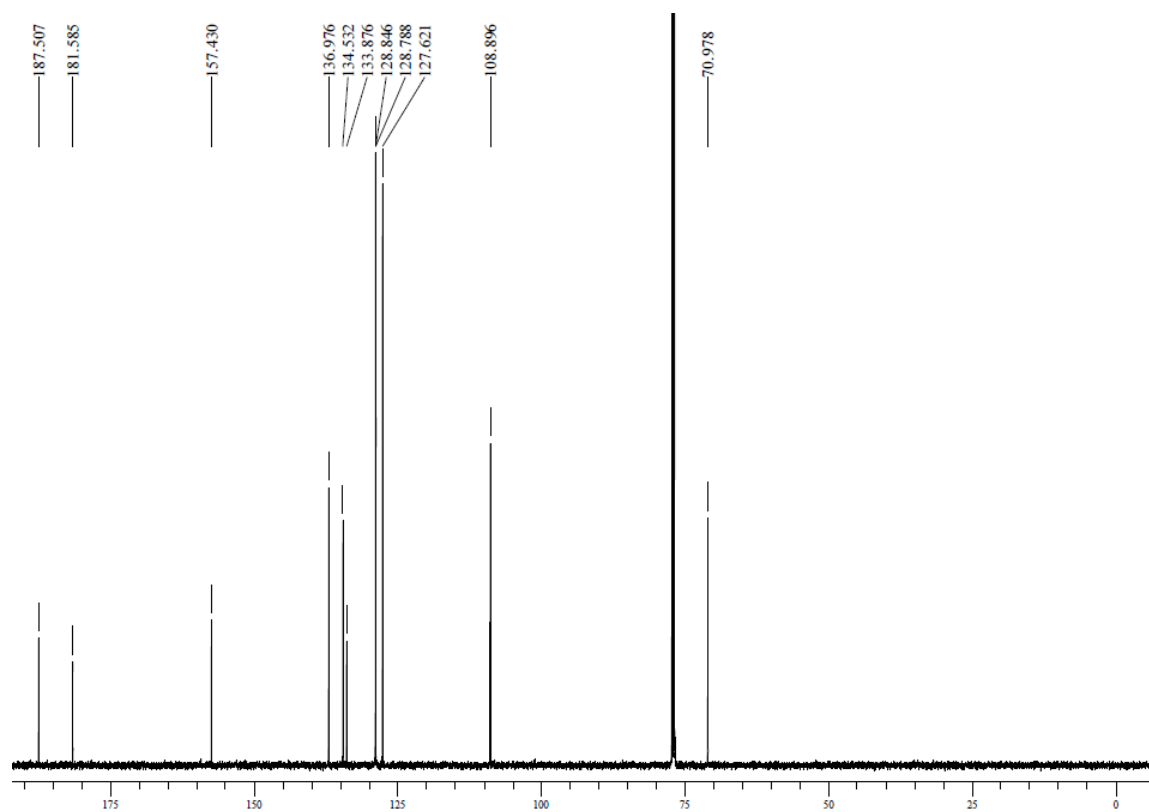
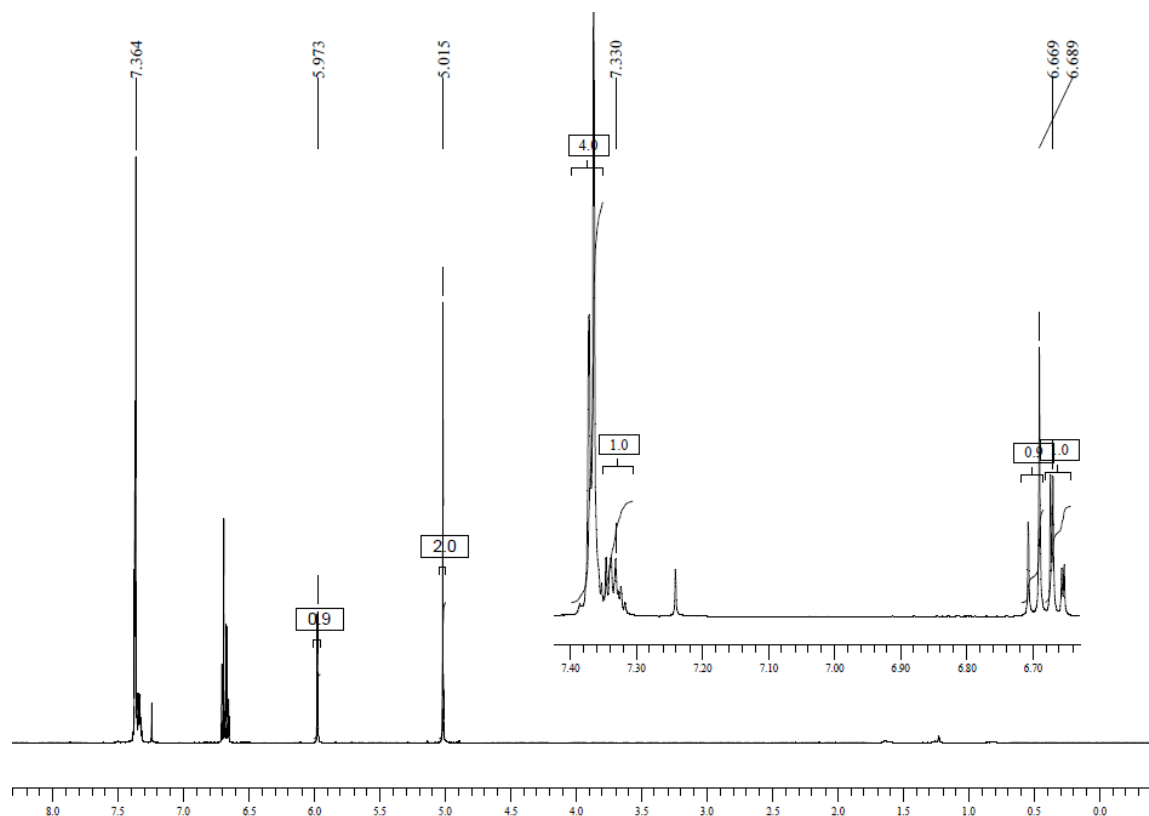
2-Phenyl-1,4-benzoquinone (5a)



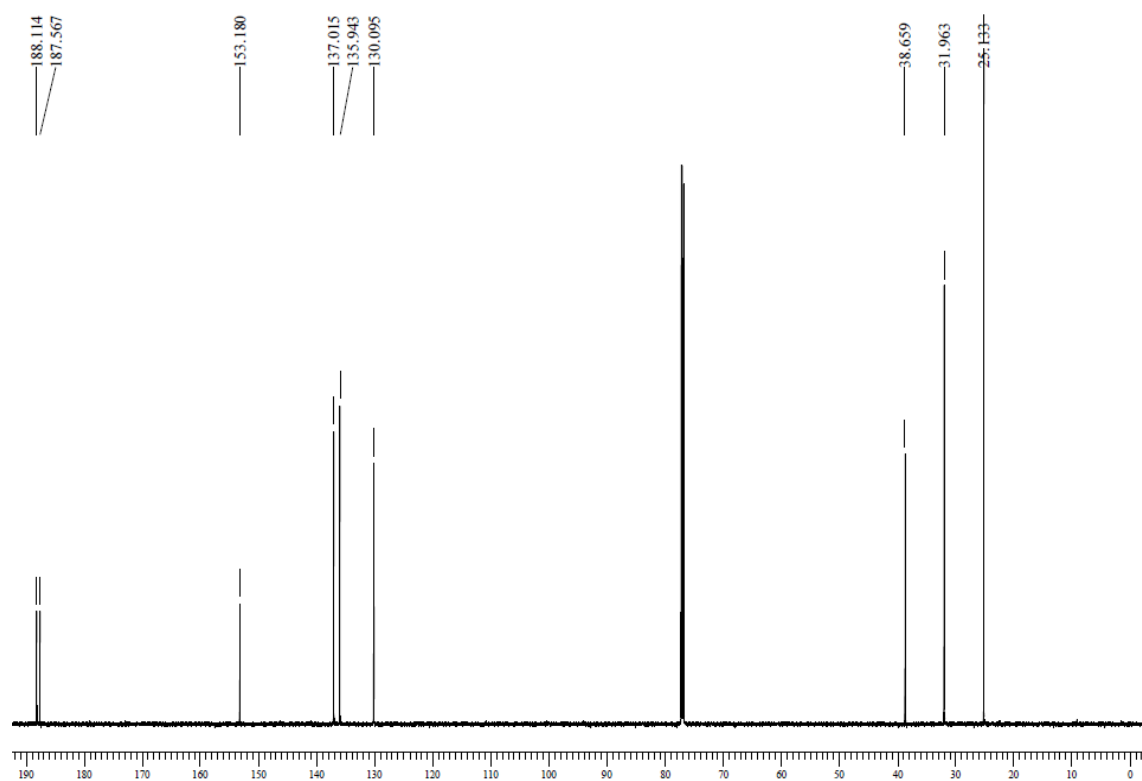
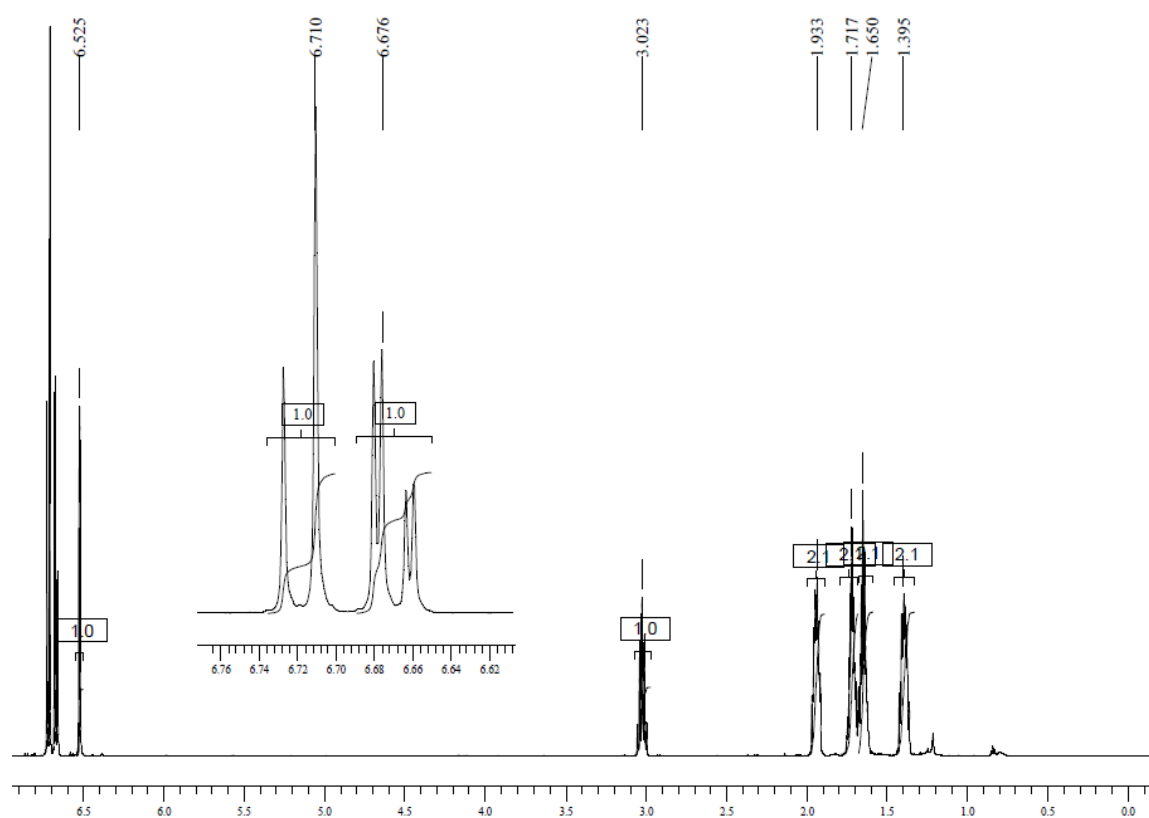
2-Benzyl-1,4-benzoquinone (5b)



2-Benzyloxy-1,4-benzoquinone (5c)



2-Cyclopentyl-1,4-benzoquinone (5d)



2-Phenyl-1,4-benzoquinone (5a)



Mass Spectrum SmartFormula Report



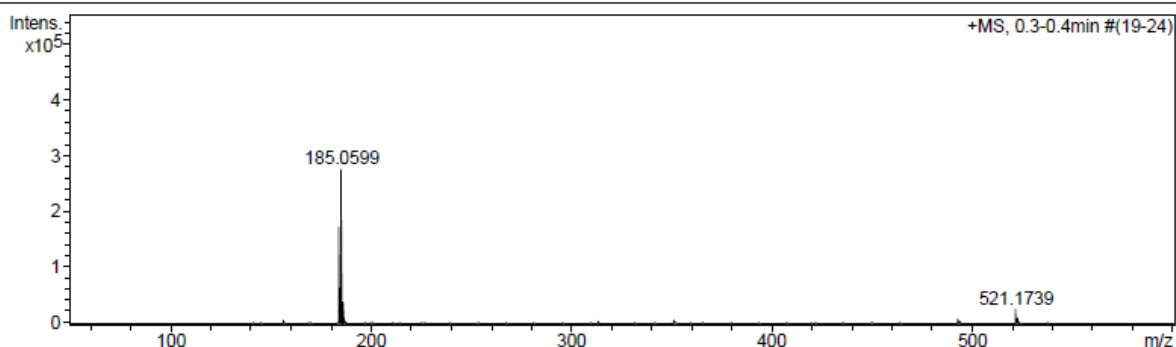
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185.0599	1	C 12 H 9 O 2	100.00	185.0597	-0.2	-1.0	2.4	8.5	ok	even

2-Benzyl-1,4-benzoquinone (5b)



Mass Spectrum SmartFormula Report



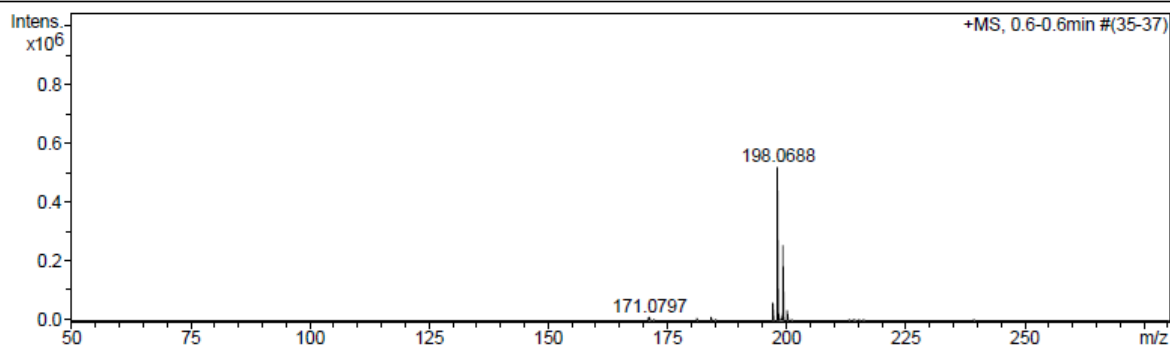
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198.0688	1	C 13 H 10 O 2	100.00	198.0675	-1.2	-6.3	204.4	9.0	ok	odd

2-Benzoyloxy-1,4-benzoquinone (5c)



Mass Spectrum SmartFormula Report



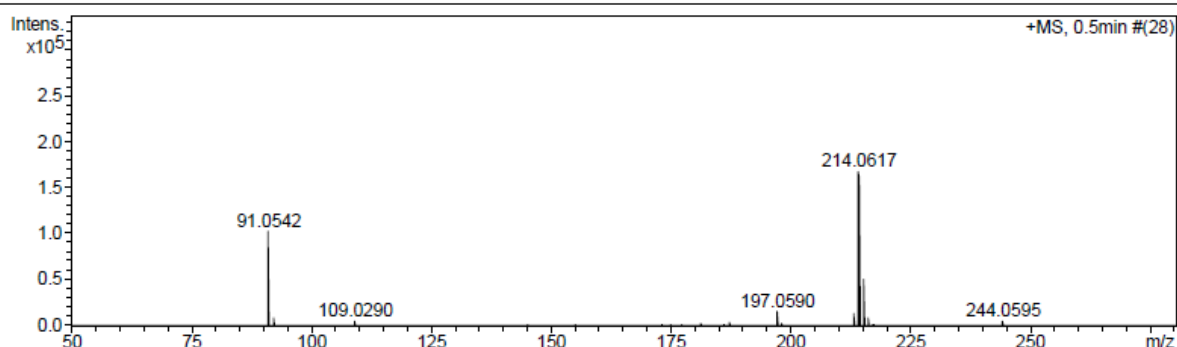
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Scan End	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste



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214.0617	1	C 13 H 10 O 3	100.00	214.0624	0.8	3.5	97.2	9.0	ok	odd

2-Cyclopentyl-1,4-benzoquinone (5d)



Mass Spectrum SmartFormula Report



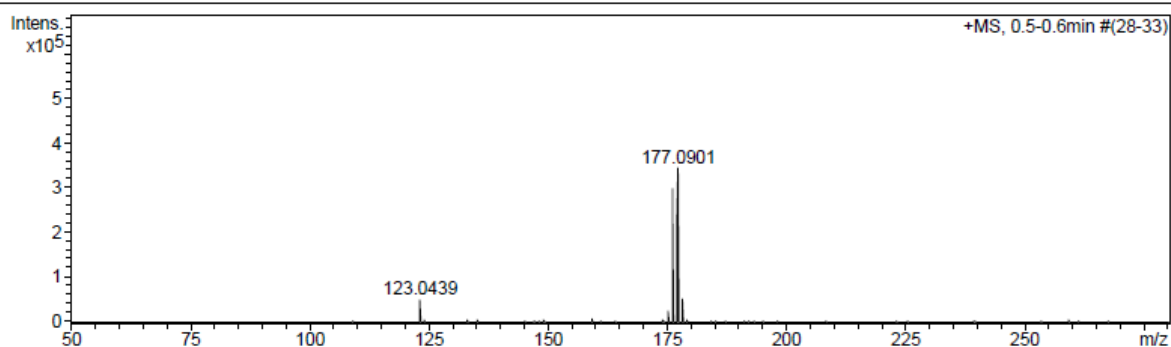
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Scan End	3000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste



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177.0901	1	C 11 H 13 O 2	100.00	177.0910	0.9	5.2	16.8	5.5	ok	even

Chapter 8

Conclusion

The purpose of this thesis was to contribute to the discovery of new MAO inhibitors and to determine structure-activity relationships of MAO inhibition by the selected classes of inhibitors. This was achieved by investigating the MAO inhibitory activities of four chemical classes of compounds with potential MAO inhibitory activity. The results were presented as four articles, chapters 4-7. The four classes of compounds investigated were:

- ❖ A synthetic series of 1-indanones (Chapter 4).
- ❖ A synthetic series of benzoxathiol-2-one derivatives (Chapter 5).
- ❖ A series of natural and synthetic 1,4-naphthoquinones (Chapter 6).
- ❖ A small synthetic series of 1,4-benzoquinones (Chapter 7).

The first article titled “*Indanones as high potency reversible inhibitors of monoamine oxidase*” focused on the synthesis of a series of 1-indanones, substituted on the C5 and C6 positions. Also included was a related series of indane derivatives. The results have shown that substitution on these positions with the benzyloxy moiety leads to much improved MAO inhibition compared to the unsubstituted parent compounds. It has also been found that further substitution with a halogen on the benzyloxy phenyl ring enhances the inhibition potencies towards both MAO isoforms. It was further shown that C6 substituted 1-indanones are the most potent MAO inhibitors of the three series, with IC_{50} values ranging from 0.001-0.030 μ M for the inhibition of MAO B and 0.032-1.348 μ M for the inhibition of MAO A. The C6 substituted 1-indanones also were more selective towards MAO B compared to the other series, with the most selective compound (**3i**) displaying a selectivity index of 1348. The C6 substituted 1-indanones are also more potent than the reference inhibitor, lazabemide, which inhibits MAO B with an IC_{50} value of 0.091 μ M. Although displaying a lower degree of potency, the C5 substituted 1-indanone and indane series may also be considered as potent inhibitors of MAO. Although all compounds of these three series are selective inhibitors of MAO B, certain compounds also are highly potent MAO-A inhibitors and may thus be classified as dual MAO inhibitors. Compound **3d** is an example of a dual MAO inhibitor and displays IC_{50} values of 0.032 μ M and 0.002 μ M for the inhibition of MAO A and MAO B, respectively. These compounds may find application in the treatment of PD patients whom also exhibit signs of depression. An important finding was that a selected 1-indanone acts as a reversible inhibitor for MAO A, while displaying potential tight-binding to MAO B. Since reversible MAO A inhibitors (in contrast to irreversible inhibitors) are not associated with tyramine-induced changes in blood-pressure, the selected 1-indanone should possess an acceptable safety profile with respect to the “cheese reaction”. This study suggests that 1-indanones are high potency MAO inhibitors and may act as promising leads in the design of therapies for neurodegenerative and neuropsychiatric disorders such as PD and depression.

The second article titled *"Inhibition of monoamine oxidase by benzoxathiolone analogues"* reported that this chemical class represents high potency inhibitors of MAO B with IC_{50} values ranging from 0.003 to 0.051 μ M. Two compounds, 6-(3-chlorobenzyloxy)-1,3-benzoxathiol-2-one (**7b**) and 6-(4-chlorobenzyloxy)-1,3-benzoxathiol-2-one (**7c**) were also potent MAO A inhibitors with IC_{50} values of 0.424 and 0.189 μ M, respectively. This study found that substitution on the C6 position of the benzoxathiolone moiety is essential for potent MAO inhibition since the parent compound, 6-hydroxy-1,3-benzoxathiol-2-one, displays weak MAO B inhibition. The MAO B inhibitory potencies of the benzoxathiolones compare well with compounds which share structural similarities such as isatin and phthalimide derivatives. It is also noteworthy that benzoxathiolone analogues share structural similarity with 1-indanones, and also bind reversibly to both MAO isoforms. Therefore, this study suggests that benzoxathiolone analogues may act as leads for the future design of therapies for PD. Some benzoxathiolones, which also inhibited MAO A, may be proposed a potential leads in the design of antidepressant drugs. It should, however, be kept in mind that lipophilicity and aqueous solubility of this chemical class requires optimisation.

The third article *"The evaluation of natural and synthetic 1,4-naphthoquinones as inhibitors of monoamine oxidase"* investigated the inhibition properties of 1,4-naphthoquinones. The lead compound for this study, 2,3,6-trimethyl-1,4-naphthoquinone (TMN), is a known MAO non-selective inhibitor and was first isolated from cured tobacco leaves. MAO inhibitors may find application in various disease states such as PD, depression, cancer and congestive heart failure. Among the 1,4-naphthoquinones, 5,8-dihydroxy-1,4-naphthoquinone was found to be the most potent inhibitor with an IC_{50} value of 0.860 μ M for the inhibition of MAO B. A related compound, shikonin, inhibits both the MAO A and MAO B isoforms with IC_{50} values of 1.50 and 1.01 μ M, respectively. Interestingly, MAO inhibition is abolished when a hydroxyl group is present on the C2 position of the 1,4-naphthoquinone moiety. It is further shown that MAO-A and MAO-B inhibition by selected 1,4-naphthoquinones is reversible and competitive. With these findings, this thesis makes a meaningful contribution to the structure-activity relationships of MAO inhibition by the 1,4-naphthoquinone class of compounds.

The final article *"The evaluation of 1,4-benzoquinones as inhibitors of human monoamine oxidase"* investigated the MAO inhibitory properties of a small series of 1,4-benzoquinones. These compounds are related to the 1,4-naphthoquinone class, which as mentioned above, are known to inhibit the MAOs. Only one report of the MAO inhibition properties of 1,4-benzoquinone exist. The results presented in this thesis showed that the series of 1,4-benzoquinones are moderate inhibitors of MAO A and MAO B with IC_{50} values of 5.03-13.2 μ M (MAO A) and 3.69-23.2 μ M (MAO B). These values are in a similar range of the MAO A inhibition potency of toloxatone (IC_{50} = 3.92 μ M), a clinically used MAO A inhibitor. Of significant interest is the finding that 1,4-benzoquinones are irreversible inhibitors of MAO A, while MAO B inhibition appears to be reversible, at least

partly. It is proposed that 1,4-benzoquinones are affinity labelling MAO A inhibitors and covalently modify the MAO A active site. A potential site of covalent interaction is the reduced flavin cofactor. Further investigation is, however, necessary to clarify this point. To the best of our knowledge, this is the first report of the irreversible inhibition of a MAO isoform by a quinone compound.

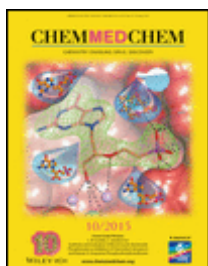
These four articles were prepared for publication in academic journals of which the first article has already been published. In conclusion, this thesis discovered several 1-indanone and benzoxathiolone derivatives which exhibit high potency inhibition of the MAOs. In all instances these compounds were selective inhibitors of the MAO-B isoform. Some compounds inhibited both MAO isoforms and may thus be viewed as dual MAO inhibitors. Such compounds may be advantageous in the treatment of depression comorbid with PD, in addition to addressing the motor symptoms of PD. This thesis also significantly contributes to the structure-activity relationships of MAO by the 1,4-naphthoquinone class of compounds and discovers 5,8-dihydroxy-1,4-naphthoquinone and shikonin as reasonably potent MAO inhibitors. Lastly, an investigation of the MAO inhibitory properties of 1,4-benzoquinones shows for the first time that this class of compounds acts as irreversible inhibitors of MAO A.

Addendum A

Author guidelines ChemMedChem

ChemMedChem

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Reviews should be divided into (numbered) sections. Cross-references in the text refer to these section numbers. The review starts with a **Lead-in** (1000 characters excl. spaces). This text should not be a mere summary; rather, it should arouse the readers' interest. The **Introduction** should introduce the nonspecialist to the subject as clearly as possible. A Review should conclude with a **Summary** and **Outlook** section, in which the achievements and new challenges for the subject are presented succinctly. In addition, a biographical sketch (500–700 characters excl. spaces) and a portrait-quality black-and-white photograph of each author, as well as a graphical suggestion for a full-page picture (**Frontispiece**) to face the first page of the review article, should be submitted. Please note that publication of a color frontispiece carries a charge of €495 (including tax).

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As a Minireview is much more focused than a Review, it is shorter (approximately 25000 characters excl. spaces) and does not contain a frontispiece or biographical sketch(es). However, it should still have an **Abstract** (1000 characters excl. spaces) that should similarly arouse the readers' interest.

3. Highlights

In the Highlights section, very important new results of original research are described, in general by a third person, with a view to highlight their significance. The results should be presented clearly, but as succinctly as possible, without the comprehensive details required for an original article. Highlights may be organized as the author wishes, but should not be more than approximately 8500 characters (excl. spaces) in length. Highlights should contain a brief **Abstract** (600–800 characters excl. spaces), which should summarize the work being highlighted and give the reader an indication of *why* it is worthy of highlight. Chemical formulae, figures, and schemes should be restricted to important examples, and the number of references kept to a minimum.

4. Concepts

Concepts are short articles that emphasize the general concepts that have guided important developments in a specific area and their implications for future research. The reference section should only include the key papers that have contributed to conceptual advances in the field under review, rather than being fully comprehensive. The author should aim to provide the nonspecialist reader with a useful guide and the expert with a new angle on a familiar problem. Concept articles may be organized as the author wishes, but should include a short **Abstract** (approximately 600–800 characters excl. spaces) that succinctly describes the concepts under discussion. Articles should consist of approximately 17000 characters, including references, tables, and legends. The liberal use of schemes and figures is encouraged.

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Inclusive of all references, footnotes, and tables, a **Communication** should be no longer than approximately 15000 characters. **Communications require an Abstract**, which should be concise (1000 characters excl. spaces) and not too technical, and should stand alone. Chemical formulae, figures, and schemes may also be added. Longer Communications will be accepted only if their quality warrants special consideration, and a written justification of their length is provided. Communications should not be divided into sections. However, experimental details can be succinctly summarized separately under the heading **Experimental Section**. Please note, only pertinent experimental information should be included in this section, and any additional experimental data should be detailed in the Supporting Information. Click [here for details about Experimental Section requirements](#). The main text of a Communication should start by giving an introduction to enable readers unfamiliar with the subject to become acquainted with the importance of the results presented. In the final paragraph the results should be summarized succinctly, and one sentence should be devoted to their significance and, if appropriate, to remaining challenges.

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6. Book Reviews

Reviews of books are written on invitation. Suggestions for books to be reviewed and for reviewers are welcome. Publishers should send brochures or, preferably, the books directly to the Editorial Office. Unsolicited books will not be returned.

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Reports on recent events and comments on publications in *ChemMedChem* are welcome if they contribute to the scientific discussion in the field. Authors interested in preparing such a contribution should contact the Editor to discuss this further (chemmedchem@wiley-vch.de).

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To ensure discoverability of an article when published, authors should take care when drafting their Abstract and deciding on the wording of their Title.

The title should be as succinct as possible and without a reference. Words such as 'new', 'novel', and 'first' in the title should be avoided. The title should be restricted to no more than 180 characters and be as informative as possible without being over complicated. Key words and phrases should be used in the title and repeated in the abstract as appropriate.

For Full Papers and Communications, **the Abstract** should describe the **motivation** for the work, the **methods** applied, the key **results**, and the **conclusions** drawn. The Abstract for other contribution types should summarize the topic under discussion, the main findings and arguments, and place the article into context. Abstracts for Full Papers, Communications, Reviews and Minireviews should be approximately 1000 characters (excl. spaces) in length, while the Abstracts of Concepts, Essays, Highlights and Viewpoints should be around 600–800 characters. In all cases, when preparing **the Abstract**, please keep the following aspects in mind:

- 1) The abstract **should awaken the curiosity** of as many readers as possible.
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Supporting Information should not include crystallographic or sequence data that are available from the relevant databases. Also, please ensure that all Greek characters are in the 'Symbol' font. Color can be used without restriction in the Supporting Information. Animated multimedia applications, Excel sheets containing database entries, or other supplementary materials are also welcome as Supporting Information.

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A Keyword List has been developed for the readers of Wiley-VCH journals.

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1. At least two of the maximum of five keywords assigned to an article must come from this list.
2. Named reactions will be incorporated only in exceptional cases. Generally the reaction type is selected instead. For example, Diels–Alder reactions will be found under "cycloadditions" and Claisen rearrangements under "rearrangements".
3. Heteroanalogues of compounds are mainly classified under the C variants, for example, (hetero)cumulenes, (hetero)dienes. A few aza and phospho derivatives are exceptions.
4. Compounds with inorganic components that are central to the article are listed under the element, for instance, iron complexes under "iron" and, if appropriate, the ligand type. Some group names such as "alkali metals" exist alongside the names of important members of the group, for example, "lithium". In such cases the group name is used for these members only when comparative studies are described. The members that do not appear separately are also categorized under the group name.
5. A keyword in the form "N ligand" is only chosen if a considerable portion of the paper deals with the coordination of any ligand that coordinates through the atom concerned (in the example, nitrogen).
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7. "Structure elucidation" is intended only if the crux of the paper is a structural elucidation or if a combination of several spectroscopic techniques were needed for conclusive solution of the structure.

8. An attempt has been made to avoid synonyms and to select more general concepts rather than specialized terms. Thus the term "double-decker complexes" is excluded in favor of "sandwich complexes". See also points 3 and 4 above.

9. Enzymes should be assigned to one of the six main enzyme classes (hydrolases, isomerases, ligases, lyases, oxidoreductases, transferases).

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Compound Characterization and Purity

For all new compounds reported in the Experimental Section, **sufficient characterization data should be provided to unambiguously identify the structure** and to allow identification through comparison by others.

In general, we encourage authors to **include as much characterization data as possible** for all compounds described, including but not limited to any/all of the following: ^1H NMR, ^{13}C NMR, IR, UV/Vis, MS and HRMS data, elemental (combustion) analysis, melting point ranges for solids, R_f values with solvent details. Specific rotation ($[\alpha]_D^{20}$) values should be given for all optically active compounds described.

Those **compounds used in biological testing**, whether synthesized or obtained from other sources (e.g., commercial), **should possess a purity of no less than 95%**. Purity may be determined by any established method (e.g., HPLC, elemental analysis with accuracy within $\pm 0.4\%$). The purity should be stated, along with the method used to determine it, in the Experimental Section and if appropriate, evidence can be provided in the Supporting Information.

Equipment and Software

Equipment (including make, model, and manufacturer if uncommon), and details of any software used (including version, release date if applicable, and developer) and conditions used for the measurement of physical data, as well as any organisms, proteins, or nucleic acids used, should be described at the beginning of the Experimental Section. Sources of less-common starting materials must be given, and solvent details should also be described.

Compound names

In so far as is practical, authors should use a systematic name for each title compound in the Experimental Section (as suggested by IUPAC, IUBMB, or Chemical Abstracts) followed by the compound number. Please do not use computer programs to generate elaborate systematic names or use extremely long compound names.

When describing the protocol for the synthesis of a given title compound, the full name followed by the compound number in parentheses should be given in **bold**. When referring to the compound in the running text of the protocol, parentheses for the compound number should be used *only* if the name identifies the compound uniquely and unambiguously (for example: "...2-ethyl-4-cyanobenzoate (**7**) was added to..." or "...cyanobenzoate **7** was added to..."). For the sake of clarity general descriptors such as compound **1**, dendrimer **2**, or alcohol **3** should be used in the running text rather than the full systematic name.

General Comments and Format

Quantities of reactants, solvents, etc. should be included in parentheses rather than in the running text (e.g., "Triphenylstannyl chloride (0.964 g, 2.5 mmol) in toluene (20 mL)..."). Physical data (in SI units whenever possible) should be quoted with decimal points and negative exponents (e.g., 25.8 JK⁻¹mol⁻¹). Products should be described, and yields should be given as both a quantity (mol or g) and in percent (e.g., "...compound **7** as a white powder (34 mg, 89%)...").

Data in the Experimental Section should be **carefully and consistently formatted** according to the journal style. **Examples:** "...gave compound **7** as a white powder (34 mg, 89%): $R_f=0.38$ (CHCl₃/MeOH 9:1); mp: 70–71°C; $[\alpha]_D^{20}=-13.5$ ($c=0.2$ in acetone); ¹H NMR (400 MHz, CDCl₃): $\delta=0.97$ (t, $J=8.2$ Hz, 3H), 1.35 ppm (q, $J=8.1$ Hz, 2H); ¹³C NMR (75 MHz, [D₆]DMSO): $\delta=8.9$, 27.3, 56.8, 64.2, 170.3 ppm; IR (KBr): $\tilde{\nu}=3248$, 3056, 1790, 1780, 1506, 1493 cm⁻¹; UV/Vis (CH₂Cl₂): $\lambda_{max}(\epsilon)=320$ (5000), 270 nm (12000); MS (EI, 70 eV) m/z (%): 173 (32), 171 (100) [$M+H$]⁺; HRMS-FAB m/z [$M+H$]⁺ calcd for C₈H₇ClO₂: 171.0135, found: 171.0142; Anal. calcd for C₈H₇ClO₂: C 56.32, H 4.14, O 18.76, found: C 56.35, H 4.11, O 18.79. **Please give data in this order.**

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Manuscripts containing animal experiments must include a statement that permission was obtained from the relevant national or local authorities. The institutional committees that have approved the experiments must be identified and the accreditation number of the laboratory or of the investigator given where applicable. If no such rules or permissions are in place in the country where the experiments were performed, then this must also be clearly stated.

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Prior to manuscript submission, the author(s) must deposit the following information in the appropriate databases: the data of X-ray structure analyses of inorganic and organic compounds, proteins, or nucleic acids; structure determination of proteins and nucleic acids by NMR spectroscopy (together with a list of NEOs), and sequence determinations of proteins or nucleic acids. The name of the database and the deposition number(s) assigned by this database must be stated in the part of the manuscript where the respective structure or sequence determination(s) is described so that referees can retrieve the information electronically. Detailed instructions for data submission can be found on the WWW homepages of the databases listed below.

Structure Data

For papers describing structural data, atomic coordinates and the associated experimental data should be deposited in the appropriate databank (see below).

Please note that the data in databanks must be released, at the latest, upon publication of the article. We trust in the cooperation of our authors to ensure that atomic coordinates and experimental data are released on time.

Organic and organometallic compounds: Crystallographic data should not be sent as Supporting Information, but should be deposited with the *Cambridge Crystallographic Data Centre* (CCDC) at http://www.ccdc.cam.ac.uk/services/structure_deposit.

Inorganic compounds: *Fachinformationszentrum Karlsruhe* (FIZ; <http://www.fiz-karlsruhe.de>).

Proteins and nucleic acids: *Protein Data Bank* (<http://www.rcsb.org/pdb>).

NMR spectroscopy data: *BioMagResBank* (<http://www.bmrb.wisc.edu>).

Sequence Data

Nucleic acids: *GenBank* (WWW: <http://www.ncbi.nlm.nih.gov/>) or *EMBL Nucleotide Sequence Database* (<http://www.ebi.ac.uk/embl/index.html>).

Proteins: *Protein Information Resource* (PIR; <http://pir.georgetown.edu/>) or *SWISS-PROT* (<http://www.expasy.ch/sprot/sprot-top.html>).

Manuscript Preparation for Submission

The following remarks aim to assist our authors in preparing their manuscript **for submission** to *ChemMedChem*. We strongly encourage our authors to adhere closely to these guidelines as it **facilitates the evaluation of an article by the Editors and Referees**. Manuscripts that are not suitable for consideration by the referees because of poor presentation or language issues will be returned to the authors for improvement prior to being sent out for peer evaluation.

Please note, authors of accepted articles looking for guidelines on preparing their production materials should consult the section below entitled "[Guidelines for Accepted Manuscripts: Production Material Preparation](#)" for specific requirements in addition to the general comments given below.

General Remarks

Spelling may be either UK or US standard English, but consistency should be maintained within a manuscript. Authors are asked to make their manuscripts suitable for a heterogeneous readership of biologists and chemists and to be considerate to our many readers for whom English is a foreign language—please use a simple, clear style and avoid jargon.

Language should be clear and easily understood. Authors may choose to seek the assistance of a colleague who is a native or highly proficient English speaker when preparing their manuscript for submission. For authors seeking professional assistance, **Wiley Editing Services** offers a variety of options, from language polishing to figure preparation.

Abbreviations and acronyms should be used sparingly and consistently, following the system of abbreviations and symbols recommended by the International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Biochemistry and Molecular Biology (IUBMB).

Where they first appear in the text, they should—apart from the most common ones such as NMR, IR, or UV—be defined. Authors may prefer to explain large numbers of abbreviations and acronyms in a **Glossary** at the end of the text. Names of organisms should comply with genetic conventions, with genus and species names written in italics and spelled out in full on first appearance. Abbreviations for genes should be written in lower-case letters and italicized, those of the corresponding protein products should start with a capital letter and should not be italicized (e.g., *hsp70* and Hsp70, respectively). Enzyme names should be accompanied by the respective Enzyme Commission (EC) numbers.

With the exception of Book Reviews, all other types of contribution should include a short text and color graphic for the **Table of Contents**. The text should be about five lines (approximately 300 characters excl. spaces) in length and formulated to arouse curiosity. Repetition or paraphrasing of the title and presentation of experimental details should be avoided. The color graphic should be kept small (ideally 5 × 5.5 cm) with minimum detail. We advise restricting the use of text in the graphic and avoiding large complicated schemes. Note, the use of **color in the Table of Contents is free of charge and strongly encouraged**.

When preparing the manuscript for initial submission and evaluation, **we encourage the use of color where necessary to facilitate the scientific understanding** and aid in the overall presentation of the manuscript. Should the manuscript be accepted for publication, we ask that authors carry part of the additional costs incurred for production of **color graphics**. If the author does not have access to funding and color is essential to the scientific understanding, we are able to assist authors in meeting the associated costs in full or in part. Authors of accepted manuscripts should contact the Editorial Office upon acceptance to discuss this matter further with our Managing Editor. Please note that the online and print versions of *ChemMedChem* are identical; we are unable to publish graphics in color online for a given article while publishing the same graphics in greyscale in print.

A maximum of five **keywords** should be given. In order to aid online searching, at least two of the keywords should be taken from the Keyword List. Please also see our Keyword List guidelines for details.

Detailed facts of importance to specialist readers can be submitted as **Supporting Information** and will be made accessible to reviewers and, if accepted, to readers via Wiley Online Library. There is no charge for the use of color in the Supporting Information, and animated multimedia applications are welcome also.

If a **crystal structure analysis** or **sequence determination** is not an essential part of a manuscript, only a footnote is required indicating where the detailed results can be found. This can be a separate publication or a freely accessible database. Further, more specific guidelines, are given elsewhere on the requirements for structural data.

If a manuscript is not accepted, all original material is returned to the correspondence author. However, if a manuscript is accepted for publication, graphical material and electronic storage media will only be returned upon request.

Manuscript Organization

A manuscript can be evaluated more efficiently if it is arranged as described below. Unless stated otherwise, the following instructions apply to all categories of contributions.

Title page (in order): title; authors names with academic titles, alphabetical footnotes (^[a], ^[b], ...) referring to addresses, and an asterisk to denote the corresponding author(s); footnotes containing affiliations of all authors including the full postal address, and e-mail address of the corresponding author(s); series title, number, and reference to the previous paper in the series, if applicable; dedication, if applicable.

Keywords: A maximum of five keywords to appear in the printed and online indexes should be given **in alphabetical order**. At least two keywords should be taken from the Keyword List to aid online searching. As mentioned above, please refer to our Keyword List guidelines for details.

In the text body, compound numbers should be used in ascending order. Compound numbers are always in **bold**; parentheses for the compound number should be used *only* if the name identifies the compound uniquely and unambiguously (for example: "...2-ethyl-4-cyanobenzoate (**7**) was used...", or: "...cyanobenzoate **7** was used..."). Please do not use computer programs to generate elaborate systematic names or use extremely long compound names. For the sake of clarity general descriptors such as compound **1**, dendrimer **2**, or alcohol **3** are recommended for the body text, reserving longer IUPAC nomenclature for the Experimental Section.

Experimental Section (applicable to Full Papers and Communications only) should be given in sufficient detail to enable others to repeat the work described. Please see the Experimental Section guidelines for specific information about formatting and requirements for compound purity and characterization data.

References: In the text numbers corresponding to the appropriate reference should be typed **in square brackets as superscript** (e.g., Blobel^[2]) and **after any punctuation**, where applicable. References must be listed in order of their appearance in the text. Please do not format the references section with the Numbering function in the word-processing program. If an automatic reference collation system of the word-processing program (Footnotes, EndNote) is used, we ask that an author converts the references into normal, typed text before submission of the final manuscript, otherwise they may disappear when typeset. Journal titles should be abbreviated according to the Chemical Abstracts Service Source Index (CASSI). Unpublished results and lectures should only be cited for exceptional reasons. When discussing/mentioning a crystal structure taken from the Protein Data Bank (PDB), or any other databank, the appropriate ID number should be quoted, and whenever possible the primary citation as given in the databank should be included.

Please follow the examples below. Either the first page or the page range may be given, but the presence or absence of the last page should be consistent throughout any given manuscript.

Journals: [1] a) J. A. Pitcher, N. J. Freedman, R. J. Lefkowitz, *Annu. Rev. Biochem.* **1998**, 67, 653–692; b) P. Sears, C.-H. Wong, *Angew. Chem. Int. Ed.* **1999**, 38, 2300–2324; *Angew. Chem.* **1999**, 111, 2446–2471.

[2] a) W. D. Wagner, *Ann. N.Y. Acad. Sci.* **1985**, 454, 52–68, and references therein; b) J. C. Wang, *Sci. Am.* **1982**, 247(1), 94–97.

Books (Without editor): [3] E. Wingender, *Gene Regulation in Eukaryotes*, VCH, Weinheim, **1993**, p. 215.

Books (With editor): [4] T. D. Tullius in *Comprehensive Supramolecular Chemistry*, Vol. 5 (Eds.: J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vögtle, K. S. Suslick), Pergamon, Oxford, **1996**, pp. 317–343.

Miscellaneous: [5] a) C. R. A. Botta (Bayer AG, Berlin, Germany), German Pat. DE-B 2235093, **1973** [*Chem. Abstr.* **1974**, 80, 55356 c]; b) A. Student, PhD thesis, University of Newcastle (UK), **1991**; c) "Synthesis in Biochemistry": R. Robinson, *J. Chem. Soc.* **1936**, 1079.

Figures, Schemes, Tables and other elements: When preparing a manuscript for evaluation by the Editor and Reviewers, **graphical elements should appear**, with their legend or footnotes if appropriate, **within the main text of the article where first mentioned**. Each figure and scheme should have a legend. These should be listed immediately below the graphic, within the main body of the manuscript. **Tables** must have a brief title and should only be subdivided by three horizontal

lines (head rule, neck rule, foot rule). Footnotes in tables are denoted [a], [b], [c], etc. and should contain all additional details. **For tables reporting biological data**, details of the number of independent experiments, the number of replicates, and errors (SD or SEM) should be given either in the table body or as a general statement in the footnote. Furthermore, given growing concerns over the reproducibility of biological data reported in the literature, **every effort should be made to evaluate reference/control agents in parallel with the newly reported compounds**; any comparisons to literature data should be given due consideration and treated with care.

Illustrations (structural formulae, figures, schemes) should, if possible, be designed for reduction to a one-column format (8.5 cm wide). The maximum width is the two-column format (17.5 cm wide). Further guidelines on the graphical representation standards for chemical structure diagrams have been published by IUPAC. **To allow the Editor and Referees to easily interpret the graphics, care should be taken to ensure all text is legible and all images are clear and of high quality.** We recommend: a sans serif font (e.g., Helvetica) for script; size of lettering, 3–3.5 mm; total maximum width, 14 cm (or 28 cm for two-column width). **Please use only one size of writing in any one diagram**; if two sizes are absolutely necessary, please keep the font sizes to within 2 pt. **Please also use only one style of font**; the journal style requires a sans serif font (e.g., Helvetica). Writing above the arrow in a scheme may be a little smaller.

Computer-aided image enhancement is often unavoidable. However, such manipulation cannot result in data that are less relevant or unrepresentative being shown and/or genuine and significant signals being lost. A clear relationship must remain between the original data and the electronic images that result from those data. If an image has been electronically modified, the form of the modification must be given in the Figure caption. If computer-aided processing or modification of an image is a fundamental part of the experimental work, then the form that this processing takes must be clearly described in the Experimental Section. A common example of this is the presentation of Western blots; authors should state clearly in the figure legend how a gel was modified, and if appropriate, provide the full gel(s) as Supporting Information for consideration by the Editor and Reviewers. Please **italicize** symbols of physical quantities in both graphics and the text, but not their units (e.g., *T* for temperature, in contrast to T for the unit Tesla; *J*, but Hz; *a*, but nm). Stereochemical information (*cis*, *Z*, *R*, etc.), locants (*N*-methyl, α -amino), and symmetry designations (C_{2v}) should also be italicized. Chemical formulae should be numbered with boldface Arabic numerals (e.g., **1**). Labels of axes should be separated from their units by a slash (e.g., *T* / K). Abbreviations such as Me, Et, *n*Bu, *i*Pr, *s*Bu, *t*Bu, and Ph (not ϕ) may be used. General substituents should be indicated by R^1 , R^2 (not R_2 , which means 2R), or R, R'. The spatial arrangement of the substituents should be indicated by hatched lines or a wedge. A minus sign must be as long as the crossbar of a plus sign.

Mathematical formulae should not be incorporated into the text as graphic files. Please type mathematical formulae as normal text in the body of the text as far as is possible.

Guidelines for Accepted Manuscripts: Production Material Preparation

The guidelines below are **applicable only to authors of accepted manuscript** who are preparing the final revised version. Authors preparing to submit an article to *ChemMedChem* as a new submission should consult the section above entitled "Manuscript Preparation".

The final revised version of the manuscript along with the rest of the production materials (graphics, etc.; see below for instructions) should be submitted after the paper has been accepted for publication via our online system or by e-mail. **Please note however, that the preferred method of submission of the final versions of manuscript is through Editorial Manager.**

Please consult the checklist provided as an attachment upon acceptance to ensure that the manuscript complies with the journal requirements. If appropriate, a response letter should be provided along with the production materials outlining the changes made to the manuscript in

response to the comments of the referees, or if an author disagrees with their suggestion(s), outlining the arguments against their remarks.

Once the final modifications to the manuscript have been made, **to facilitate the typesetting process and preparation of the galley proofs**, authors should follow these simple steps:

1. Please remove the text from the submission template and instead present the text as a standard document (single-column, no page header or section breaks). The text should be typed as "continuous text", that is, with carriage returns only at the end of a paragraph, title, heading, and similar features. The text should be provided either as a Word document (.doc) or in rich text format (.rtf).
2. Remove all graphics from the text; these should be provided as separate files (see below). Our typesetter will insert the graphics where they are mentioned in the text, therefore, please ensure all Figures and Schemes are mentioned in sequence.
3. **Tables** are edited in the text and therefore should not be sent as graphical elements but rather **should be generated in Word and provided at the end of the manuscript text file**. The tables should be set up with tabulators, not with the space bar or line breaks.
4. For Tables containing structures, these may be left in the word document for reference but should also be provided as a graphic file. When supplying these structures in the original file format (i.e., ChemDraw or ISISDraw), all structures may be contained within a single file if easier.
5. Each Scheme, Figure, or Formula, as well as the graphic for the Table of Contents, should be provided in their original file format (e.g., PowerPoint, ChemDraw, etc.) or as high-resolution image files (.tiff, .jpeg, etc.). Graphics embedded in word documents are **not** useable due to the loss of resolution when extracted for the purpose of typesetting. Graphics should be revised to adhere to the journal house style outlined in the Checklist provided upon acceptance. To ensure trouble-free reproduction of the manuscript, please adhere closely to the guidelines given here and in the Checklist.
6. Any Supporting Information should be provided as a separate document in PDF format; **Author/Title information for Supporting Information files should not be included**, as a cover sheet containing this information is generated automatically during the production process.
7. Preferred word-processing programs are: Microsoft Word for Windows 6.0, 7.0, and updates, WordPerfect, and Macintosh files, preferably stored in Word format. Documents prepared with other word-processing programs should be converted if possible. ChemTex files, for example, cannot be used.
8. Please avoid end-of-line word divisions; if necessary, these are included automatically by our typesetter and can be reviewed prior to publication in the galley proofs. Please use only one font type, except for Greek letters, which should be typed in the Symbol font.
9. Formula numbers, and in the reference section, the year of publication (but not headings such as "Table 1" or "Figure 1") should be in boldface or doubly underlined. The symbols for 1 (one) and l ("ell"), 0 (zero) and O ("oh") should be distinct.
10. The manuscript should not be fragmented into separate files. Please save only the graphics and the Supporting Information as separate files.

Structure and Sequence Data

If not already done prior to the original manuscript submission, authors of accepted papers **must** deposit structural and sequence information in the appropriate databases, as indicated above in the [Experimental Section guidelines](#). Detailed instructions for data submission can be found on the WWW homepages of the databases listed in that section as well.

Please remember that the data in databanks must be released, at the latest, upon publication of the manuscript. We trust in the cooperation of our authors in ensuring that data is released in a timely fashion.

Supporting Information

After manuscript acceptance, succinct text and the necessary graphics should be submitted as a **single separate PDF file** along with the final revised version of the manuscript. Only in the Supporting Information should the graphics be present as imports in the PDF file and not as separate files. The Supporting Information should be exactly as an Author wishes it to appear to readers; as such, Authors should check the PDF carefully to ensure the graphics are clearly and appropriately presented. **Please do NOT include a Title/Authors/Affiliations page, as a cover page for the Supporting Information including these details is generated automatically during production.** Supporting Information should not include crystallographic or sequence data that are available from the relevant databases. Also, please ensure that all Greek characters are in the 'Symbol' font. There is no charge for the use of color in the Supporting Information. Animated multimedia applications are welcome as Supporting Information.

Editorial Policy on Computational Work

In general terms, manuscripts reporting primarily computational medicinal chemistry results must fall into one of the following categories (see below for more in-depth details): 1) manuscripts describing a novel technique with explicit application to medicinal chemistry and related fields; 2) manuscripts reporting experimentally validated results that provide insight into a topical problem within the field of medicinal chemistry; 3) manuscripts detailing the application of statistics or data mining, providing provocative and interesting results of importance to the field.

Those manuscripts that fail to meet these criteria will not be considered for publication in the journal.

Molecular Modeling and Binding Modes

Molecular modeling has become an increasingly popular tool in the study of drug–target binding modes. As such, there is an ever-present risk of relying too heavily on computational results in drawing conclusions. A careful balance must be maintained between computational and experimental work; it is important that the results of modeling studies be used to establish hypotheses that are followed up by experimental work, or to help confirm and interpret previously acquired experimental data.

Therefore, we ask authors—as well as referees of manuscripts in peer review—to carefully assess the need for molecular modeling work: Is it essential for the research in understanding exactly how and where a compound might bind its target? Is a computational model or study truly needed? What are the key messages resulting from the calculations? If there are none, authors should consider whether the manuscript might be more appropriate for a different journal.

If the modeling aspects are essential, they should be experimentally validated. For example, a proposed binding mode can be experimentally checked, but a molecular dynamics study that proposes a mechanism for ligand entry into a binding pocket cannot, and thus a manuscript on

such a topic remains a purely theoretical exercise, and should be submitted to a more specialized journal.

Wherever possible, experimental data should accompany the modeling work. **If no experimental evidence is provided to corroborate the theoretical study, then the following should be clearly stated:** 1) what is prediction and what is solid experimental evidence, and 2) what experiments could be conducted to assess the hypothesis. Furthermore, the outcome of "automated" molecular design tools such as docking programs should always be critically and visually examined.

QSAR/QSPR Studies

Topics in quantitative structure–activity relationships appear frequently in *ChemMedChem*, and in light of the recent broadening of this field, it is important that prospective authors are aware of our editorial policy toward QSAR/QSPR manuscripts. Manuscripts must meet the following strict criteria to be considered for publication in the journal:

1. If a new method or theory is reported, it should be compared with at least one other commonly used method, and validated against three datasets, at least two of which should be published (i.e., publicly available). All QSAR/QSPR models must be validated using external data. Data used in the development of the model are NOT appropriate for use in model validation.
2. All QSAR/QSPR models generated through standard or well-known computational techniques must be tested with regard to their predictive power using experimental results. Biological data may be taken from the literature; however, they should be taken from a single source to ensure accuracy and to avoid discrepancies arising through variations in experimental conditions, etc.
3. All data used in performing the QSAR study should be reported in the manuscript itself, provided in the Supporting Information, or accessible elsewhere. In short, data should be readily available without restriction.

Furthermore, the novelty of the QSAR/QSPR study should be clearly stated, preferably in the article's Abstract and Introduction.

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Journal: ChemMedChem

Author names: Mostert, S., Petzer, A. & Petzer, J.P.

Corresponding author: Petzer, J.P.

Email address: jacques.petzer@nwu.ac.za

Title: Indanones as high-potency reversible inhibitors of monoamine oxidase

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Addendum B

Author guidelines Chemical Biology & Drug Design

Chemical Biology & Drug Design

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Author Guidelines

Aims and Scope

Chemical Biology & Drug Design is a peer-reviewed scientific journal that is dedicated to the advancement of innovative science, technology and medicine with a focus on the multidisciplinary fields of chemical biology and drug design. It is the aim of *Chemical Biology & Drug Design* to capture significant research and drug discovery that highlights new concepts, insight and new findings within the scope of chemical biology and drug design.

The following scientific topics will exemplify the scope of the journal:

- Protein, peptide, peptidomimetic, nucleic acid, lipids, carbohydrate and natural product chemical biology and drug design
- Receptor agonist/antagonist, protease substrate/inhibitor, and signal transduction modulator chemical biology and drug design
- Small-molecule diversity and synthetic methods important to investigate chemical space relationships to drug discovery
- Structure-based drug design, molecular modeling.
- Virtual screening of drug targets and related in silico chemoinformatics and bioinformatics technologies
- NMR, X-ray crystallography, calorimetry and related biophysical technologies
- Therapeutic target proteomics, chemical genomics and molecular screening technologies
- Emerging chemical, biological and drug design concepts of significance to pharmacology, ADMET and drug delivery
- Novel proof-of-concept ligands and breakthrough medicines (CB&DD thematic issues)

Types of paper

Chemical Biology & Drug Design welcomes correspondence in the following categories:

- **Reviews and perspectives** will be focused on a significantly important theme to chemical biology and drug design. Reviews should incorporate a judicious use of tables, figures, schemes and references. Perspectives are short reviews on a narrow topic.
- **Research Articles** will provide detailed description of scientific findings within the scope of the journal and formatted according to guidance highlighted below with respect to subheadings, inclusion of tables and/or figures, reference style, etc.
- **Research Letters** will be short version of a Research Article, but should be continuous text (subheadings for 'Results and Discussion' and 'Conclusions and Future Directions' are optional).
- **Commentaries** will be invited by the Editors to provide focused viewpoints of specific topics of interest, and written as continuous text with no subheadings.

ONLINE SUBMISSION

To submit a manuscript for *Chemical Biology & Drug Design* please visit <http://mc.manuscriptcentral.com/cbdd>.

If you have any problems with the electronic submission, please contact Andrea Lewis at the central Editorial Office: cbddeditorial@wiley.com

Initial submission

At initial submission, we encourage authors to submit their text, figures and tables as one PDF file for the purposes of peer review. Alternatively the following files will be converted into a single PDF:

- The manuscript (including title page, abstract, main text, references and tables) in Word format.
- Figures can be included with the main text for reviewing purposes, or provided as a Powerpoint or PDF file.
- Supplementary figures and tables for publication should be included for reviewing purposes (see **Supplementary material**).

Please note that high quality images in the correct format for Production will be required should your manuscript be accepted.

Submission of a revised manuscript

Please follow instructions in the revision letter provided in the editorial decision letter. You will need to:

- Provide a point-by-point response to the reviewers' comments online.
- Upload a revised version of the text in Word format.
- Upload separate print quality figure files in TIFF or EPS format. Information on our digital illustration standards can be obtained here: <http://authorservices.wiley.com/bauthor/illustration.asp>

Guidelines for Research Article Manuscript Preparation

Articles should be assembled as below:

Title page:

Should include the following:

- Title- less than 50 words
- short running title - less than 50 characters,
- 5-10 keywords,
- complete names of institutions for each author, and the name, address, phone number, fax number and e-mail address for the corresponding author.

Abstract: Less than 200 words. Abstracts should include a sentence that clarifies the novelty of the manuscript and its place in the field. No references may be included in the abstract.

Introduction: Should be focused; exhaustive literature overviews are not appropriate.

Methods and Materials: Must contain sufficient detail such that, in combination with the references cited, all experiments reported can be fully reproduced. "Nested" methods are not acceptable.

Results should be kept distinct from the discussion.

Discussion: This section discusses and interprets the results with regard to the previous work in the field and as reported in the literature.

Conclusions Acknowledgments: All sources of institutional, private and corporate financial support for the work within the manuscript must be fully acknowledged.

Conflict of Interest: All authors must declare financial/commercial conflicts of interest, or declare if there is none,

References should be cited in the text parenthetically by number, listed at the end of the manuscript in the order cited. "In press" references or "personal communication" are not permitted. CB&DD uses the Vancouver style of references that is outlined below. In all cases, the author list should be abbreviated to et al. after 15 authors.

Journal Article:

1. Sawyer, T.K. (2004) Cancer metastasis therapeutic targets and drug discovery: emerging small-molecule protein kinase inhibitors. *Expert Opin Investig Drugs* 13: 1-19.

Article in Book:

2. Sawyer, T.K. (1997) Peptidomimetic and nonpeptide drug discovery: Impact structure-based drug design. In: Veerapandian, P., editor. *Structure-Based Drug Design: Diseases, Targets, Techniques and Developments*. Marcel Dekker, New York, USA, p. 559-634.

Complete Book

3. Borchardt, R.T., Freidinger, R.M., Sawyer, T.K., Smith, P.L. (1998) *Integration of Pharmaceutical Discovery and Development: Case Histories*. Plenum Publishing Corp., New York, USA.

We recommend the use of a tool such as EndNote or Reference Manager for reference management and formatting.

Notes should be listed in the text as superscript lower case letters, a, b, c, etc and listed at the end of the manuscript (following the References section) in the order cited in the text. Websites should also be listed in the Notes section and not within the reference list. Other usage of the Notes section may be permitted as dependent on editorial review.

Abbreviations: The use of unnecessary abbreviations is strongly discouraged. CB&DD adheres to the conventions outlined in Units, Symbols and Abbreviations: A guide for medical and scientific editors and authors. Non-standard abbreviations must be used 5 or more times and written in full when first used.

Figures: All figures should be planned to fit within either 1 column width (8 cm), 1.5 column widths (13 cm) or 2 column widths (17 cm), and must be suitable for photocopy reproduction from the typeset version of the manuscript. These should be at a resolution of at least 300 dpi at final size.

The editors recommend using the detailed guidelines
<http://authorservices.wiley.com/bauthor/illustration.asp>.

If preferred, authors may submit figures and schemes in PDF format on submission. However, please note that TIFF or EPS files will be requested should the manuscript be accepted.

Chemical structures should be prepared using a chemical drawing program (e.g., ChemDraw) as standard practice and saved as TIFF or EPS files in submitting along with Tables and manuscript (text and references).

Figure Legends and Tables should be a separate section of the manuscript. Tables should be double spaced with no vertical rulings, with a single bold ruling beneath the column titles. Units of measurement must be included in the column title.

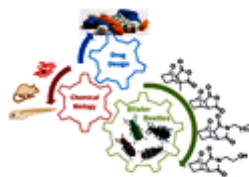
Supporting Information: The availability of Supporting Information should be indicated in the main manuscript by a paragraph, to appear after the References, headed 'Supporting Information' and providing titles of figures, tables, etc. The Supporting Information should be submitted in its final form. No changes can be made subsequently by the Editorial Office or the Publisher. When submitting Supporting Information via the electronic submission website authors should choose 'Supplementary File' from the file designation drop down list on the File Upload page. Further details on Supporting Information are available [here](#).

In general, Supplemental Information is limited to data and other materials that directly support the main conclusions of a paper but are considered additional or secondary support for the main conclusions, or cannot be included in the main paper for reasons such as space or file format restrictions. Supplemental Information should be within the conceptual scope of the main paper and not extend beyond it. The quality and presentation of Supplemental Information should be on par with that in the main paper and is subject to the same scientific standards.

Please note it is not acceptable to move material from the main paper to Supporting Information to avoid page charges.

Graphical Table of Contents

CBDD's table of contents will be presented in graphical form with a brief abstract.



Chemical and pharmacological information on cantharidin-based SMs was analyzed. The review summarizes new facts about blister beetles metabolites for the period 2006-2012. General synthetic approaches to cantharidin-based small molecules as well as its chemical transformations and potential biological applications were discussed in this mini review that could help to design new SMs modulators from other biological models.

The table of contents entry must include the article title, the authors' names (with the corresponding author indicated by an asterisk), no more than 80 words or 3 sentences of text summarising the key findings presented in the paper and a figure that best represents the scope of the paper. Table of contents entries should be submitted to ScholarOne, uploaded as 'Graphical Abstract' during the manuscript submission process.

The image should be supplied as a tif or a pdf and fit within the dimensions of 50mm x 60mm, and be fully legible at this size.

Language

CB & DD publishes in English. Non-native speakers are advised to have their manuscripts checked for grammar and style by a native English speaker before submission. A list of independent suppliers of editing services can be found [here](#). All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication.

Guidelines for Research Letter Manuscript Preparation

The same as Research Articles with the exception that headings are not required and Letters are no more than 4 typeset pages in length. There are no page charges for Letters.

Guidelines for 'Review' and 'Perspective' Manuscript Preparation

These are the same as for Research Articles, but without a methods section. Sub-headings can be used as required.

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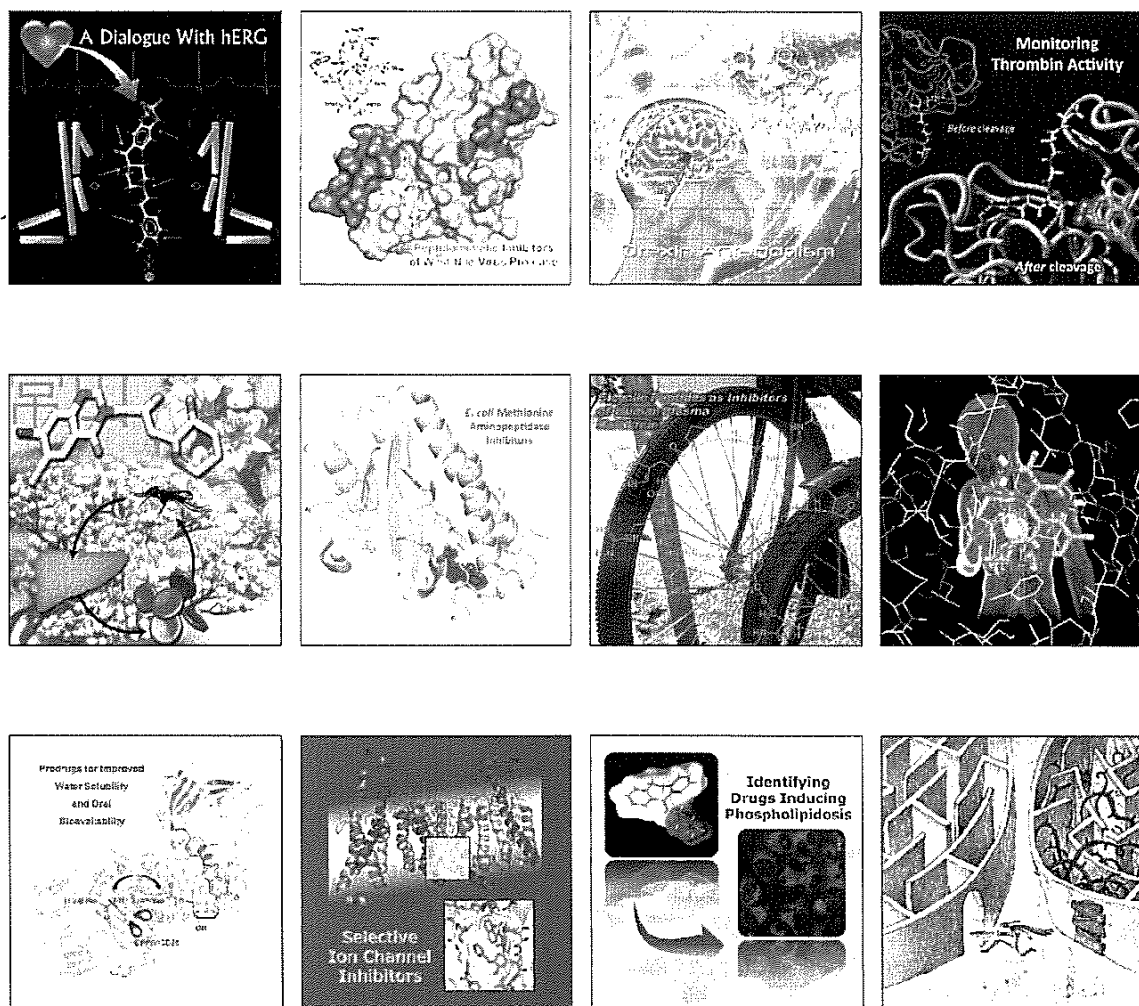
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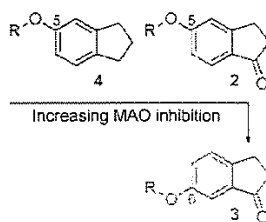
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Indanones As High-Potency Reversible Inhibitors of Monoamine Oxidase



Location, location, location: C6-substituted indanones **3** are highly potent and selective MAO-B inhibitors, with IC_{50} values ranging from 0.001–0.030 μM . C5-substituted indanone (**2**) and indane (**4**) derivatives are comparatively weaker MAO-B inhibitors. C6-substituted indanones are thus promising leads for the design of therapies for Parkinson's disease.

Indanones As High-Potency Reversible Inhibitors of Monoamine Oxidase

Samantha Mostert,^[a, b] Anél Petzer,^[a] and Jacobus P. Petzer^{*,[a, b]}

Recent reports document that α -tetralone (3,4-dihydro-2H-naphthalen-1-one) is an appropriate scaffold for the design of high-potency monoamine oxidase (MAO) inhibitors. Based on the structural similarity between α -tetralone and 1-indanone, the present study involved synthesis of 34 1-indanone and related indane derivatives as potential inhibitors of recombinant human MAO-A and MAO-B. The results show that C6-substituted indanones are particularly potent and selective MAO-B inhibitors, with IC_{50} values ranging from 0.001 to 0.030 μ M. C5-Substituted indanone and indane derivatives are comparatively

weaker MAO-B inhibitors. Although the 1-indanone and indane derivatives are selective inhibitors of the MAO-B isoform, a number of homologues are also potent MAO-A inhibitors, with three homologues possessing IC_{50} values < 0.1 μ M. Dialysis of enzyme-inhibitor mixtures further established a selected 1-indanone as a reversible MAO inhibitor with a competitive mode of inhibition. It may be concluded that 1-indanones are promising leads for the design of therapies for neurodegenerative and neuropsychiatric disorders such as Parkinson's disease and depression.

Introduction

The monoamine oxidase (MAO) enzymes are mitochondrial-bound flavoproteins and catalyze the α -carbon two-electron oxidation of amine substrates.^[1,2] MAO consists of two separate isoforms, MAO-A and MAO-B, which are encoded by distinct genes.^[3] Although the substrate specificities of MAO-A and MAO-B often overlap, certain substrates are specifically metabolized by one MAO isoform. In this regard, serotonin is preferentially metabolized by MAO-A, whereas the false neurotransmitter β -phenylethylamine is a MAO-B-specific substrate. The dietary amine tyramine, dopamine, epinephrine, and norepinephrine are considered to be substrates for both MAO isoforms.^[4] The MAO enzymes are pharmacologically important, as they terminate the physiological actions of amine neurotransmitters in the brain and peripheral tissues.^[5,6] Inhibitors of MAO-A and MAO-B have therefore been extensively used in the treatment of disease states that result from deficient neurotransmitter levels. For example, decreased serotonin levels in the brain are associated with depressive illness, and MAO-A inhibitors are thus used as antidepressants.^[7,8] For Parkinson's disease, MAO-B inhibitors are used to inhibit the MAO-catalyzed breakdown of dopamine,^[9] which relieves dopamine depletion in the basal ganglia. MAO-B inhibitors are often combined with L-DOPA, the metabolic precursor of dopamine, in the treatment of Parkinson's disease in order to prolong the

action of dopamine derived from L-DOPA.^[10,11] Interestingly, clinical evidence supports a role for MAO-B inhibitors as an aid in smoking cessation.^[12]

Notably, MAO-B inhibitors are considered to possess neuroprotective properties in Parkinson's disease. Although the mechanism is unclear, this neuroprotective effect of MAO-B inhibitors may be associated with a decrease in the central levels of hydrogen peroxide and aldehydes.^[5,13] H_2O_2 and aldehyde intermediates are formed by the MAO catalytic cycle, and may injure neuronal cells if not adequately inactivated.^[5,13] In this regard, MAO-B inhibitors are considered more effective than MAO-A inhibitors in treating Parkinson's disease, as MAO-B activity, and not MAO-A activity, increases as the human brain ages. This suggests that injurious species produced by MAO in the aged Parkinsonian brain is more likely to be a result of the action of MAO-B than MAO-A.^[14] H_2O_2 and aldehyde intermediates of the MAO catalytic cycle may also play roles in cardiovascular pathophysiology. By decreasing the concentrations of these species in the myocardium, MAO inhibitors may improve cardiac function in patients with congestive heart failure.^[15]

Based on the various pharmacological roles of MAO inhibitors, the discovery of novel classes and scaffolds is of value. Considering their isoform selectivities, MAO inhibitors may be categorized as MAO-A selective, MAO-B selective, and MAO-A/B nonselective inhibitors. MAO inhibitors may be further categorized as irreversible and reversible. Whereas MAO-B-selective inhibitors, including irreversible inhibitors, have an excellent safety profile, MAO-A inhibitors and MAO-A/B nonselective inhibitors are used with caution in the clinical setting. This is due to the ability of MAO-A inhibitors to cause a severe hypertensive response when taken with dietary tyramine.^[16] Tyramine is, to a large degree, metabolized in the intestine by MAO-A, which limits its entry into the systemic circulation. Inhibition of

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MAO-A therefore results in high plasma levels of tyramine. Because tyramine induces the release of norepinephrine from peripheral neurons, excessive amounts of this amine result in severe hypertension.^[16] Importantly, this effect is associated with irreversible MAO-A inhibition, and reversible MAO-A inhibitors do not cause tyramine-induced hypertension.^[17,18] In the design of MAO inhibitors, a reversible mode of inhibition is therefore desirable, particularly for the MAO-A isoform.

We recently reported a series of α -tetralone (3,4-dihydro-2H-naphthalen-1-one) derivatives **1**, which act as highly potent MAO-B inhibitors; all fifteen compounds exhibited IC_{50} values in the nanomolar range (Figure 1).^[19] The α -tetralones were

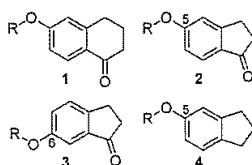


Figure 1. Structures of compounds 1–4 discussed in the text.

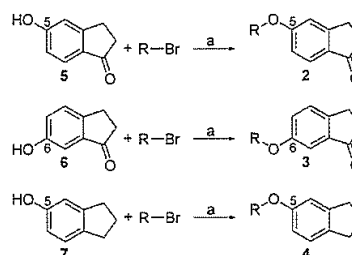
also found to be potent MAO-A inhibitors, with ten compounds showing IC_{50} values in the nanomolar range. With the objective of discovering additional high-potency MAO inhibitors, the present study involved the synthesis of a series of 1-indanone derivatives (**2** and **3**) as potential MAO inhibitors. 1-Indanone may be viewed as the five-membered-ring analogue of α -tetralone and thus represents a suitable scaffold for MAO inhibitor design. For the purpose of this study, 1-indanone was substituted at the C5- or C6-positions with a benzyloxy moiety, which has been shown to be a suitable substituent for high-potency MAO inhibition by α -tetralone derivatives when placed at the C6-position. Because halogen substitution enhances both MAO-A and MAO-B inhibitory potencies of such α -tetralones, the 1-indanone derivatives were substituted at the *meta* and *para* positions of the benzyloxy phenyl ring with halogen atoms (Cl, Br). To further derive structure–activity relationships (SARs) for MAO inhibition by the 1-indanone compound class, homologues substituted at C5 or C6 with cyclohexylmethoxy, cyclohexylethoxy, phenylpropoxy, 4-methylpentoxy, and 2-phenoxyethoxy moieties were included. In addition, derivatives substituted at the *para* positions of the 2-phenoxyethoxy phenyl ring with halogens (Cl, Br) were also considered. We also synthesized a homologous series of indane derivatives **4** for comparison with the 1-indanones. From the analysis above, it is evident that 1-indanone and indane derivatives should act as reversible MAO inhibitors, particularly when the MAO-A isoform is inhibited. Active MAO inhibitors may be leads for the design of new therapies for a variety of disorders in which MAO is implicated in the pathophysiology. The proposal that 1-indanone and indane derivatives may act as MAO inhibitors is supported by the observation that rasagiline, a MAO-B inhibitor in clinical use, is an indane

derivative. In addition, a number of rasagiline analogues, also indane derivatives, have been found to act as MAO inhibitors.^[20]

Results and Discussion

Chemistry

The 1-indanone (**2** and **3**) and indane **4** derivatives were synthesized in poor to good yields (6–85%) according to a published procedure (Scheme 1).^[21] Commercially available 5-hy-



Scheme 1. Synthetic route to the 1-indanone (**2** and **3**) and indane (compound **4**) derivatives. Reagents and conditions: a) DMF, K_2CO_3 , RT, 5–24 h.

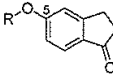
droxy-1-indanone (**5**), 6-hydroxy-1-indanone (**6**), or 5-indanol (**7**) was dissolved in *N,N*-dimethylformamide (DMF) and treated with an appropriately substituted alkyl bromide in the presence of potassium carbonate. After stirring the reaction for 5–24 h at room temperature, ethyl acetate was added to the reaction. The ethyl acetate phase was washed with water and brine and dried over magnesium sulfate. The crude obtained after evaporation of the solvent was purified by recrystallization, and the structures of the target compounds were verified by ^1H NMR, ^{13}C NMR, and mass spectrometry.

MAO inhibition

The 1-indanone and indane derivatives were evaluated as inhibitors of recombinant human MAO-A and MAO-B. The inhibitory potencies of the derivatives are expressed as IC_{50} values, which were calculated from sigmoidal plots of enzyme catalytic rate versus inhibitor concentration. The MAO-A and MAO-B catalytic activities were measured by using the MAO-A/B mixed substrate kynuramine, and measuring the MAO-catalyzed formation of 4-hydroxyquinoline by fluorescence spectrophotometry.^[22] By measuring the fluorescence of 4-hydroxyquinoline in the absence and presence of the test inhibitors, it was established that the 1-indanone and indane derivatives do not fluoresce under the specific assay conditions and concentrations used, and thus do not interfere with the fluorescence measurements.

The MAO inhibition potencies of the 1-indanones (**2**, **3**) and indane derivatives **4** are given as the corresponding IC_{50} values in Tables 1–3. From the inhibition data it is evident that the de-

Table 1. Inhibition of recombinant human MAO-A and MAO-B by the 1-indanone derivatives 2.

 R	IC ₅₀ [μM] ^[a]		SI ^[b]
	MAO-A	MAO-B	
2a C ₆ H ₅ CH ₂ –	10.75 ± 0.799	0.135 ± 0.007	79.6
2b 3-ClC ₆ H ₄ CH ₂ –	2.56 ± 0.159	0.015 ± 0.002	170.6
2c 3-BrC ₆ H ₄ CH ₂ –	3.068 ± 0.193	0.019 ± 0.005	161.5
2d 4-ClC ₆ H ₄ CH ₂ –	0.504 ± 0.162	0.058 ± 0.002	8.7
2e 4-BrC ₆ H ₄ CH ₂ –	0.659 ± 0.066	0.052 ± 0.013	12.7
2f C ₆ H ₁₁ (CH ₂) ₂ –	1.607 ± 0.081	0.053 ± 0.023	30.3
2g C ₆ H ₅ (CH ₂) ₃ –	2.296 ± 0.346	0.04 ± 0.007	57.4
2h (CH ₃) ₂ CH(CH ₂) ₃ –	0.537 ± 0.061	0.081 ± 0.024	6.6
2i C ₆ H ₅ O(CH ₂) ₂ –	1.583 ± 0.136	0.113 ± 0.01	14.0
2j 4-ClC ₆ H ₄ O(CH ₂) ₂ –	0.812 ± 0.129	0.298 ± 0.083	2.7
2k 4-BrC ₆ H ₄ O(CH ₂) ₂ –	0.86 ± 0.309	0.16 ± 0.038	5.4
2l C ₆ H ₅ CH ₂ –	0.971 ± 0.191	0.05 ± 0.025	19.4

[a] Values are the mean ± SD of triplicate determinations. [b] Selectivity index for the MAO-B isoform, given as the ratio: (IC₅₀MAO-A)/(IC₅₀MAO-B).

Table 2. Inhibition of recombinant human MAO-A and MAO-B by the 1-indanone derivatives 3.

	IC ₅₀ [μM] ^[a]		SI ^[b]	
	R	MAO-A		MAO-B
3a	C ₆ H ₅ CH ₂ –	0.555 ± 0.096	0.015 ± .003	37.0
3b	3-ClC ₆ H ₄ CH ₂ –	0.084 ± 0.009	0.002 ± 0.00003	42.0
3c	3-BrC ₆ H ₄ CH ₂ –	0.116 ± 0.013	0.002 ± 0.0002	58.0
3d	4-ClC ₆ H ₄ CH ₂ –	0.032 ± 0.004	0.002 ± 0.0001	16.0
3e	4-BrC ₆ H ₄ CH ₂ –	0.039 ± 0.001	0.003 ± 0.0005	13.0
3f	C ₆ H ₁₁ (CH ₂) ₂ –	0.757 ± 0.11	0.03 ± 0.006	25.2
3g	C ₆ H ₅ (CH ₂) ₃ –	0.203 ± 0.022	0.019 ± 0.006	10.7
3h	(CH ₃) ₂ CH(CH ₂) ₃ –	0.364 ± 0.027	0.02 ± 0.005	18.2
3i	C ₆ H ₅ O(CH ₂) ₂ –	1.348 ± 0.043	0.001 ± 0.0005	1348
3j	4-ClC ₆ H ₄ O(CH ₂) ₂ –	0.161 ± 0.011	0.007 ± 0.001	23.0
3k	4-BrC ₆ H ₄ O(CH ₂) ₂ –	0.323 ± 0.03	0.016 ± 0.001	20.2

[a] Values are the mean ± SD of triplicate determinations. [b] Selectivity index for the MAO-B isoform, given as the ratio: (IC₅₀MAO-A)/(IC₅₀MAO-B).

derivatives 2–4 are selective inhibitors of MAO-B, with selectivity index (SI) values > 2.7. Compound 3i is a particularly selective inhibitor (Table 2), and was found to be 1348-fold more selective for MAO-B over the MAO-A isoform. The 1-indanone derivatives 3 substituted at the C6-position proved to be the most potent MAO-B inhibitors of the series evaluated, with all homologues possessing IC₅₀ values < 0.030 μM. All of the C6-substituted 1-indanones may therefore be considered as highly potent MAO-B inhibitors. Compared with the reference MAO-B inhibitor lazabemide (IC₅₀ = 0.091 μM), the C6-substituted 1-indanones are thus higher-potency MAO-B inhibitors.^[23] Although generally less potent, the C5-substituted 1-indanones 2 are also potent MAO-B inhibitors, with eight of twelve homologues possessing IC₅₀ values < 0.10 μM (Table 1). Among the three series, the indane derivatives 4 are the least potent, with three of eleven homologues possessing IC₅₀ values < 0.10 μM (Table 3).

Table 3. Inhibition of recombinant human MAO-A and MAO-B by the indane derivatives 4.

 R	MAO-A	MAO-B	SI ^[b]	
IC ₅₀ [μM] ^[a]				
4a	C ₆ H ₅ CH ₂ –	35.315 ± 0.530	0.353 ± 0.056	100.0
4b	3-ClC ₆ H ₄ CH ₂ –	3.14 ± 0.247	0.041 ± 0.009	76.6
4c	3-BrC ₆ H ₄ CH ₂ –	3.966 ± 0.406	0.081 ± 0.016	49.0
4d	4-ClC ₆ H ₄ CH ₂ –	4.086 ± 0.561	0.512 ± 0.13	8.0
4e	4-BrC ₆ H ₄ CH ₂ –	3.329 ± 0.411	0.148 ± 0.016	22.5
4f	C ₆ H ₁₁ (CH ₂) ₂ –	3.916 ± 2.268	0.059 ± 0.012	66.4
4g	C ₆ H ₅ (CH ₂) ₃ –	9.158 ± 0.313	1.179 ± 0.055	7.7
4h	(CH ₃) ₂ CH(CH ₂) ₃ –	7.588 ± 1.564	0.413 ± 0.076	18.4
4i	C ₆ H ₅ O(CH ₂) ₂ –	9.312 ± 0.384	0.309 ± 0.1	30.1
4j	4-ClC ₆ H ₄ O(CH ₂) ₂ –	> 100	> 100	–
4k	4-BrC ₆ H ₄ O(CH ₂) ₂ –	25.677 ± 0.43	5.918 ± 2.041	4.3

[a] Values are the mean ± SD of triplicate determinations. [b] Selectivity index for the MAO-B isoform, given as the ratio: (IC₅₀MAO-A)/(IC₅₀MAO-B).

Some SARs may be derived from the inhibition data: For both series of 1-indanone derivatives 2 and 3, substitution on the benzyloxy phenyl ring with halogens (Cl, Br) enhanced MAO-B inhibitory potency relative to the unsubstituted homologues 2a and 3a. For the indane derivatives 4, chlorine and bromine substitution at the *meta* position, and bromine substitution at the *para* position of the benzyloxy phenyl ring led to enhanced MAO-B inhibitory potency. In contrast, Cl and Br substitution on the 2-phenoxyethoxy phenyl ring did not enhance MAO-B inhibition for any of the three series relative to the unsubstituted homologues 2i, 3i, and 4i. Also notable is that for all three series, the cyclohexyl-containing homologues may be considered highly potent MAO-B inhibitors, with IC₅₀ values < 0.059 μM. This suggests that a terminal cyclohexyl moiety is beneficial for MAO-B inhibition, and in certain instances (as for the C5-substituted 1-indanones) may even lead to enhanced MAO-B inhibition relative to the phenyl-substituted homologues (compare 2l and 2f versus 2a). For the series of 1-indanone derivatives 2 and 3, the 4-methylpentoxo substituent is also well tolerated, with homologues containing this substituent displaying IC₅₀ values < 0.081 μM. Similarly, for the series of 1-indanone derivatives, but not the indanes, substitution with the phenylpropoxo moiety also yields compounds with potent MAO-B inhibitory activity, with IC₅₀ values < 0.04 μM.

Similar to MAO-B, the 1-indanone (2, 3) and indane 4 derivatives were also found to be inhibitors of MAO-A, with the 1-indanone derivatives 3 substituted at the C6-position displaying greater inhibitory potency than the C5-substituted 1-indanones and indanes. In fact, among the C6-substituted 1-indanones, with the exception of one homologue, all compounds possess IC₅₀ values in the sub-micromolar range for the inhibition of MAO-A. The C5-substituted 1-indanones, in turn, are more potent MAO-A inhibitors than the indanes. The most potent MAO-A inhibitors among the compounds evaluated are 3b, 3d, and 3e, which display IC₅₀ values < 0.084 μM. Because these compounds are also highly potent MAO-B inhibitors, they may be viewed as MAO-A/B nonselective inhibitors. These

inhibitors are also significantly more potent than the reversible MAO-A inhibitor tolloxatone, which inhibits human MAO-A with an IC_{50} value of $3.92 \mu\text{M}$.^[23] Interesting SARs may also be derived from the MAO-A inhibition data. Among all three series, substitution on the benzyloxy phenyl ring with halogens (Cl, Br) enhances MAO-A inhibitory potency. For the 1-indanones **2** and **3**, halogen substitution at the *para* position is more beneficial than at the *meta* position. Chlorine or bromine substitution on the 2-phenoxyethoxy phenyl ring of the 1-indanones **2** and **3** also enhances MAO-A inhibitory potency. For all three series, the 4-methylpentoxy substituent enhances MAO-A inhibitory potency relative to the benzyloxy-substituted homologues, whereas for the C5-substituted 1-indanones and indanes, the cyclohexyl-containing homologues are more potent than the corresponding benzyloxy-substituted homologues. For all three series, substitution with the phenylpropoxy moiety also enhances MAO-A inhibitory potency relative to the benzyloxy-substituted homologues, which suggests that elongation of the C5/6 side chains of 1-indanone and indane derivatives may be a strategy to enhance MAO-A inhibitory potency.

To show that the C5 and C6 substituents of the 1-indanone (**2**, **3**) and indane **4** derivatives are indeed structural requirements for MAO inhibition, 5-hydroxy-1-indanone (**5**), 6-hydroxy-1-indanone (**6**), or 5-indanol (**7**) were examined as potential inhibitors of human MAO. The results, listed in Table 4,

Table 4. Inhibition of recombinant human MAO-A and MAO-B by compounds 5–7.			
	MAO-A	MAO-B	S ^[b]
5	61.7 ± 3.87	> 100	–
6	64.7 ± 4.28	85.4 ± 5.82	0.8
7	> 100	87.8 ± 4.71	–

[a] Values are the mean $\pm$ SD of triplicate determinations. [b] Selectivity index for the MAO-B isoform, given as the ratio: $(IC_{50\text{MAO-A}})/(IC_{50\text{MAO-B}})$.

document these compounds as being weak MAO inhibitors, with IC_{50} values ranging from 61.7 to $>100 \mu\text{M}$. Compared with the benzyloxy-substituted derivatives **2a**, **3a**, and **4a**, these compounds are thus weak MAO-A and MAO-B inhibitors. This result demonstrates that appropriate C5 and C6 substitution is a requirement for the MAO inhibitory activities of the 1-indanone and indane derivatives investigated in this study.

Reversibility of MAO inhibition

Based on the finding that 1-indanone derivatives are potent inhibitors of MAO, with the C6-substituted homologues **3b**, **3d**, and **3e** displaying highly potent inhibition of both MAO isoforms, the reversibility of MAO-A and MAO-B inhibition was examined. For this purpose, compound **3d** was selected as a rep-

resentative inhibitor. To determine if **3d** is a reversible MAO inhibitor, this compound (at a concentration of $4 \times IC_{50}$) was incubated with MAO-A and MAO-B for a period of 15 min and subsequently dialyzed for 24 h. The residual MAO catalytic activities were measured and compared with the MAO activities of control samples, which consisted of dialysis experiments carried out in the absence of the test inhibitor.^[24] For reversible inhibition it is expected that, following dialysis of enzyme-inhibitor mixtures, enzyme activity will recover to levels equivalent to those of the control values. As shown in Figure 2, after

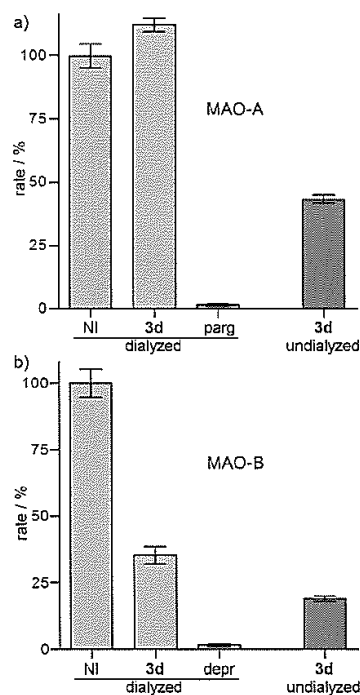


Figure 2. Reversibility of inhibition of human a) MAO-A and b) MAO-B by compound **3d**. The MAO enzymes and **3d** (at $4 \times IC_{50}$) were pre-incubated for 15 min, dialyzed for 24 h, and the residual enzyme activities were measured (**3d** dialyzed). The enzymes were similarly pre-incubated in the absence (no inhibitor [NI]) and presence of the irreversible inhibitors, pargyline (parg) or (*R*)-deprenyl (depr), and dialyzed. For comparison, the residual MAO activities of undialyzed mixtures of the MAOs with **3d** are also shown (**3d** undialyzed).

dialysis of MAO-A-**3d** mixtures, MAO-A activity is completely reversed with the catalytic activity at 112% of the control value (recorded in the absence of inhibitor). In contrast, the MAO-A activity of undialyzed mixtures of MAO-A and **3d** is 43% of the control value. This result suggests that compound **3d** interacts reversibly with MAO-A. After similar treatment and dialysis of mixtures of MAO-A and pargyline, an irreversible MAO-A inhibitor, the enzyme activity is not recovered, with the residual enzyme activity at a level of only 1.6% of the control value.

The results also document that after dialysis of MAO-B-3d mixtures, MAO-B activity is recovered to a small degree after dialysis for 24 h, with the catalytic activity at 36% of the control value. For comparison, the enzyme activity of undialyzed mixtures of MAO-B and 3d is 19% of the control value. This result suggests that the inhibition of MAO-B by 3d cannot be readily reversed by dialysis. This behavior was previously observed for the inhibition of MAO-B by a variety of inhibitor classes, including α -tetralones.^[19] Based on these published reports, a possible explanation may be that 3d exhibits tight binding to MAO-B. For example, oxadiazolones are reported to bind reversibly and time-dependently to rat brain MAO-B,^[25] whereas other reports describe this compound class to be reversible and extremely tight-binding MAO-B specific inhibitors.^[26] Further investigation is required to determine the exact mechanism of MAO-B inhibition by 1-indanone derivatives. As expected, after dialysis of mixtures of MAO-B and (*R*)-deprenyl, an irreversible MAO-B inhibitor, enzyme activity is not recovered, with only 1.6% activity (of the control value) remaining.

To investigate the modes of MAO-A and MAO-B inhibition by the representative inhibitor 3d, sets of Lineweaver–Burk plots for the inhibition of each MAO isoform were constructed. Each set of Lineweaver–Burk plots was constructed by measuring MAO activity at eight different kynuramine concentrations (15–250 μM), in the absence of inhibitor, and presence of five different concentrations of the representative inhibitor. The Lineweaver–Burk plots for the inhibition of MAO-A and MAO-B by 3d are shown in Figure 3. For the inhibition of both MAO isoforms, the lines of the Lineweaver–Burk plots are linear and intersect at the y-axis. This suggests that compound 3d is a competitive inhibitor of both MAO-A and MAO-B, and may be viewed as further evidence that 1-indanones are reversible MAO inhibitors. From plots of the slopes of the Lineweaver–Burk plots versus the concentration of 3d, K_i values of 0.047 and 0.0017 μM for the inhibition of MAO-A and MAO-B are estimated, respectively. Global (shared) fitting of the inhibition data directly to the Michaelis–Menten equation yielded similar K_i values of $0.040 \pm 0.00029 \mu\text{M}$ ($r^2 = 0.99$) and $0.0011 \pm$

$0.0001 \mu\text{M}$ ($r^2 = 0.99$) for MAO-A and MAO-B, respectively. Fitting of the MAO-B inhibition data to the modification of the Michaelis–Menten equation previously reported, yielded a $K_{i(\text{red})}$ value of $0.0061 \pm 0.001 \mu\text{M}$ and a $K_{i(\text{ox})}$ value of $0.0033 \pm 0.001 \mu\text{M}$ ($r^2 = 0.99$).^[27] This approach takes into account that the inhibitor binds to both the oxidized and reduced enzyme and may better describe the observed MAO-B kinetic data.

Molecular modeling

Molecular docking experiments often provide insight into potential binding orientations and interactions of inhibitors with the active sites of enzymes. Such information may assist in the future design of inhibitors, as well as to explain the inhibition properties of existing inhibitors. A molecular docking study was thus performed in an attempt to determine potential modes of binding of the 1-indanone (2, 3) and indane 4 derivatives to MAO-A and MAO-B. Representative compounds of each series, compounds 2a, 3a, and 4a, were selected to identify any major differences in the orientations and interactions with the MAOs between the three series. For the modeling studies, the structures of human MAO-A co-crystallized with harmine (PDB ID: 2Z5X)^[28] and human MAO-B co-crystallized with safinamide (PDB ID: 2V5Z)^[29] were used. Preparation of the models and docking calculations were carried out with the Windows-based Discovery Studio version 3.1 modeling software package^[30] according to published procedures.^[31] Docking of the inhibitors into the MAO models was performed with the CDOCKER protocol, and the structural integrity of the highest-ranked docking solutions was further examined with short (500 ps) molecular dynamics (MD) simulations of the resulting enzyme–inhibitor complexes. Frames of the production run were recorded every 1 ps to yield 500 frames. Because the CDOCKER protocol has the ability to correctly dock the co-crystallized inhibitors—harmine and safinamide—into the MAO active sites, this protocol appears to be suitable for predicting the binding orientations of inhibitors in the active sites of MAO-A and MAO-B.^[31] For harmine and safinamide, the ten

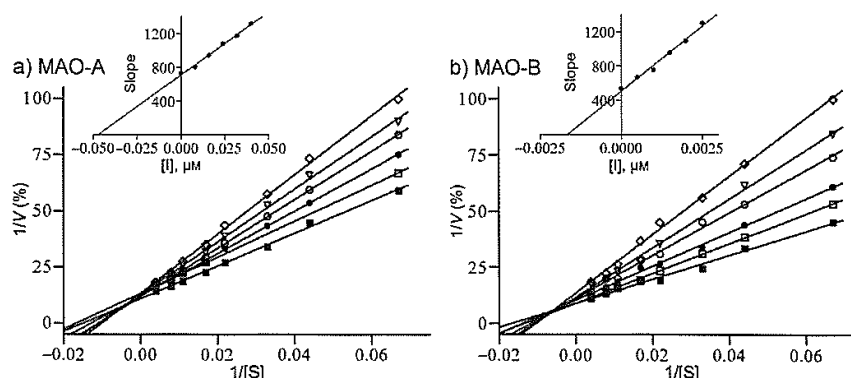


Figure 3. Lineweaver–Burk plots for human a) MAO-A and b) MAO-B in the absence (■) and presence of 3d at various concentrations. The concentrations of 3d used were equal to $1/4 \times \text{IC}_{50}$, $1/2 \times \text{IC}_{50}$, $3/4 \times \text{IC}_{50}$, $1 \times \text{IC}_{50}$ and $1/4 \times \text{IC}_{50}$. The insets are graphs of the slopes of the Lineweaver–Burk plots versus inhibitor concentration [$K_{i(\text{MAO-A})} = 0.047 \mu\text{M}$, $K_{i(\text{MAO-B})} = 0.0017 \mu\text{M}$].

best-ranked docked orientations exhibit respective root-mean-square deviation (RMSD) values of only 1.91 ± 0.93 and 1.48 ± 0.26 Å from the orientations of the co-crystallized ligands.

The best-ranked solutions for the binding of compounds **2a**, **3a**, and **4a** to the active site of MAO-A are shown in Figure 4. The results show that the 1-indanone and indane moieties of

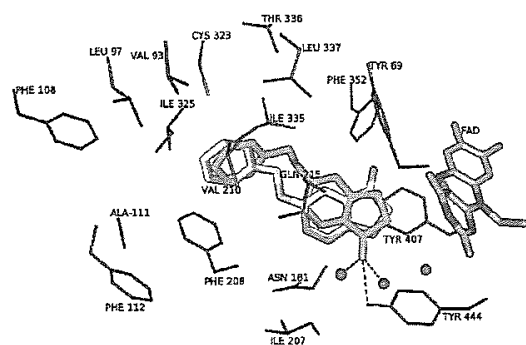


Figure 4. The best-ranked docking solutions of **2a** (green), **3a** (yellow), and **4a** (magenta) for binding to the active site of MAO-A.

these inhibitors bind in the vicinity of the FAD cofactor of MAO-A, while the C5 or C6 substituents project toward the entrance of the active site cavity. Whereas the 1-indanone moiety of **3a** and the indane moiety of **4a** exhibit similar orientations, the 1-indanone moiety of **2a** is rotated by $\sim 180^\circ$, with the carbonyl group projecting to the region in MAO-A lined by Tyr444 and Tyr407. The carbonyl group of compound **3a**, in turn, is oriented in the opposite direction toward the space lined by Tyr69, Phe352, and the FAD cofactor. These differing binding modes of the 1-indanone moieties of **2a** and **3a** are most likely guided by the optimal placement of the C5 and C6 substituents, which occupies nearly the same space in the MAO-A active site. An analysis of the trajectories of **2a**, **3a**, and **4a** shows that their orientations and placement in the MAO-A active site is relatively stable with average RMSDs from the initial docked orientation of 0.83 ± 0.36 , 1.57 ± 0.51 , and 1.75 ± 0.62 Å, respectively. For compound **2a**, hydrogen bonding occurs between the 1-indanone carbonyl oxygen and the phenolic hydrogen of Tyr444. This interaction appears to play a relatively large role in the stabilization of this inhibitor in MAO-A, as it is present in 429 frames (of a total of 500 frames). Compound **2a** also undergoes hydrogen bonding with two active site water molecules, interactions which are present in 296 and 223 frames (of a total of 500 frames) for the two waters HOH739 and HOH718, respectively. Compound **3a**, in turn, undergoes hydrogen bonding with one active site water molecule (HOH710), an interaction which is only present in 84 frames (of a total of 500 frames). No hydrogen bonding is observed between **4a** and MAO-A. The hydrogen bond interactions contribute to 9.7 and 4.3% of the total interaction energy between **2a** and **3a**, respectively, and the MAO-A active site. The most prominent van der Waals interactions of

2a, **3a**, and **4a** with MAO-A occur with Ile180, Asn181, Phe208, Gln215, Ile335, and Tyr407. These interactions contribute to 48.5, 47.6, and 51.7% of the total interaction energy between **2a**, **3a**, and **4a**, respectively, and the MAO-A active site. For **2a**, a significant van der Waals interaction also occurs with Tyr444, which contributes 6.0% of the total interaction energy. Based on this analysis, it may be concluded that **2a**, **3a**, and **4a** are stabilized to similar extents by van der Waals interactions, and mostly interact with the same residues in the MAO-A active site (Table 5). For compounds **3a** and **4a**, the in-

Table 5. Interaction energies between key MAO-A residues and compounds **2a**, **3a**, and **4a**.^[a]

Residue	2a	3a	4a
Ile 180	-3.32 ± 0.63	-3.08 ± 0.52	-2.89 ± 0.41
Asn 181	-2.30 ± 0.67	-2.02 ± 0.50	-1.92 ± 0.46
Phe 208	-3.49 ± 1.10	-3.25 ± 1.15	-4.29 ± 0.86
Gln 215	-4.79 ± 0.63	-4.65 ± 0.77	-4.25 ± 0.60
Ile 335	-2.28 ± 0.51	-2.02 ± 0.42	-2.23 ± 0.66
Tyr 407	-1.89 ± 0.62	-1.96 ± 0.67	-2.13 ± 0.67

[a] Values are the mean $\pm$ SD of 500 frames.

hibitory potencies are thus principally due to stabilization by van der Waals interactions. In spite of this, **3a** and other C6-substituted indanones are, in general, more potent MAO-A inhibitors than **4a** and the other indane derivatives. A possible molecular explanation for the improved inhibitory potencies of the C6-substituted indanones **3** relative to the indane derivatives **4** may be that although limited, the former undergoes hydrogen bonding with the MAO-A active site, whereas the indane derivatives do not. An explanation for the improved inhibitory potency of **3a** over **2a**, however, is not apparent, particularly in considering that **2a** undergoes more extensive hydrogen bonding than **3a**.

The best-ranked solutions for the binding of compounds **2a**, **3a**, and **4a** to the active site of MAO-B are shown in Figure 5. These inhibitors bind with their 1-indanone and indane moieties in the substrate cavity of MAO-B, in close proximity to the FAD cofactor, while the C5 or C6 substituents project toward

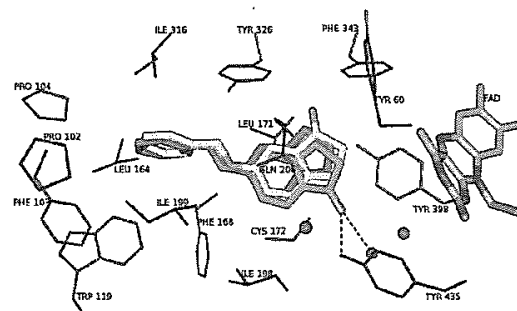


Figure 5. The best-ranked docking solutions of **2a** (green), **3a** (yellow), and **4a** (magenta) for binding to the active site of MAO-B.

the entrance cavity. The trajectory analysis for **2a**, **3a**, and **4a** shows that their orientations and placements in MAO-B are stable, with respective RMSDs of 0.76 ± 0.17 , 0.76 ± 0.17 , and 0.71 ± 0.18 Å. The most significant van der Waals interactions occur with Phe168, Leu171, Ile198, Ile199, Gln206, and Tyr326, which together contribute to 57.3, 58.0, and 61.1% of the total interaction energy between **2a**, **3a**, and **4a**, respectively, and the MAO-B active site. These interactions remain significant throughout the production run (20.3–82.5% of the total interaction energy). As in MAO-A, the 1-indanone moiety of **3a** and the indane moiety of **4a** exhibit similar orientations, while the 1-indanone moiety of **2a** is rotated by $\sim 180^\circ$. The carbonyl group of **2a** thus projects toward the space lined by Tyr398 and Tyr435. The carbonyl group of compound **3a**, in turn, projects in the opposite direction, toward a niche in the substrate cavity of MAO-B, lined by Tyr60, Tyr326, and Gln206.^[29] The result of these differing binding modes is that the carbonyl oxygen atom of **2a** is able to undergo hydrogen bonding with waters HOH1155, HOH1351 and HOH1170. The interaction with HOH1155 appears to be important, as it is observed in 362 of 500 frames, whereas hydrogen bonding with HOH1351 and HOH1170 is only present in 53 and 40 frames. Hydrogen bonding also occurs between **2a** and Tyr435 (133 of 500 frames). No hydrogen bonding is observed between the MAO-B active site and **3a** and **4a**. From these results it may be concluded that hydrogen bonding cannot explain the enhanced MAO-B inhibitory potency of **3a** compared with **2a** and **4a**. Similarly, differing van der Waals interactions also do not explain the differences between the inhibitory potencies of **2a**, **3a**, and **4a**, as the binding orientations and interactions with key residues are highly similar. As shown in Table 6, the

Table 6. Interaction energies between key MAO-B residues and compounds **2a**, **3a**, and **4a**.^[a]

Residue	2a	3a	4a
Phe-168	-2.49 ± 0.47	-2.31 ± 0.45	-2.48 ± 0.47
Leu-171	-4.16 ± 0.79	-4.61 ± 0.69	-4.29 ± 0.78
Ile-198	-2.43 ± 0.29	-2.36 ± 0.32	-2.25 ± 0.27
Ile-199	-5.28 ± 0.64	-5.29 ± 0.62	-5.26 ± 0.60
Gln-206	-3.36 ± 0.52	-4.06 ± 0.40	-3.11 ± 0.47
Tyr-326	-3.68 ± 0.39	-4.04 ± 0.45	-3.71 ± 0.36

[a] Values are the mean $\pm$ SD of 500 frames.

interaction energies between key MAO-B residues and **2a**, **3a**, and **4a** are similar. An explanation for the improved inhibitory potency of **3a** over **2a** and **4a** is thus not apparent. Interestingly, in crystal structures of rasagiline in complex with MAO-B, the indane moiety of rasagiline adopts a similar binding orientation to that of indane derivative **4a**. For both structures, the indane moieties are located in the substrate cavity. For rasagiline, the C2 atom of the five-membered ring of the indane moiety is, however, directed toward the top of the substrate cavity, toward the niche lined by Tyr60, Tyr326, and Gln206. In contrast, C2 of **4a** is directed toward FAD. These differing binding modes are most likely guided by the placement of the

benzyloxy side chain of **4a** in the entrance cavity and the formation of a covalent flavocyanine adduct between rasagiline and MAO-B.^[20]

Conclusions

In summary, the present study shows that C6-substituted 1-indanones **3** and to a lesser extent the C5-substituted homologues **2** are highly potent inhibitors of the human MAOs. All compounds from these two series proved to be selective MAO-B inhibitors, with compound **3i** being the most selective ($SI = 1348$). In conjunction with L-DOPA, selective MAO-B inhibitors are an appropriate treatment for Parkinson's disease. While a clear explanation for the selectivity of the study compounds for MAO-B over the MAO-A isoform is not apparent, it should be noted that MAO-B accommodates larger inhibitors better than MAO-A. The reason for this is that the entrance and substrate cavities of MAO-B may fuse after rotation of the side chain of Ile199 from its normal position in the cavity. In contrast, the increased size of Phe208, the residue in MAO-A that corresponds to Ile199 in MAO-B, prevents it from occupying an alternative conformation, and this residue may hinder larger inhibitors from binding to MAO-A.^[32] The 1-indanones and indanes may therefore possess selectivity for MAO-B in part because their relatively larger structures are not well accommodated in the MAO-A active site. As exemplified by compounds **3b**, **3d**, and **3e**, certain 1-indanones also are potent MAO-A/B nonselective inhibitors, drugs which may find application in the treatment of both Parkinson's disease and depression. In this regard, MAO-A/B nonselective inhibitors may be useful for patients of Parkinson's disease who also suffer from depression.^[33] As discussed, an important consideration for MAO-A inhibitors is the reversibility of inhibition. This study shows that a representative inhibitor, compound **3d**, is a reversible MAO-A inhibitor, a desirable property, as reversible MAO-A inhibitors do not cause tyramine-induced hypertension.^[17,18] Interestingly, MAO-B inhibition by **3d** is not readily reversed by dialysis. Although the molecular basis for this behavior is not apparent, it is unlikely that this would be of concern from a drug safety point of view, because irreversible MAO-B inhibitors in clinical use are reported to possess excellent safety profiles.^[34] A similar result has been obtained with the previously reported α -tetralone derivatives,^[19] which served as leads for the current study. α -Tetralones also act as reversible MAO-A inhibitors, while MAO-B inhibition by is partially reversible. The C6-substituted 1-indanones **3** may be considered to be more potent MAO-B inhibitors than the previously reported α -tetralone derivatives.^[19] For example, the α -tetralones possess IC_{50} values for the inhibition of MAO-B of 0.0045–0.078 μM , whereas compounds **3a–k** exhibit IC_{50} values of 0.001–0.030 μM . The α -tetralone derivatives are also promising MAO-A inhibitors with one compound in particular, 6-(3-cyanobenzyloxy)-3,4-dihydro-2H-naphthalen-1-one (**8**), possessing an IC_{50} value

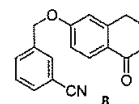


Figure 6. Structure of the α -tetralone derivative, 6-(3-cyanobenzyloxy)-3,4-dihydro-2H-naphthalen-1-one (**8**).

of 0.024 μM (Figure 6). This compound is also a MAO-A/B non-selective inhibitor, as it also inhibits MAO-B with an IC_{50} value of 0.078 μM . In this study it was shown that several compounds, such as **3b**, **3d**, and **3e**, are high-potency MAO-A inhibitors and excellent candidates for MAO-A/B nonselective inhibition. Based on this study, it may be concluded that 1-indanones are promising MAO inhibitors for the treatment of depression and Parkinson's disease.

Experimental Section

Reagents and instrumentation: Unless otherwise noted, all starting materials and reagents were obtained from Sigma-Aldrich and were used without further purification. ^1H and ^{13}C NMR spectra were recorded with a Bruker Avance III 600 spectrometer at frequencies of 600 and 150 MHz, respectively, with CDCl_3 as the solvent. Chemical shifts (δ) are reported in parts per million downfield from the internal standard, tetramethylsilane. Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), qn (quintet), sept (septet), or m (multiplet). High-resolution mass spectra (HRMS) were recorded on a Bruker microTOF-Q II mass spectrometer in atmospheric pressure chemical ionization (APCI) mode. Melting points (mp) were determined with a Büchi M-545 melting point apparatus, and are uncorrected. Thin-layer chromatography (TLC) was carried out using silica gel 60 (Merck) with UV₂₅₄ fluorescent indicator. Fluorescence spectrophotometry was carried out with a Varian Cary Eclipse fluorescence spectrophotometer. Microsomes from insect cells containing recombinant human MAO-A and MAO-B (5 mg mL⁻¹) and kynuramine-2HBr were obtained from Sigma-Aldrich. Slide-A-Lyzer dialysis cassettes with a molecular weight cutoff of 10000 Da and a sample volume capacity of 0.5–3 mL were obtained from Thermo Scientific. HPLC analyses were carried out to determine the purities of the synthesized compounds. HPLC analyses were performed with 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, and the mobile phase consisted initially of 30% CH₃CN and 70% Milli-Q water at a flow rate of 1 mL min⁻¹. At the start of each HPLC run a solvent gradient program was initiated by linearly increasing the composition of the CH₃CN in the mobile phase to 85% over a period of 5 min. Each HPLC run lasted 15 min, and a period of 5 min was allowed for equilibration between runs. Solutions (20 μL) of the test compounds in CH₃CN (1 mM) were injected into the HPLC system, and the eluent was monitored at λ 254 nm.

Synthesis of 1-indanone and indane derivatives: Commercially available 5-hydroxy-1-indanone (**5**), 6-hydroxy-1-indanone (**6**), or 5-indanol (**7**) (3.375 mmol) was dissolved in DMF (5 mL), and the reaction was placed under an atmosphere of argon. To the reaction solution was added K₂CO₃ (7.424 mmol) followed by the appropriately substituted alkyl bromide (3.712 mmol). After stirring the reaction for 5–24 h at room temperature, EtOAc (30 mL) was added to the reaction, and the resulting mixture was washed with H₂O (30 mL) and brine (2×30 mL). The EtOAc phase was dried over MgSO₄ and removed under reduced pressure. The crude obtained after evaporation of the solvent was purified by recrystallization from a mixture of cyclohexane (40 mL) and EtOAc (3 mL).^[21]

5-Benzyloxy-2,3-dihydro-1H-inden-1-one (2a): Beige crystals; yield: 560 mg, 70%; mp: 107 °C; ^1H NMR (600 MHz, CDCl_3): δ = 2.65 (t, J = 5.8 Hz, 2H), 3.05 (t, J = 5.8 Hz, 2H), 5.12 (s, 2H), 6.95 (m, 2H), 7.32–7.34 (m, 1H), 7.37–7.42 (m, 4H), 7.67 ppm (m, 1H); ^{13}C NMR

(150 MHz, CDCl_3): δ = 25.8, 36.4, 70.2, 110.7, 115.8, 125.3, 127.4, 128.2, 128.7, 130.6, 136.0, 158.1, 164.3, 205.2 ppm; APCI-HRMS m/z [$M+H$]⁺ calcd for C₁₆H₁₅O₂: 239.1072, found 239.1068; purity [HPLC]: 99%.

5-(3-Chlorobenzyloxy)-2,3-dihydro-1H-inden-1-one (2b): Beige crystals; yield: 487 mg, 53%; mp: 116 °C; ^1H NMR (600 MHz, CDCl_3): δ = 2.65 (t, J = 6.0 Hz, 2H), 3.05 (t, J = 6.0 Hz, 2H), 5.08 (s, 2H), 6.93 (m, 2H), 7.27–7.31 (m, 3H), 7.41 (s, 1H), 7.66 ppm (d, J = 8.3 Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ = 25.8, 36.4, 69.3, 110.8, 115.7, 125.3, 125.4, 127.3, 128.3, 130.0, 130.8, 134.6, 138.1, 158.1, 164.0, 205.2 ppm; APCI-HRMS m/z [$M+H$]⁺ calcd for C₁₆H₁₄O₂Cl: 273.0682, found 273.0682; purity [HPLC]: 96%.

5-(3-Bromobenzyloxy)-2,3-dihydro-1H-inden-1-one (2c): Beige crystals; yield: 900 mg, 85%; mp: 121.5 °C; ^1H NMR (600 MHz, CDCl_3): δ = 2.65 (t, J = 5.8 Hz, 2H), 3.06 (t, J = 5.8 Hz, 2H), 5.08 (s, 2H), 6.93 (m, 2H), 7.23–7.26 (m, 1H), 7.32 (d, J = 7.9 Hz, 1H), 7.45 (d, J = 7.9 Hz, 1H), 7.57 (s, 1H), 7.67 ppm (d, J = 7.9 Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ = 25.8, 36.4, 69.3, 110.8, 115.7, 122.8, 125.4, 125.8, 130.2, 130.8, 131.3, 138.3, 158.1, 163.9, 205.2 ppm; APCI-HRMS m/z [$M+H$]⁺ calcd for C₁₆H₁₄O₂Br: 317.0177, found 317.0155; purity [HPLC]: 97%.

5-(4-Chlorobenzyloxy)-2,3-dihydro-1H-inden-1-one (2d): Beige solid; yield: 579 mg, 63%; mp: 127 °C; ^1H NMR (600 MHz, CDCl_3): δ = 2.64 (t, J = 6.0 Hz, 2H), 3.05 (t, J = 6.0 Hz, 2H), 5.08 (s, 2H), 6.93 (m, 2H), 7.34 (s, 4H), 7.67 ppm (d, J = 8.3 Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ = 25.8, 36.4, 69.4, 110.7, 115.8, 125.4, 128.7, 128.9, 130.7, 134.1, 134.2, 158.0, 164.0, 205.2 ppm; APCI-HRMS m/z [$M+H$]⁺ calcd for C₁₆H₁₄O₂Cl: 273.0682, found 273.0664; purity [HPLC]: 99%.

5-(4-Bromobenzyloxy)-2,3-dihydro-1H-inden-1-one (2e): Beige crystals; yield: 503 mg, 47%; mp: 128.5 °C; ^1H NMR (600 MHz, CDCl_3): δ = 2.64 (t, J = 6.0 Hz, 2H), 3.05 (t, J = 6.0 Hz, 2H), 5.06 (s, 2H), 6.92 (m, 2H), 7.29 (d, J = 8.3 Hz, 2H), 7.49 (d, J = 8.3 Hz, 2H), 7.66 ppm (d, J = 8.3 Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ = 25.8, 36.4, 66.4, 110.7, 115.8, 122.2, 125.4, 129.0, 130.8, 131.8, 135.1, 158.0, 164.0, 205.2 ppm; APCI-HRMS m/z [$M+H$]⁺ calcd for C₁₆H₁₄O₂Br: 317.0177, found 317.0145; purity [HPLC]: 99%.

5-(2-Cyclohexylethoxy)-2,3-dihydro-1H-inden-1-one (2f): Beige crystals; yield: 315 mg, 36%; mp: 65.3 °C; ^1H NMR (600 MHz, CDCl_3): δ = 0.93–0.98 (m, 2H), 1.12–1.25 (m, 3H), 1.45–1.51 (m, 1H), 1.62–1.74 (m, 7H), 2.63 (t, J = 6.0 Hz, 2H), 3.04 (t, J = 6.0 Hz, 2H), 4.03 (t, J = 6.8 Hz, 2H), 6.86 (m, 2H), 7.63 ppm (d, J = 9.0 Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ = 25.8, 26.2, 26.4, 33.2, 34.5, 36.4, 36.4, 66.4, 110.2, 115.6, 125.3, 130.1, 158.1, 164.8, 205.2 ppm; APCI-HRMS m/z [$M+H$]⁺ calcd for C₁₇H₂₃O₂: 259.1698, found 259.1686; purity [HPLC]: 99%.

5-(3-Phenylpropoxy)-2,3-dihydro-1H-inden-1-one (2g): Beige solid; yield: 273 mg, 30%; mp: 74 °C; ^1H NMR (600 MHz, CDCl_3): δ = 2.13 (qn, J = 7.5 Hz, 2H), 2.65 (t, J = 6.0 Hz, 2H), 2.81 (t, J = 7.5 Hz, 2H), 3.05 (t, J = 6.0 Hz, 2H), 4.01 (t, J = 6.4 Hz, 2H), 6.85 (s, 1H), 6.87 (dd, J = 8.7, 2.3 Hz, 1H), 7.18–7.20 (m, 3H), 7.28 (t, J = 7.5 Hz, 2H), 7.66 ppm (d, J = 8.7 Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ = 25.8, 30.5, 32.0, 36.4, 67.2, 110.2, 115.6, 125.3, 126.0, 128.4, 128.4, 130.3, 141.1, 158.1, 164.7, 205.2 ppm; APCI-HRMS m/z [$M+H$]⁺ calcd for C₁₈H₁₉O₂: 267.1385, found 267.1360; purity [HPLC]: 97%.

5-[(4-Methylpentyl)oxy]-2,3-dihydro-1H-inden-1-one (2h): Orange oil; yield: 398 mg, 51%; ^1H NMR (600 MHz, CDCl_3): δ = 0.90 (d, J = 6.4 Hz, 6H), 1.31–1.33 (m, 2H), 1.56–1.61 (m, 1H), 1.77–1.81 (m, 2H), 2.64 (t, J = 6.0 Hz, 2H), 3.04 (t, J = 6.0 Hz, 2H), 3.98 (t, J = 6.7 Hz, 2H), 6.85 (d, J = 6.0 Hz, 2H), 7.63 ppm (d, J = 9.4 Hz, 1H);

^{13}C NMR (150 MHz, CDCl_3): δ = 22.5, 25.8, 26.9, 27.8, 35.0, 36.4, 68.7, 110.2, 115.6, 125.2, 130.1, 158.1, 164.8, 205.2 ppm; APCI-HRMS m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{21}\text{O}_2$: 233.1542, found 233.1523; purity [HPLC]: 96%.

5-(2-Phenoxyethoxy)-2,3-dihydro-1H-inden-1-one (2i): Beige crystals; yield: 130 mg, 14%; mp: 36.7 °C; ^1H NMR (600 MHz, CDCl_3): δ = 2.65 (t, J = 6.0 Hz, 2H), 3.06 (t, J = 6.0 Hz, 2H), 4.33 (t, J = 4.0 Hz, 2H), 4.38 (t, J = 4.0 Hz, 2H), 6.90–6.98 (m, 5H), 7.29 (t, J = 7.9 Hz, 2H), 7.67 ppm (d, J = 9.0 Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ = 25.8, 36.4, 66.1, 66.8, 110.5, 114.6, 115.6, 121.3, 125.3, 129.5, 130.7, 158.0, 158.4, 164.2, 205.2 ppm; APCI-HRMS m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{17}\text{H}_{19}\text{O}_3$: 269.1178, found 269.1153; purity [HPLC]: 100%.

5-[2-(4-Chlorophenoxy)ethoxy]-2,3-dihydro-1H-inden-1-one (2j): Beige crystals; yield: 61 mg, 6%; mp: 145 °C; ^1H NMR (600 MHz, CDCl_3): δ = 2.66 (t, J = 6.0 Hz, 2H), 3.06 (t, J = 6.0 Hz, 2H), 4.30 (t, J = 5.0 Hz, 2H), 4.36 (t, J = 5.0 Hz, 2H), 6.86 (d, J = 9.0 Hz, 2H), 6.92 (m, 2H), 7.22 (m, 2H), 7.66 ppm (d, J = 9.0 Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ = 25.8, 36.4, 66.5, 66.7, 110.5, 115.9, 125.4, 126.1, 129.4, 130.8, 157.0, 158.0, 164.1, 205.2 ppm; APCI-HRMS m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{17}\text{H}_{16}\text{O}_3\text{Cl}$: 303.0788, found 303.0753; purity [HPLC]: 99%.

5-[2-(4-Bromophenoxy)ethoxy]-2,3-dihydro-1H-inden-1-one (2k): Beige solid; yield: 493 mg, 42%; mp: 148 °C; ^1H NMR (600 MHz, CDCl_3): δ = 2.66 (t, J = 6.0 Hz, 2H), 3.07 (t, J = 6.0 Hz, 2H), 4.3 (t, J = 4.3 Hz, 2H), 4.35 (t, J = 4.9 Hz, 2H), 6.81 (d, J = 8.7 Hz, 2H), 6.92 (m, 2H), 7.36 (d, J = 9.0 Hz, 2H), 7.67 ppm (d, J = 9.0 Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ = 25.8, 36.4, 66.5, 66.7, 110.5, 113.5, 115.6, 116.4, 125.4, 130.8, 132.3, 157.5, 158.1, 164.1, 205.3 ppm; APCI-HRMS m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{17}\text{H}_{16}\text{O}_3\text{Br}$: 347.0283, found 347.0245; purity [HPLC]: 99%.

5-(Cyclohexylmethoxy)-2,3-dihydro-1H-inden-1-one (2l): Beige crystals; yield: 441 mg, 53%; mp: 128.5 °C; ^1H NMR (600 MHz, CDCl_3): δ = 1.04–1.06 (m, 2H), 1.15–1.21 (m, 1H), 1.24–1.31 (m, 2H), 1.67–1.85 (m, 6H), 2.64 (t, J = 6.0 Hz, 2H), 3.04 (t, J = 6.0 Hz, 2H), 3.78 (d, J = 6.4 Hz, 2H), 6.85 (m, 2H), 7.63 ppm (d, J = 9.0 Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ = 25.7, 25.8, 26.4, 29.8, 36.4, 37.5, 73.8, 110.2, 115.6, 125.2, 130.1, 158.1, 165.0, 205.2 ppm; APCI-HRMS m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{16}\text{H}_{21}\text{O}_2$: 245.1542, found 245.1534; purity [HPLC]: 100%.

6-(Benzyloxy)-2,3-dihydro-1H-inden-1-one (3a): Light-beige solid; yield: 175 mg, 22%; mp: 96 °C; ^1H NMR (600 MHz, CDCl_3): δ = 2.70 (t, J = 6.0 Hz, 2H), 3.05 (t, J = 6.0 Hz, 2H), 5.07 (s, 2H), 7.26 (m, 2H), 7.32 (t, J = 7.5 Hz, 1H), 7.38 (m, 3H), 7.41 ppm (d, J = 7.5 Hz, 2H); ^{13}C NMR (150 MHz, CDCl_3): δ = 25.1, 36.9, 70.2, 106.1, 124.5, 127.4, 127.5, 128.1, 128.6, 136.4, 138.2, 148.2, 158.4, 206.9 ppm; APCI-HRMS m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{16}\text{H}_{15}\text{O}_2$: 239.1072, found 239.1045; purity [HPLC]: 97%.

6-(3-Chlorobenzyloxy)-2,3-dihydro-1H-inden-1-one (3b): Beige solid; yield: 267 mg, 29%; mp: 84.7 °C; ^1H NMR (600 MHz, CDCl_3): δ = 2.70 (t, J = 5.7 Hz, 2H), 3.05 (t, J = 6.0 Hz, 2H), 5.03 (s, 2H), 7.21 (m, 1H), 7.23–7.25 (m, 1H), 7.26–7.31 (m, 3H), 7.36 (d, J = 8.3 Hz, 1H), 7.41 ppm (s, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ = 25.1, 37.0, 69.3, 106.0, 124.5, 125.3, 127.4, 127.5, 128.2, 129.9, 134.5, 138.2, 138.4, 148.4, 158.1, 206.8 ppm; APCI-HRMS m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{16}\text{H}_{14}\text{O}_2\text{Cl}$: 273.0682, found 273.0653; purity [HPLC]: 95%.

6-(3-Bromobenzyloxy)-2,3-dihydro-1H-inden-1-one (3c): Beige solid; yield: 257 mg, 24%; mp: 85 °C; ^1H NMR (600 MHz, CDCl_3): δ = 2.75 (t, J = 6.0 Hz, 2H), 3.09 (t, J = 6.0 Hz, 2H), 5.07 (s, 2H), 7.26 (m, 1H), 7.27 (m, 2H), 7.36 (d, J = 7.9 Hz, 1H), 7.41 (d, J = 7.9 Hz, 1H), 7.47 (d, J = 7.9 Hz, 1H), 7.61 ppm (s, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ = 25.1, 36.9, 69.3, 106.0, 122.7, 124.5, 125.8, 127.6, 130.3,

130.1, 131.1, 138.2, 138.7, 148.4, 158.1, 206.9 ppm; APCI-HRMS m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{16}\text{H}_{14}\text{O}_2\text{Br}$: 317.0177, found 317.0144; purity [HPLC]: 96%.

6-(4-Chlorobenzyloxy)-2,3-dihydro-1H-inden-1-one (3d): Beige solid; yield: 381 mg, 41%; mp: 114.5 °C; ^1H NMR (600 MHz, CDCl_3): δ = 2.69 (t, J = 5.6 Hz, 2H), 3.04 (t, J = 5.8 Hz, 2H), 5.02 (s, 2H), 7.21–7.23 (m, 2H), 7.32–7.36 ppm (m, 5H); ^{13}C NMR (150 MHz, CDCl_3): δ = 25.1, 36.9, 69.4, 106.0, 124.5, 127.5, 128.8, 128.8, 133.9, 134.9, 138.2, 148.3, 158.1, 206.8 ppm; APCI-HRMS m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{16}\text{H}_{14}\text{O}_2\text{Cl}$: 273.0682, found 273.0681; purity [HPLC]: 95%.

6-(4-Bromobenzyloxy)-2,3-dihydro-1H-inden-1-one (3e): Beige solid; yield: 618 mg, 58%; mp: 120.8 °C; ^1H NMR (600 MHz, CDCl_3): δ = 2.69 (t, J = 5.6 Hz, 2H), 3.04 (t, J = 5.6 Hz, 2H), 5.01 (s, 2H), 7.20–7.23 (m, 2H), 7.28 (d, J = 8.3 Hz, 2H), 7.35 (d, J = 7.9 Hz, 1H), 7.47 ppm (d, J = 8.3 Hz, 2H); ^{13}C NMR (150 MHz, CDCl_3): δ = 25.1, 36.9, 69.4, 106.0, 122.0, 124.5, 127.5, 129.1, 131.7, 135.4, 138.2, 148.3, 158.1, 206.8 ppm; APCI-HRMS m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{16}\text{H}_{14}\text{O}_2\text{Br}$: 317.0177, found 317.0176; purity [HPLC]: 100%.

6-(2-Cyclohexylethoxy)-2,3-dihydro-1H-inden-1-one (3f): Light-beige solid; yield: 273 mg, 31%; mp: 91 °C; ^1H NMR (600 MHz, CDCl_3): δ = 0.91–0.97 (m, 2H), 1.10–1.17 (m, 1H), 1.20–1.25 (m, 2H), 1.46 (m, 1H), 1.61–1.73 (m, 7H), 2.67 (t, J = 5.6 Hz, 2H), 3.03 (t, J = 5.6 Hz, 2H), 3.98 (t, J = 6.8 Hz, 2H), 7.14–7.15 (m, 2H), 7.31–7.33 ppm (m, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ = 25.1, 26.2, 26.5, 33.2, 34.5, 36.4, 37.0, 66.4, 105.5, 124.4, 127.2, 138.1, 147.7, 158.8, 207.1 ppm; APCI-HRMS m/z $[\text{M} + \text{H}]^+$ calcd for 259.1698, found 259.1702; purity [HPLC]: 100%.

6-(3-Phenylpropoxy)-2,3-dihydro-1H-inden-1-one (3g): Light-beige solid; yield: 293 mg, 33%; mp: 70 °C; ^1H NMR (600 MHz, CDCl_3): δ = 2.10 (qn, J = 7.2 Hz, 2H), 2.70 (t, J = 5.7 Hz, 2H), 2.79 (t, J = 7.5 Hz, 2H), 3.04 (t, J = 5.7 Hz, 2H), 3.96 (t, J = 6.4 Hz, 2H), 7.15 (m, 1H), 7.17–7.20 (m, 4H), 7.24 (t, J = 7.2 Hz, 2H), 7.34 ppm (d, J = 8.3 Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ = 25.1, 30.6, 32.0, 37.0, 67.2, 105.6, 124.3, 126.0, 127.3, 128.4, 128.4, 138.1, 141.3, 147.9, 158.7, 207.0 ppm; APCI-HRMS m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{18}\text{H}_{19}\text{O}_2$: 267.1385, found 267.1360; purity [HPLC]: 100%.

6-[(4-Methylpentyl)oxy]-2,3-dihydro-1H-inden-1-one (3h): Orange oil; yield: 525 mg, 67%; ^1H NMR (600 MHz, CDCl_3): δ = 0.88 (d, J = 6.7 Hz, 6H), 1.29 (m, 2H), 1.57 (sept, J = 6.7 Hz, 1H), 1.75 (m, 2H), 2.68 (t, J = 5.6 Hz, 2H), 3.03 (t, J = 5.8 Hz, 2H), 3.93 (t, J = 6.8 Hz, 2H), 7.14–7.16 (m, 2H), 7.31 ppm (d, J = 9.0 Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ = 22.5, 25.0, 27.0, 27.8, 35.0, 37.0, 68.7, 105.5, 124.4, 127.3, 138.1, 147.7, 158.8, 207.1 ppm; APCI-HRMS m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{21}\text{O}_2$: 233.1542, found 233.1557; purity [HPLC]: 98%.

6-(2-Phenoxyethoxy)-2,3-dihydro-1H-inden-1-one (3i): Light-beige solid; yield: 288 mg, 32%; mp: 160.4 °C; ^1H NMR (600 MHz, CDCl_3): δ = 2.70 (t, J = 5.6 Hz, 2H), 3.05 (t, J = 5.6 Hz, 2H), 4.33 (s, 4H), 6.93–7.00 (m, 3H), 7.21–7.24 (m, 2H), 7.28 (t, J = 8.0 Hz, 2H), 7.35 ppm (d, J = 7.5 Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ = 25.1, 37.0, 66.3, 66.9, 105.7, 114.6, 121.1, 124.6, 127.5, 129.5, 138.2, 148.3, 158.3, 158.5, 206.9 ppm; APCI-HRMS m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{17}\text{H}_{19}\text{O}_3$: 269.1178, found 269.1153; purity [HPLC]: 95%.

6-[2-(4-Chlorophenoxy)ethoxy]-2,3-dihydro-1H-inden-1-one (3j): Beige solid; yield: 171 mg, 17%; mp: 127 °C; ^1H NMR (600 MHz, CDCl_3): δ = 2.70 (t, J = 5.6 Hz, 2H), 3.05 (t, J = 5.6 Hz, 2H), 4.27–4.32 (m, 4H), 6.86 (m, 2H), 7.20–7.23 (m, 4H), 7.35 ppm (d, J = 8.3 Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3): δ = 25.1, 37.0, 66.6, 66.7, 105.6, 116.0, 124.5, 126.0, 127.5, 129.3, 138.2, 148.4, 157.1, 158.2,

206.9 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $C_{17}H_{16}O_2Cl$: 303.0788, found 303.0772; purity [HPLC]: 99%.

6-[2-(4-Bromophenoxy)ethoxy]-2,3-dihydro-1H-inden-1-one (3k): Beige solid; yield: 165 mg, 14%; mp: 141.5 °C; ^1H NMR (600 MHz, $CDCl_3$): δ = 2.71 (t, J = 5.6 Hz, 2H), 3.05 (t, J = 5.6 Hz, 2H), 4.27–4.32 (m, 4H), 6.80–6.81 (m, 2H), 7.20–7.22 (m, 2H), 7.35–7.37 ppm (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 25.1, 37.0, 66.6, 66.7, 105.6, 113.3, 116.5, 124.5, 127.5, 132.3, 138.2, 148.4, 157.6, 158.2, 206.9 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $C_{17}H_{16}O_2Br$: 347.0283, found 347.0254; purity [HPLC]: 98%.

5-Benzyloxy-2,3-dihydro-1H-indene (4a): Light-orange oil; yield: 498 mg, 66%; ^1H NMR (600 MHz, $CDCl_3$): δ = 2.08 (qn, J = 7.5 Hz, 2H), 2.86 (t, J = 7.5 Hz, 2H), 2.89 (t, J = 7.5 Hz, 2H), 5.05 (s, 2H), 6.79 (dd, J = 8.0, 2.3 Hz, 1H), 6.89 (s, 1H), 7.12 (d, J = 8.3 Hz, 1H), 7.33 (t, J = 7.2 Hz, 1H), 7.39 (t, J = 7.9 Hz, 2H), 7.44 ppm (d, J = 7.2 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 25.8, 32.0, 33.2, 70.2, 110.9, 112.8, 124.7, 127.4, 127.8, 128.5, 136.4, 137.4, 145.7, 157.7 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $C_{16}H_{17}O$: 225.1279, found 225.1253; purity [HPLC]: 95%.

5-(3-Chlorobenzyloxy)-2,3-dihydro-1H-indene (4b): Light-orange oil; yield: 571 mg, 65%; ^1H NMR (600 MHz, $CDCl_3$): δ = 2.07 (qn, 7.5 Hz, 2H), 2.84 (t, J = 7.5 Hz, 2H), 2.88 (t, J = 7.5 Hz, 2H), 5.0 (s, 2H), 6.75 (dd, J = 7.9, 2.3 Hz, 1H), 6.85 (s, 1H), 7.11 (d, J = 7.9 Hz, 1H), 7.29 (m, 3H), 7.44 ppm (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 25.8, 32.0, 33.1, 69.4, 110.9, 112.8, 124.8, 125.3, 127.3, 127.9, 129.8, 134.4, 136.8, 139.5, 145.8, 157.4 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $C_{16}H_{16}OCl$: 259.0890, found 259.0851.

5-(3-Bromobenzyloxy)-2,3-dihydro-1H-indene (4c): Light-beige oil; yield: 81 mg, 8%; ^1H NMR (600 MHz, $CDCl_3$): δ = 2.07 (qn, J = 7.5 Hz, 2H), 2.84 (t, J = 7.5 Hz, 2H), 2.87 (t, J = 7.5 Hz, 2H), 4.99 (s, 2H), 6.75 (dd, J = 7.9, 2.3 Hz, 1H), 6.84 (s, 1H), 7.11 (d, J = 8.3 Hz, 1H), 7.23 (m, 1H), 7.33 (d, J = 8.3 Hz, 1H), 7.43 (d, J = 7.9 Hz, 1H), 7.59 ppm (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 25.8, 32.0, 33.1, 69.3, 110.9, 112.8, 122.6, 124.8, 125.8, 130.1, 130.2, 130.8, 136.8, 139.8, 145.8, 157.4 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $C_{16}H_{16}OBr$: 303.0385, found 303.0355; purity [HPLC]: 100%.

5-(4-Chlorobenzyloxy)-2,3-dihydro-1H-indene (4d): White crystals; yield: 137 mg, 16%; mp: 79 °C; ^1H NMR (600 MHz, $CDCl_3$): δ = 2.08 (qn, J = 7.5 Hz, 2H), 2.84 (t, J = 7.5 Hz, 2H), 2.88 (t, J = 7.5 Hz, 2H), 5.0 (s, 2H), 6.75 (dd, J = 7.9, 1.9 Hz, 1H), 6.85 (s, 1H), 7.1 (d, J = 7.9 Hz, 1H), 7.34–7.37 ppm (m, 4H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 25.8, 32.0, 33.1, 69.4, 110.9, 112.8, 124.8, 128.7, 128.7, 133.5, 135.9, 136.7, 145.8, 157.4 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $C_{16}H_{16}OCl$: 259.0890, found 259.0869; purity [HPLC]: 100%.

5-(4-Bromobenzyloxy)-2,3-dihydro-1H-indene (4e): White crystals; yield: 420 mg, 41%; mp: 82 °C; ^1H NMR (600 MHz, $CDCl_3$): δ = 2.06 (qn, J = 7.5 Hz, 2H), 2.83 (t, J = 7.5 Hz, 2H), 2.87 (t, J = 7.5 Hz, 2H), 4.98 (s, 2H), 6.74 (dd, J = 8.3, 2.3 Hz, 1H), 6.83 (s, 1H), 7.1 (d, J = 8.3 Hz, 1H), 7.30 (d, J = 8.3 Hz, 2H), 7.48 ppm (d, J = 8.3 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 25.8, 32.0, 33.1, 69.4, 110.9, 112.8, 121.6, 124.8, 129.0, 131.6, 136.4, 136.7, 145.8, 157.4 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $C_{16}H_{16}OBr$: 303.0385, found 303.0367; purity [HPLC]: 100%.

5-(2-Cyclohexylethoxy)-2,3-dihydro-1H-indene (4f): Light-yellow oil; yield: 598 mg, 73%; ^1H NMR (600 MHz, $CDCl_3$): δ = 0.95–1.01 (m, 2H), 1.14–1.29 (m, 3H), 1.5 (m, 1H), 1.7–1.78 (m, 7H), 2.07 (qn, J = 7.5 Hz, 2H), 2.84 (t, J = 7.5 Hz, 2H), 2.88 (t, J = 7.5 Hz, 2H), 3.97 (t, J = 6.5 Hz, 2H), 6.70 (d, J = 7.9 Hz, 1H), 6.80 (s, 1H), 7.10 ppm (d, J = 8.3 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 25.8, 26.3, 26.6, 32.0, 33.2, 33.3, 34.6, 36.7, 66.1, 110.5, 112.5, 124.6, 135.9, 145.6,

158.0 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $C_{17}H_{22}O$: 245.1905, found 245.1916; purity [HPLC]: 95%.

5-(3-Phenylpropoxy)-2,3-dihydro-1H-indene (4g): Yellow oil; yield: 542 mg, 64%; ^1H NMR (600 MHz, $CDCl_3$): δ = 2.05–2.13 (m, 4H), 2.82 (t, J = 7.9 Hz, 2H), 2.85 (t, J = 7.5 Hz, 2H), 2.88 (t, J = 7.5 Hz, 2H), 3.95 (t, J = 6.4 Hz, 2H), 6.71 (dd, J = 7.9, 2.3 Hz, 1H), 6.8 (s, 1H), 7.11 (d, J = 8.3 Hz, 1H), 7.19–7.23 (m, 3H), 7.3 ppm (t, J = 7.5 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 25.8, 30.9, 32.0, 32.2, 33.1, 67.0, 110.6, 112.6, 124.7, 125.8, 128.4, 128.5, 136.1, 141.6, 145.6, 157.9 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $C_{18}H_{22}O$: 253.1592, found 253.1597; purity [HPLC]: 96%.

5-[(4-Methylpentyl)oxy]-2,3-dihydro-1H-indene (4h): Yellow oil; yield: 539 mg, 73%; ^1H NMR (600 MHz, $CDCl_3$): δ = 0.92 (d, J = 9.0 Hz, 6H), 1.34 (m, 2H), 1.61 (m, 1H), 1.78 (qn, J = 8.2 Hz, 2H), 2.07 (qn, J = 7.5 Hz, 2H), 2.84 (t, J = 7.5 Hz, 2H), 2.88 (t, J = 7.5 Hz, 2H), 3.92 (t, J = 6.7 Hz, 2H), 6.69 (d, J = 7.9 Hz, 1H), 6.79 (s, 1H), 7.10 ppm (t, J = 7.9 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 22.6, 25.8, 27.3, 27.8, 32.0, 33.2, 35.2, 68.5, 110.6, 112.5, 124.7, 135.9, 145.6, 158.0 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $C_{19}H_{22}O$: 219.1749, found 219.1756; purity [HPLC]: 96%.

5-(2-Phenoxyethoxy)-2,3-dihydro-1H-indene (4i): White solid; yield: 507 mg, 59%; mp: 91.3 °C; ^1H NMR (600 MHz, $CDCl_3$): δ = 2.09 (qn, J = 7.5 Hz, 2H), 2.86 (t, J = 7.5 Hz, 2H), 2.9 (t, J = 7.5 Hz, 2H), 4.31 (s, 4H), 6.77 (dd, J = 8.3, 2.3 Hz, 1H), 6.86 (s, 1H), 6.96–7.00 (m, 3H), 7.13 (d, J = 8.3 Hz, 1H), 7.31 ppm (t, J = 8.0 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 25.8, 31.0, 33.1, 66.4, 66.7, 110.8, 112.7, 114.7, 121.0, 124.7, 129.4, 136.6, 145.7, 157.5, 158.6 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $C_{17}H_{19}O_2$: 255.1385, found 255.1580; purity [HPLC]: 98%.

5-[2-(4-Chlorophenoxy)ethoxy]-2,3-dihydro-1H-indene (4j): White solid; yield: 162 mg, 17%; mp: 128.8 °C; ^1H NMR (600 MHz, $CDCl_3$): δ = 2.12 (qn, J = 7.5 Hz, 2H), 2.89 (t, J = 7.5 Hz, 2H), 2.93 (t, J = 7.5 Hz, 2H), 4.31 (m, 4H), 6.78 (dd, J = 8.0, 1.9 Hz, 1H), 6.88 (s, 1H), 6.93 (d, J = 9.0 Hz, 2H), 7.16 (d, J = 7.9 Hz, 1H), 7.28 ppm (d, J = 9.0 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 25.8, 32.0, 33.1, 66.7, 66.9, 110.8, 112.7, 116.0, 124.8, 125.9, 129.3, 136.7, 145.8, 157.3, 157.4 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $C_{17}H_{18}O_2Cl$: 289.0995, found 289.0987; purity [HPLC]: 100%.

5-[2-(4-Bromophenoxy)ethoxy]-2,3-dihydro-1H-indene (4k): White solid; yield: 765 mg, 68%; mp: 135.4 °C; ^1H NMR (600 MHz, $CDCl_3$): δ = 2.07 (qn, J = 7.5 Hz, 2H), 2.84 (t, J = 7.5 Hz, 2H), 2.87 (t, J = 7.5 Hz, 2H), 4.27 (m, 4H), 6.73 (dd, J = 8.3, 2.3 Hz, 1H), 6.81–6.83 (m, 3H), 7.11 (d, J = 8.3 Hz, 1H), 7.37 ppm (d, J = 8.7 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 25.8, 32.0, 33.1, 66.7, 66.9, 110.8, 112.7, 113.2, 116.5, 124.7, 132.2, 136.7, 145.8, 157.4, 157.8 ppm; APCI-HRMS m/z $[M+H]^+$ calcd for $C_{17}H_{18}O_2Br$: 333.0490, found 333.0473; purity [HPLC]: 99%.

MAO inhibition: IC_{50} values for the inhibition of MAO-A and MAO-B were measured by using the nonselective MAO substrate, kynuramine. The recombinant human MAOs served as enzyme sources.^[33] Reactions were carried out in white 96-well microtiter plates (Eppendorf) in potassium phosphate buffer (100 mM, made isotonic with KCl) at pH 7.4. The final volume of the reactions was 200 μ L and contained the MAO-A/B mixed substrate kynuramine (50 μ M) and various inhibitor concentrations spanning at least three orders of magnitude. Each reaction also contained DMSO as co-solvent (4%). The reactions were initiated by the addition of MAO-A or MAO-B [0.0075 (mg protein) mL^{-1}] and incubated for 20 min at 37 °C in a convection oven. After termination with the addition of 80 μ L NaOH (2N), the concentrations of 4-hydroxyquinoline were

measured by fluorescence spectrophotometry ($\lambda_{\text{ex}}=310$; $\lambda_{\text{em}}=400$ nm).^[22] A linear calibration curve was constructed using authentic 4-hydroxyquinoline (0.047–1.56 μM). The catalytic rates were calculated and fitted to the one-site competition model incorporated into the Prism 5 software package (GraphPad). The IC_{50} values were determined in triplicate and are expressed as the mean $\pm$ standard deviation (SD).

Reversibility of MAO inhibition: The reversibility of MAO inhibition by **3d** was examined by dialysis.^[24] MAO-A or MAO-B (0.03 mg mL^{-1}) and the test inhibitor, at a concentration of $4 \times \text{IC}_{50}$ for the inhibition of the MAOs, were placed in dialysis cassettes. These mixtures were prepared in potassium phosphate buffer (100 mM, pH 7.4, 5% sucrose) to a final volume of 0.8 mL, and contained 4% DMSO as co-solvent. These mixtures were pre-incubated for 15 min at 37 °C and subsequently dialyzed at 4 °C in 80 mL dialysis buffer (100 mM potassium phosphate, pH 7.4, 5% sucrose). The dialysis buffer was replaced with fresh buffer at 3 and 7 h after the start of dialysis. As controls, MAO-A and MAO-B were similarly pre-incubated in the presence of the irreversible inhibitors pargyline and (*R*)-deprenyl, respectively, as well as in the absence of inhibitor. The concentrations of pargyline [$\text{IC}_{50\text{MAO-A}}=13 \mu\text{M}$]^[26] and (*R*)-deprenyl [$\text{IC}_{50\text{MAO-B}}=0.079 \mu\text{M}$]^[27] used were equal to $4 \times \text{IC}_{50}$ for the inhibition of MAO-A and MAO-B, respectively. After 24 h of dialysis, samples of the dialysis mixtures (250 μL) were diluted twofold with the addition of kynuramine (250 μL , dissolved in potassium phosphate buffer, 100 mM, pH 7.4, made isotonic with KCl). This yielded a final inhibitor concentration of $2 \times \text{IC}_{50}$, and a final kynuramine concentration of 50 μM . After incubation of these enzyme reactions for 20 min at 37 °C, the residual MAO activities were subsequently measured by fluorescence spectrophotometry as described above. For comparison, undialyzed mixtures of the MAOs and the test inhibitor were maintained at 4 °C over the same time period. All reactions were carried out in triplicate, and the residual enzyme catalytic rates are expressed as the mean $\pm$ SD.

Lineweaver-Burk plots and K_i value calculations: For the inhibition of MAO-A and MAO-B by **3d**, sets consisting of six Lineweaver-Burk plots were constructed. The first plot was constructed in the absence of inhibitor, while the remaining five plots were constructed in the presence of various concentrations of the test inhibitor: $1/4 \times \text{IC}_{50}$, $1/2 \times \text{IC}_{50}$, $3/4 \times \text{IC}_{50}$, $1 \times \text{IC}_{50}$, and $1 1/4 \times \text{IC}_{50}$. The enzyme substrate kynuramine was used at concentrations ranging from 15 to 250 μM , whereas the final enzyme concentration was 0.015 mg mL^{-1} . All incubations were carried out in 1.5 mL microcentrifuge tubes to a volume of 500 μL . The MAO catalytic activities were measured by fluorescence spectrophotometry as described for the IC_{50} value determination.^[24] K_i values were estimated from plots of the slopes of the Lineweaver-Burke plots versus inhibitor concentration, where the x-axis intercept equals $-K_i$.

Docking and molecular dynamics simulations: All modeling calculations were carried out with the Windows-based Discovery Studio version 3.1 software package.^[20] Unless otherwise specified, all applications within Discovery Studio were set to their default values. The X-ray crystal structures of human MAO-A (PDB ID: 2Z5X)^[28] and human MAO-B (PDB ID: 2V5Z)^[29] were obtained from the Brookhaven Protein Data Bank. The pK_a values and protonation states of the ionizable amino acids were calculated, and hydrogen atoms were added at pH 7.4 to the protein models. When necessary, the valences of the FAD cofactors (oxidized state) and co-crystallized ligands were corrected, the protein models were automatically typed with the Momany and Rone CHARMM force field, and a fixed atom constraint was applied to the backbone. The models were subsequently energy minimized using the Smart Minimizer al-

gorithm with the maximum amount of steps set to 50 000. The implicit generalized Born solvation model with molecular volume was used during the minimization procedure. The co-crystallized ligands, waters, and backbone constraints were removed from the models, and the binding sites were identified from an analysis of the enzyme cavities. In each model, three active site waters are considered to be conserved and were retained. These waters are HOH710, 718, and 739 in the MAO-A active site, and HOH1155, 1170, and 1351 in the A-chain of the MAO-B active site. The structures of compounds **2a**, **3a**, and **4a** were drawn in Discovery Studio, the geometries were briefly optimized using a Dreiding-like force field (5000 iterations), and the structures were submitted to the Prepare Ligands protocol. Atom potential types and partial charges were subsequently assigned to the structures with the Momany and Rone CHARMM force field. Docking of **2a**, **3a**, and **4a** into the MAO models was carried out with the CDOCKER algorithm. For this purpose, ten random ligand conformations were generated, the heating target temperature was set at 700 K, and full potential mode was employed. The docking solutions were finally refined using in situ ligand minimization with the Smart Minimizer algorithm.

The highest-ranked docking solutions were subjected to a short (500 ps) MD simulation. Fixed atom constraints were applied to the backbone of the protein models as well as residues $>5 \text{ \AA}$ away from the docked ligand. The models were automatically typed with the Momany and Rone CHARMM force field. The standard dynamics cascade was used for this study and consisted of a steepest-descent minimization (maximum steps, 2500) followed by a conjugate gradient minimization (maximum steps, 2500; RMS gradient, 0.01). Heating was subsequently carried out in 2000 steps (time step, 0.001 ps) from 50 to 300 K, and equilibration of the system was allowed for 10 000 steps (time step, 0.001 ps). The production runs were carried out for 500 000 steps (time step, 0.001 ps) in the NVT ensemble, applying the SHAKE algorithm to all bonds involving hydrogen atoms. The equations for motion were integrated with the Leapfrog Verlet algorithm. The implicit generalized Born solvation model with simple switching was used during the dynamics calculations. The conformation of the complex was saved every 1 ps, yielding 500 frames. The illustrations were generated with PyMOL and represent the docked orientations prior to MD simulation.^[38]

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