Monoamine oxidase inhibition properties of quinolinone analogues

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Thesis submitted for the degree *Doctor Philosophiae* in Pharmaceutical Chemistry at the Potchefstroom Campus of the North-West University

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PREFACE

This thesis is submitted in article format and consists of four research articles. One of the articles was submitted to the academic journal, Mini-Reviews in Medicinal Chemistry, while a second was submitted to Drug Research. The article and author guidelines for these two articles are also included. All scientific research for this thesis was conducted by L. Meiring at the North-West University, Potchefstroom campus. Letters of agreement from the co-authors of the research articles and the publishing agreements from the editors of the stated journals are included.

I would like to take this opportunity to thank the North-West University, especially the School of Pharmacy, for the financial support and for granting me the enormous opportunity to pursue doctoral studies. I would like to thank the following people for supporting and assisting me throughout the completion of this study:

- André Joubert, for the numerous NMR and MS spectra.
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- My family and parents for their motivation and support.
- Finally, my husband for the love and support, especially during the hardships.

DECLARATION

This thesis is submitted in fulfillment of the requirements for the degree of the Philosophiae Doctor in Pharmaceutical Chemistry, at the School of Pharmacy, North-West University.

I, Letitia Meiring hereby declare that the dissertation with the title: **MONOAMINE OXIDASE INHIBITION PROPERTIES OF QUINOLINONE ANALOGUES** is my own work and has not been submitted at any other university either whole or in part.

L. Meiring

LETTER OF AGREEMENT

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To whom it may concern,

Dear Sir/Madam

CO-AUTHORSHIP ON RESEARCH PAPERS

The undersigned as co-authors of the research articles listed below, hereby give permission to L. Meiring to submit these articles as part of the degree PhD in Pharmaceutical Chemistry at the North-West University, Potchefstroom campus.

- A review of the pharmacological properties of 3,4-dihydro-2(1*H*)-quinolinones
- C6- and C7-Substituted 3,4-dihydro-2(1*H*)-quinolinones as inhibitors of monoamine oxidase.
- The evaluation of 2-phenoxyethoxy-substituted tetralones as inhibitors of monoamine oxidase
- The evaluation of N-propargylamine-2-aminotetralin as an irreversible inhibitor of monoamine oxidase

Yours sincerely,

Prof. A. Petzer **Prof. J.P. Petzer** **Dr. L.J. Legoabe**

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THE EVALUATION OF N-PROPARGYLAMINE-2-AMINOTETRALIN AS AN IRREVERSIBLE INHIBITOR OF MONOAMINE OXIDASE

ABSTRACT

Parkinson's disease (PD) is an age-related neurodegenerative disorder characterised by selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) of the brain. This leads to the loss of dopamine from the striatum, which is responsible for the motor symptoms of PD. Monoamine oxidase (MAO) plays an important role in the neurodegenerative processes and therapy of PD since dopamine is oxidised by MAO in the basal ganglia. Inhibitors, of the MAO-B isoform conserve the depleted supply of dopamine and are thus used in the therapy of PD. MAO-B inhibitors may also enhance the therapeutic efficacy of L-dopa, the metabolic precursor of dopamine, by enhancing dopamine levels derived from administered L-dopa.

In this study, three chemical classes were synthesised and evaluated as potential recombinant human MAO-A and MAO-B inhibitors. These include (1) C6- and C7-substituted 3,4-dihydro-2(1*H*)-quinolinones, (2) 2-phenoxyethoxy-substituted tetralones and (3) N-propargylamine-2-aminotetralin (2-PAT). The quinolinone and tetralone derivatives are structurally related to chemical classes that have been reported to inhibit the MAO enzymes, including α-tetralone, 1-indanone and 3-coumaranone derivatives. 2-PAT is structurally similar to rasagiline, an irreversible MAO-B inhibitor currently used in clinic.

C6- and C7-substituted 3,4-dihydro-2(1*H*)-quinolinone and 2-phenoxyethoxy tetralone derivatives were synthesised by reacting 6- or 7-hydroxy-3,4-dihydro-2(1*H*)-quinolinone and 6- or 7-hydroxytetralone, respectively, with an appropriately substituted alkyl bromide in the presence of base. 2-PAT was synthesised in low yield by dehydrating β–tetralone and propargylamine (commercially available) in the presence of sodium cyanoborohydride ($NaCNBH_4$).

To evaluate the MAO inhibitory properties (IC_{50} values) of the synthesised derivatives the recombinant human MAO-A and MAO-B enzymes were used. The reversibility of inhibition of selected derivatives was examined by employing dialysis, while the mode of MAO inhibition was determined by constructing Lineweaver-Burk plots.

To determine possible binding modes and key interactions of selected inhibitors with the MAO enzymes, the inhibitors were docked into the MAO active sites.

The results document that the 3,4-dihydro-2(1*H*)-quinolinone derivatives are highly potent and selective MAO-B inhibitors with the most potent inhibitor displaying an IC_{50} value of 0.0014 μM. Based on dialysis experiments it was concluded that a selected quinolinone derivative is a reversible MAO-B inhibitor. The Lineweaver-Burk plots constructed for the inhibition of MAO-B by the selected quinolinone derivative were linear and intersected on the y-axis. These data indicated that this compound is a competitive MAO-B inhibitor with a K_i value of 2.8 nM.

The results further document that 7-(2-phenoxyethoxy)-3,4-dihydronaphthalen-2(1*H*)-one from the second series is a highly potent MAO-B inhibitor with an IC_{50} value of 0.033 μM. Based on dialysis experiments it was concluded that this β-tetralone derivative is a reversible and competitive MAO-B inhibitor. An analysis of the Lineweaver-Burk plots indicated that this compound inhibit MAO-B with a K_i value of 0.128 μM. This is the first report of the MAO inhibition properties of a β-tetralone.

Finally, 2-PAT was found to be a reversible MAO-A (IC_{50} = 0.721μM) inhibitor, while acting as an inactivator of MAO-B (IC_{50} = 14.6 μM). 2-PAT is also approximately fivefold more potent than toloxatone (IC_{50} = 3.92 μM), a clinically used antidepressant and reversible MAO-A inhibitor.

It may thus be concluded that the synthesised compounds are promising potent MAO-B inhibitors, and thus leads for the design of therapeutic agents for PD.

UITTREKSEL

Parkinson se siekte (PS) is 'n ouderdomsverwante neurodegeneratiewe siekte wat deur die degenerasie van die neurone van die substantia nigra in die brein en die verlies van dopamien in die striatum gekenmerk word. Die gevolglike tekort aan dopamien in die striatum is verantwoordelik vir die simptome van PS. Die ensiem, monoamienoksidase (MAO), speel moontlik 'n rol in die neurodegeneratiewe prosesse van PS aangesien dopamien deur MAO geoksideer word. MAO-inhibeerders blokkeer die katabolisme van dopamien en word dus gebruik om die siekteverloop van PS te vertraag. MAO-inhibeerders kan ook in kombinasie met L-dopa gebruik word en verbeter so die terapeutiese effek van L-dopa.

In hierdie studie is drie reekse verbindings gesintetiseer en geëvalueer as inhibeerders van rekombinante menslike MAO-A en MAO-B. Hierdie reekse sluit die volgende in (1) C6- en C7-gesubstitueerde 3,4-dihidro-2(1*H*)-kinolinoonderivate, (2) 2-fenoksie-etoksie-gesubstitueerde tetraloon derivate en (3) N-propargielamien-2-aminotetralien (2-PAT). Hierdie kinolinoon- en tetraloonderivate is struktureel verwant aan verbindings wat in vorige studies as potente MAO-inhibeerders geïdentifiseer is, insluitend α-tetraloon-, 1-indanoon- en 3-kumarienoonderivate. 2-PAT is struktureel verwant aan rasagilien, 'n onomkeerbare MAO-B-inhibeerder wat tans klinies gebruik word.

C6- en C7-gesubstitueerde 3,4-dihidro-2(1*H*)-kinolinoon- en 2-fenoksie-etoksie-gesubstitueerde tetraloonderivate is gesintetiseer deur 6- of 7-hidroksie-3,4-dihidro-2(1*H*)-kinolinoon en 6- of 7-hidroksietetraloon, onderskeidelik, met 'n toepaslike alkielbromied in die teenwoordigheid van 'n basis te laat reageer. 2-PAT is met 'n lae opbrengs gesintetiseer deur β-tetraloon en propargielamien in die teenwoordigheid van natriumsianoborohidried ($NaCNBH_4$) te dehidreer. Die produk is met HCl aangesuur, gewas met diëtieleter en met dichloormetaan geëkstraheer, om die soutsuursout van 2-PAT te lewer.

Rekombinante menslike MAO-A en MAO-B is gebruik om die MAO-inhibisie eienskappe (IC_{50}-waardes) van die derivate te evalueer. Die omkeerbaarheid van inhibisie van 'n geselekteerde derivaat is deur dialise geëvalueer, terwyl die meganisme van inhibisie ondersoek is deur Lineweaver-Burk-grafieke op te stel.

Moontlike bindingsoriëntasieseninteraksies van geselekteerde inhibeerders met die MAO-ensiem is geëvalueer deur die inhibeerders in die MAO aktiewe setel te pas.

Die resultate toon dat die 3,4-dihidro-2(1*H*)-kinolinoonderivate hoogs potente en selektiewe MAO-B-inhibeerders is. Die mees potente MAO-B-inhibeerder het 'n IC_{50}-waarde van 0.0014 µM getoon. Op grond van dialise-eksperimente en Lineweaver-Burk-grafieke is tot die gevolgtrekking gekom dat 'n geselekteerde derivaat as 'n omkeerbare MAO-B-inhibeerder optree. Die resultate toon ook dat die Lineweaver-Burk-grafieke op een punt op die y-as sny. Dit dui daarop dat die spesifieke kinoloon derivaat 'n kompeterende inhibeerder van MAO-B is. Verdere analise van die Lineweaver-Burk-grafieke dui daarop dat MAO-B met 'n K_i-waarde van 2.8 nM inhibeer word.

Die resultate toon verder dat 7-(2-fenoksi-etoksie)-3,4-dihidro-2(1*H*)-naftalenoon, 'n verbinding uit die tweede reeks, 'n hoogs potente MAO-B-inhibeerders is, met 'n IC_{50}-waarde van 0.033 µM. Op grond van dialise eksperimente en Lineweaver-Burk-grafieke is tot die gevolgtrekking gekom dat hierdie verbinding 'n kompeterende en omkeerbare MAO-B-inhibeerder is. Verdere analise van die Lineweaver-Burk-grafieke dui daarop dat MAO-B met 'n K_i-waarde van 0.128 µM inhibeer word. Hierdie is die eerste berig van die MAO-inhiberende eienskappe van β-tetraloon derivate.

Laastens is gevind dat 2-PAT 'n omkeerbare MAO-A-inhibeerder (IC_{50} = 0.721µM) is, maar optree as 'n inaktiveerder van MAO-B (IC_{50} = 14.6 µM). 2-PAT is ongeveer 2750-voudig meer potent as toloksatoon (IC_{50} = 3.92 µM), 'n omkeerbare MAO-A inhibeerder wat tans gebruik word.

Uit hierdie studie kan afgelei word dat die gesintetiseerde verbindings belowende, hoogs potente MAO-B-inhibeerders is, wat as moontlike geneesmiddelkandidate vir die behandeling van PS kan dien.

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LIST OF ABBREVIATIONS

A	
ADH	Aldehyde dehydrogenase
$AlCl_3$	Aluminum trichloride
APCI	Atmospheric-pressure chemical ionization
AVP	Arginine vasopressin
AVPR1A	Arginine vasopressin receptor 1A
C	
CNS	Central nervous system
CSC	8-(3-Chlorostyryl)caffeine
D	
DA	Dopamine
F	
FAD	Flavin adenine dinucleotide
G	
GPO	Glutathione peroxidase
GSH	Glutathione
H	
12-HETE	12(S)-Hydroxyeicosatetraenoic acid
H_2O_2	Hydrogen peroxide
5- HT	Serotonin
I	
IC_{50}	Inhibitor concentration at 50% inhibition
ISA	Intrinsic sympathomimetic activity
M	
MAO	Monoamine oxidase
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MPP^+	1-Methyl-4-phenylpyridinium
MP	Melting points
2-MPAT	N-Methyl-N-propargylamine-2-aminotetralin

N

NA	Noradrenaline
$NaCNBH_4$	Sodium cyanoborohydride
NMDA	N-Methyl-D-aspartate

P

2-PAT	N-propargylamine-2-aminotetralin
PD	Parkinson's disease
PDE	Phosphodiesterase

S

SAR	Structure-activity relationships
SerT	Serotonin transporter
SNpc	Substantia nigra pars compacta

CHAPTER 1

INTRODUCTION

1.1 Background

Parkinson's disease: James Parkinson discovered an unrecognised disorder, Parkinson's disease (PD), in the 1800's. PD is regarded as the second most common neurodegenerative disease and is characterised by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) of the brain (Parkinson, 2002; Dauer & Przedborski, 2003). The clinical manifestations encountered with this disease are mostly motor dysfunctions such as tremors, rigidity, postural instability, slowness or absence of voluntary movement and freezing (Dauer & Przedborski, 2003; Lees, 2005; Prediger *et al.*, 2012; Nagatsu & Sawada, 2006; Henderson *et al.*, 2003; Yazdani *et al.*, 2006). The debilitating movement disorders observed in PD patients is a result of the degeneration of dopaminergic neurons, which leads to depletion of dopamine (DA) in the striatum (Figure 1.1) (Przedborski, 2005). Approximately 1% of the world population is affected by PD today and the incidence of this disease rises steeply with age, with a mean duration of 15 years from diagnoses to death (Lees *et al.*, 2009; Dorsey *et al.*, 2007; Von Campenhausen *et al.*, 2005; Dluzen & McDermott, 2000).

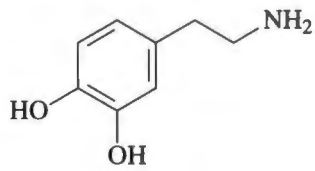


Figure 2.1: The structure of dopamine.

The prevention of degeneration of dopaminergic neurons is the ultimate goal of PD treatment (Dauer & Przedborski, 2003), but at present no such neuroprotective strategies exist (Bové *et al.*, 2005). Treatment of PD is symptomatic and involves mainly DA replacement therapy with agents such as L-dopa and DA agonists, drugs which either replace DA or stimulate DA receptors in the brain (Schapira & Olanow, 2008; Jenner *et al.*, 2009).

Monoamine oxidase: Another treatment option for PD is to inhibit the metabolism of DA in the brain. Monoamine oxidase (MAO) type B is an enzyme that is involved in the catabolism of DA in the brain. MAO-B inhibitors thus conserve the depleted supply of DA in PD and are used as adjuvants to L-dopa therapy (Nagatsu & Sawada, 2006; Youdim *et al.*, 2006; Yamada &Yasuhara, 2004).

MAO consists of two isoenzymes, MAO-A and MAO-B. The MAOs are flavin adenine dinucleotide (FAD) containing enzymes, which are tightly anchored to the mitochondrial outer membrane. DA is oxidised by mainly MAO-B within the basal ganglia. This oxidation is associated with the formation of by-products such as hydrogen peroxide and aldehydes, which may lead to oxidative stress and neurotoxicity if not inactivated by cellular protective systems (Fernandez & Chen, 2007). It has been suggested that the hydrogen peroxide and aldehydes produced by the MAO catalytic cycle may contribute to neurodegeneration in PD. By preventing the formation of these potentially neurotoxic metabolic by-products, MAO-B inhibitors may act as neuroprotective agents (Youdim *et al.*, 2006). This analysis shows that MAO-B inhibitors may be useful as symptomatic and potentially neuroprotective agents for the treatment of PD.

Two types of MAO-B inhibitors are currently available in the clinic for the treatment of PD, irreversible and reversible inhibitors. Irreversible MAO-B inhibitors, which include (R)-deprenyl (also known as selegiline) and rasagiline, are the most often used MAO inhibitors for the treatment of PD (Youdim *et al.*, 2006). These inhibitors contain the propargylamine functional group. Reversible MAO-B inhibitors, such as safinamide which has completed phase III development for the management of PD, are also under consideration as future medications (Borgohain *et al.*, 2014; Dézsi & Vécsei, 2014).

While the irreversible MAO-B inhibitors are well tolerated and exhibit good safety profiles, they may have potential disadvantages. For example, a slow enzyme recovery rate after drug withdrawal occurs with the irreversible inhibition of MAO, since *de novo* synthesis of the enzyme is required for activity to recover. The recovery of enzyme activity with reversible inhibitors is almost immediate after drug withdrawal and clearance from the tissues (Riederer *et al.*, 2004; Tipton *et al.*, 2004). This makes reversible MAO inhibitors more desirable than irreversible inhibitors (Binda *et al.*, 2003).

In spite of the risks associated with the use of irreversible inhibitors of the MAOs, they are an important class of therapeutic agents (Yamada &Yasuhara, 2004; Tipton *et al.*, 2004).

Another factor to consider when designing MAO inhibitors are adverse effects that may occur with MAO-A inhibition. Irreversible inhibition of MAO-A is associated with a dangerous elevation in blood pressure, termed the "cheese reaction". The cheese reaction occurs when MAO-A inhibitors are used in combination with tyramine and other dietary amines usually present in foods (most commonly in cheeses). Dietary amines are normally metabolised by MAO-A in the gut wall and liver, preventing them from entering the systemic circulation. When used in combination with irreversible MAO-A inhibitors, tyramine and other dietary amines are free to enter the systemic circulation where they release noradrenaline (NA) from peripheral adrenergic neurons, leading to a hypertensive response which can be fatal (Youdim & Bakhle, 2006; Youdim *et al.*, 2006; Youdim & Weinstock, 2004; Hasan *et al.,* 1988; Finberg & Tenne, 1982; Chen & Swope, 2005). Reversible MAO-A inhibitors do not cause the cheese reaction because dietary amines can displace the reversible inhibitor from MAO-A and can thus be normally metabolised in the intestine (Haefely *et al.*, 1992).

Based on the observation that MAO-B inhibitors are useful agents for the treatment of PD, the aim of this study is to discover inhibitors of MAO-B. Such compounds may enhance the levels of DA in the brain and therefore provide symptomatic relief for PD patients. MAO-B inhibitors may also represent potential neuroprotective treatment for PD.

1.2 Rationale

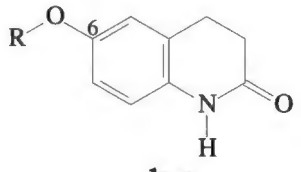
In this study three chemical classes were investigated as potential MAO-B inhibitors. These include (1) C6- and C7-substituted 3,4-dihydro-2(1*H*)-quinolinones, (2) 2-phenoxyethoxy-substituted tetralones and (3) N-propargylamine-2-aminotetralin.

1.2.1 C6- and C7-Substituted 3,4-dihydro-2(1*H*)-quinolinones as inhibitors of monoamine oxidase.

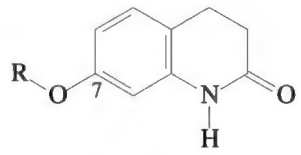
Based on our interest in the discovery of reversible inhibitors with specificity for MAO-B, we have recently reported that, among a series of ten 3,4-dihydro-2(1*H*)-quinolinone derivatives, are high potency MAO-B inhibitors, which possess specificity for the MAO-B isoform (Meiring *et al.*, 2013).

To expand on these results, a series of fourteen 3,4-dihydro-2(1*H*)-quinolinone derivatives will be synthesised (**1a–g** and **2a–g**) and substituted on C6 and C7 with a variety of substituents (Table 1). Substitution with the benzyloxy, phenylethoxy and 2-phenoxyethoxy moieties on both the C6 (compounds **1**) and C7 (compounds **2**) positions will be considered.

Table 1. The structures of the C6- and C7-substituted quinolinones (**1a–g** and **2a–g**) that will be synthesised in this study.



1a-g



2a-g

	R		R
1a	4-$ClC_6H_4CH_2$–	**2a**	4-$ClC_6H_4CH_2$–
1b	4-$BrC_6H_4CH_2$–	**2b**	4-$BrC_6H_4CH_2$–
1c	3-$CH_3C_6H_4CH_2$–	**2c**	3-$CH_3C_6H_4CH_2$–
1d	4-$CH_3C_6H_4CH_2$–	**2d**	4-$CH_3C_6H_4CH_2$–
1e	3-$CH_3C_6H_4(CH_2)_2$–	**2e**	4-$CH_3C_6H_4(CH_2)_2$–
1f	4-$CH_3C_6H_4(CH_2)_2$–	**2f**	$C_6H_5O(CH_2)_2$–
1g	$C_6H_5O(CH_2)_2$–	**2g**	4-$ClC_6H_4O(CH_2)_2$–

It is expected that the synthesised compounds will act as reversible MAO-B inhibitors since the previously synthesised series of 3,4-dihydro-2(1*H*)-quinolinone derivatives were reported to interact reversibly with the MAO enzymes (Meiring *et al.*, 2013). As mentioned, with reversible MAO-B inhibitors enzyme activity is recovered as soon as the inhibitor is cleared, which is a major advantage over irreversible inhibitors (Tipton *et al.*, 2004; Binda *et al.*, 2003).

1.2.2 The evaluation of 2-phenoxyethoxy-substituted tetralones as inhibitors of monoamine oxidase.

Tetralones are often used as starting material for the synthesis of heterocyclic compounds, active pharmaceutical ingredients, natural products and their derivatives (Carreño *et al.*, 2006). The tetralone moiety is also frequently encountered in compounds displaying pharmacological activities (Manvar *et al.*, 2015).

Previous studies have shown that α-tetralone (3,4-dihydro-2*H*-naphthalen-1-one) derivatives are highly potent MAO inhibitors with substitution on both the C6- and C7-positions (**3** and **4**) (Legoabe *et al.*, 2014; Legoabe *et al.*, 2015).

Other chemical classes closely related to α-tetralone, including 1-indanone (**5** and **6**), 3-coumaranone (**7**) and 3,4-dihydro-2(1*H*)-quinolinone derivatives (**8** and **9**) have also been reported to inhibit the MAOs (Figure 1.2) (Meiring *et al.*, 2013; Mostert *et al.*, 2015; Van Dyk *et al.*, 2015)

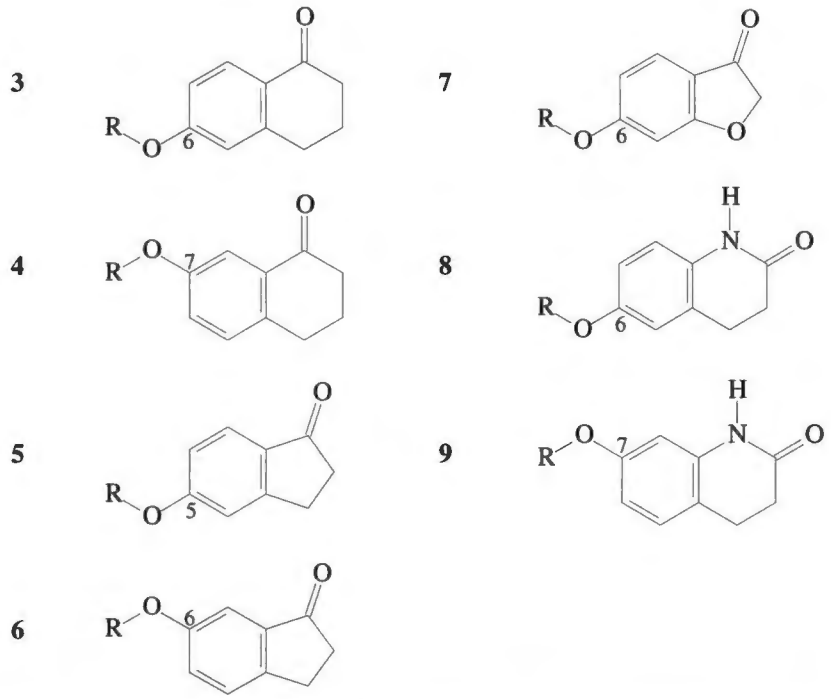


Figure 1.2: The structures of α-tetralone (**3** and **4**), 1-indanone (**5** and **6**), 3-coumaranone (**7**) and 3,4-dihydro-2(1*H*)-quinolinone derivatives (**8** and **9**) (R = alkyl/arylalkyl substituent).

The present study will investigate the possibility that β-tetralone (3,4-dihydro-1*H*-naphthalen-2-one), the regioisomer of α-tetralone, may also represent a useful scaffold for the design of MAO inhibitors (Figure 1.3). β-Tetralone is structurally related to α-tetralone, 1-indanone, 3-coumaranone and 3,4-dihydro-2(1*H*)-quinolinone derivatives (figure 1.2) which, as mentioned above, have been used as scaffolds for MAO inhibitor design.

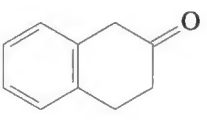


Figure 1.3: The structure of β-tetralone.

For the purpose of this study, three β-tetralone derivatives will be synthesised: 6-(2-phenoxyethoxy)-3,4-dihydronaphthalen-1(2*H*)-one (**10**), 7-(2-phenoxyethoxy)-3,4-dihydronaphthalen-1(2*H*)-one (**11**) and 7-(2-phenoxyethoxy)-3,4-dihydronaphthalen-2(1*H*)-one (**12**) (Figure 1.4).

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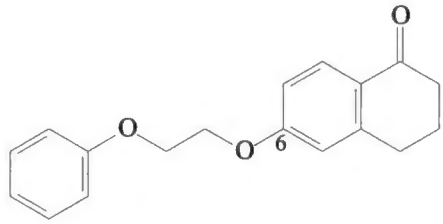

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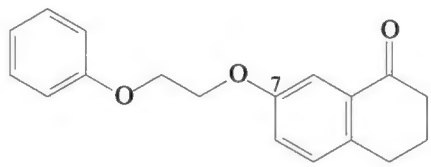

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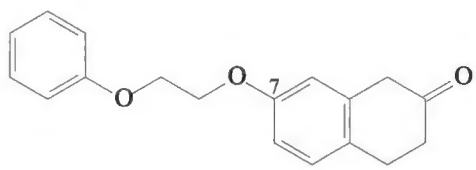


Figure 1.4: The structures of 6-(2-phenoxyethoxy)-3,4-dihydronaphthalen-1(2*H*)-one (**10**), 7-(2-phenoxyethoxy)-3,4 dihydronaphthalen-1(2*H*)-one (**11**) and 7-(2-phenoxyethoxy)-3,4-dihydronaphthalen-2(1*H*)-one (**12**).

It is also expected that these synthesised compounds will act as reversible MAO-B inhibitors since the previously synthesised and closely related α-tetralone derivatives were reported to interact reversibly with the MAO enzymes (Legoabe *et al.*, 2014; Legoabe *et al.*, 2015). As will be shown in this thesis, the selection of the phenoxyethoxy side chain was based, in part, on synthetic feasibility.

The phenoxyethoxy side chain is also found in experimental MAO inhibitors. In particular, a series of 8-(2)-phenoxyethoxycaffeine derivatives (Figure 1.5) have been shown to act as high potency MAO-B inhibitors (Strydom *et al.*, 2012).

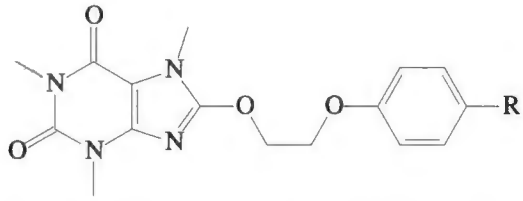


Figure 1.5: The structures of 8-(2)-phenoxyethoxycaffeine derivatives (R = H, Cl, Br, F, CF_3, CH_3, OCH_3, I, CN, NO_2) that have been shown to inhibit MAO.

1.2.3 The evaluation of N-propargylamine-2-aminotetralin as an irreversible inhibitor of monoamine oxidase.

Irreversible inhibitors of the MAOs are an important class of therapeutic agents, in spite of the risks associated with their use. These include the slow recovery of enzyme activity after the inhibitor is withdrawn, and loss of isoform selectivity with repeated administration of the drug (Tipton *et al.*, 2004). Irreversible MAO inhibitors that are currently used in clinic include (R)-deprenyl, rasagiline, tranylcypromine, phenelzine, isocarboxazid, mofegiline and lazabemide (Figure 1.6) (Edmondson *et al.*, 2004a; Edmondson *et al.*, 2004b; Binda *et al.*, 2004; Binda *et al.*, 2005; Milczek *et al.*, 2008). A transdermal delivery system of selegiline [(R)-deprenyl)] has also been developed and reported to be effective for the treatment of major depressive disorder (Pae *et al.*, 2014).

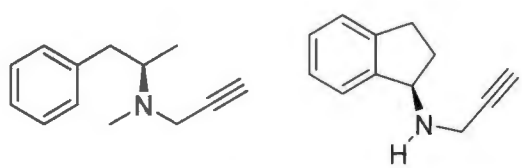
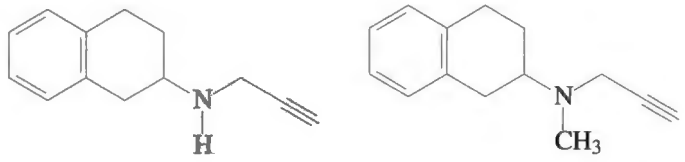


Figure 1.6: The structures of the irreversible MAO-B inhibitors, selegiline and rasagiline.

Motivated by the academic challenge of designing and discovering high potency and isoform-specific MAO inhibitors that may be useful therapeutic agents, the final study of this thesis synthesises a rasagiline related structure, N-propargylamine-2-aminotetralin (2-PAT) (Figure 1.7). 2-PAT is structurally very similar to rasagiline, and also contains the propargylamine functional group. The MAO inhibition properties of 2-PAT may, however, be very different from that of rasagiline. According to literature, N-methylation of rasagiline significantly affects the inactivation kinetics, inhibition potency and isoform selectivity (Hubálek *et al.*, 2004) and the MAOs are much less sensitive to inhibition by the *S*-enantiomer of rasagiline.



2-PAT 2-MPAT

Figure 1.7: The structures of N-propargylamine-2-aminotetralin (2-PAT) and N-methyl-N-propargylamine-2-aminotetralin (2-MPAT).

N-Methyl-N-propargylamine-2-aminotetralin (2-MPAT), the N-methyl analogue of 2-PAT, has previously been studied. *In vitro* studies have shown that 2-MPAT displays potent inhibition of both MAO isoforms (Hazelhoff *et al.*, 1985), however the (+)-enantiomer displayed the highest MAO-B inhibition as well as superior isoform selectivity (for MAO-B over MAO-A) compared to the (-)-enantiomer. The present study will therefore investigate the human MAO inhibition properties of 2-PAT and compare it with those reported for 2-MPAT. As with rasagiline and its N-methyl analogue, the MAO inhibition properties of 2-PAT and 2-MPAT may be very different.

1.3 Hypotheses of this study

Based on literature it is postulated that highly potent and MAO-B specific inhibitors may be designed using 3,4-dihydro-2(1*H*)-quinolinone and β-tetralone as scaffolds. In fact, previous studies have already demonstrated the MAO inhibition potential of the 3,4-dihydro-2(1*H*)-quinolinone and α-tetralone classes of compounds (Meiring *et al.*, 2013; Legoabe *et al.*, 2014; Legoabe *et al.*, 2015).

It is further postulated that 2-PAT will act as an irreversible MAO inhibitor. Although 2-PAT is structurally very similar to rasagiline and the previously studied 2-MPAT, the MAO inhibition properties of 2-PAT may be very different. Based on motivation by the academic challenge of designing and discovering high potency and isoform-specific MAO inhibitors that may be useful therapeutic agents, 2-PAT will thus be synthesised and evaluated as a MAO inhibitor.

1.4 Aim and objectives of this study

The aim of this study is to discover new MAO inhibitors with specificity for the MAO-B isoform. Such compounds may represent useful adjuvants to L-dopa in the treatment of PD. In this study a series of substituted 3,4-dihydro-2(1*H*)-quinolinones and 2-phenoxyethoxy-substituted tetralones will be examined as potential MAO inhibitors. N-propargylamine-2-aminotetralin will also be synthesised and its MAO inhibition properties will be determined.

The objectives of this study are:

- This study will synthesise (1) a series of C6- and C7-substituted 3,4-dihydro-2(1*H*)-quinolinones, (2) a series of 2-phenoxyethoxy-substituted tetralones and (3) N-propargylamine-2-aminotetralin.
- The synthesised analogues will be investigated as potential MAO-A and MAO-B inhibitors using the recombinant human MAO enzymes. IC_{50} values (concentration of the inhibitor that produces 50% inhibition) will be used to express the inhibition potencies of the analogues.
- The reversibility of inhibition of MAO by selected derivatives will be assessed by employing dialysis.
- For reversible inhibition, sets of Lineweaver-Burk plots will be constructed to determine the modes of inhibition of selected inhibitors.
- Finally, the inhibitors will be docked into the MAO active sites using the CDOCKER docking algorithm of Discovery Studio 3.1. This will be done to gain insight into the interactions between the enzyme and selected inhibitors.

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CHAPTER 2

LITERATURE BACKGROUND

2.1 Monoamine oxidase

2.1.1 General background

Over 50 years ago, MAO inhibitors were developed as antidepressant drugs by Zeller's laboratory. Patients receiving treatment for tuberculosis evoked further development of MAO inhibitors after experiments revealed that the antituberculosis drug, isoniazid, was also a potent MAO inhibitor. The first antidepressant to be developed was a MAO inhibitor related to isoniazid, iproniazid.

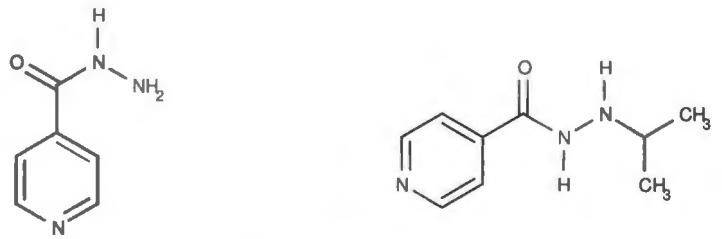


Figure 2.1: The structures of isoniazid (left) and iproniazid (right), respectively.

MAO-A and MAO-B have since received extensive attention, with more than 14 000 MAO related published papers (Youdim *et al.*, 1988). In 1928, MAO was first described by Mary Hare-Bernheim as catalysing the oxidative deamination of tyramine. Since coming to England in the 1930s, Hugh Blaschko's primary interest in research was MAO and he later discovered that tyramine oxidase, noradrenaline oxidase and aliphatic amine oxidase is the same enzyme, able to metabolise primary, secondary and tertiary amines, but not diamines. The enzyme, mitochondrial monoamine oxidase, was eventually named by Albert Zeller in 1938 (MAO; EC1.4.3.4) (Schnaitman *et al.*, 1967; Youdim *et al.*, 1988; Shih *et al.*, 1999; Tipton *et al.*, 2004).

MAO is present in most mammalian tissues and consists of two isoenzymes, MAO-A and MAO-B, varying in proportion from tissue to tissue (Shih *et al.*, 1999; Tipton *et al.*, 2004; Nicotra *et al.*, 2004; Tsang *et al.*, 1986; Strolin Benedetti *et al.*, 1992).

Gene cloning and sequencing for MAO-A and MAO-B was the breakthrough in demonstrating that MAO-A and MAO-B are two separate enzymes, sharing similar structures and possessing covalently attached flavin adenine dinucleotide (FAD) cofactors, with an amino acid sequence identity of ~70% (Bach *et al.*, 1988; Youdim *et al.*, 2006). Both MAO-A and MAO-B are associated with the mitochondrial outer membrane, with MAO-B being the main form in the basal ganglia, blood platelets and liver, and MAO-A being mostly present in the placenta and gastrointestinal tract (Youdim *et al.*, 2006). MAO-A and MAO-B have different substrate specificities, biological functions, pH optima and sensitivity to heat inactivation, as well as different specificities for inhibitors (Youdim *et al.*, 2006; Youdim *et al.*, 1988; Shih *et al.*, 1999; Nicotra & Parvez, 1999).

In 1968, JP Johnston distinguished between MAO-A and MAO-B in discovering that MAO-A is inhibited by low concentrations of clorgyline and preferably oxidises serotonin (5-HT) and noradrenaline (NA), while MAO-B is inhibited by low concentrations of selegiline [(R)-deprenyl] and preferably oxidises benzylamine and 2-phenylethylamine (Knoll, 1978). DA, tyramine and tryptamine are oxidised by both MAO-A and MAO-B.

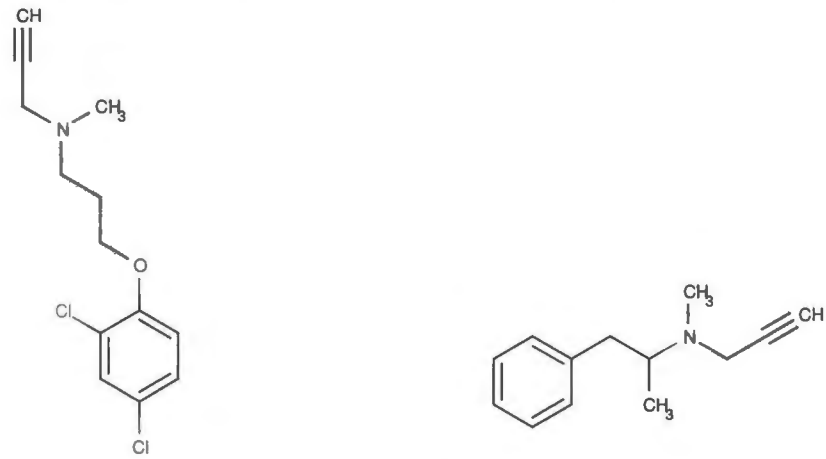


Figure 2.2: The structures of clorgyline (left) and selegiline (right), respectively.

2.1.2 The therapeutic applications of MAO-A and MAO-B

MAO inhibitors for the treatment of Parkinson's disease: MAO exerts a protective action and serves as metabolic barrier by oxidising potentially harmful amines already present in the blood and preventing exogenous amines from entering the systemic circulation. Besides acting as metabolic barriers, the MAOs also terminate the action of certain neurotransmitters (Youdim *et al.*, 2006). Since MAO-B is predominantly present in the basal ganglia, in the brain, the metabolism of DA in this region is largely due to MAO-B.

MAO-B thus participates in the degradation of DA and also deaminates β-phenylethylamine, an endogenous amine which stimulates DA release and inhibits neuronal DA uptake (Glover *et al.*, 1977; Fernandez & Chen, 2007). Because of MAO-B's role in the metabolism of DA in the brain, MAO-B inhibitors have been used for the treatment of Parkinson's disease. In Parkinson's disease the nigrostriatal neuronal pathway degenerates, which results in a deficiency of DA in the striatum. Symptomatic therapy is aimed at replacing DA in this brain region with the most effective therapy being L-dopa, the metabolic precursor of DA. To further enhance DA levels after L-dopa treatment, MAO-B inhibitors may be used. MAO-B inhibitors act by inhibiting DA metabolism in the brain. In early Parkinson's disease, MAO-B inhibitors may be used as monotherapy (Dauer & Przedborski, 2003; Carlsson, 1959; Rascol *et al.*, 2002a; Lees *et al.*, 2009)

The oxidative deamination of DA catalysed by MAO-B produces hydrogen peroxide (H_2O_2), the corresponding aldehyde (dopanal - RCHO), either ammonia or a substituted amine and the reduced FAD cofactor (Binda *et al.*, 2002a; Edmondson *et al.*, 2004).

In high concentrations these products can be harmful and may contribute to neurodegeneration (Riederer *et al.*, 2004). Dopanal has been implicated in α-synuclein aggregation, while increased H_2O_2 levels promote apoptotic cell death (Burke *et al.*, 2008). The aldehyde (RCHO) is oxidised by aldehyde dehydrogenase (ADH), releasing the final excreted metabolite, a carboxylic acid (RCOOH) (Youdim & Bakhle, 2006). ADH deficiency could result in accumulation of neurotoxic aldehydes derived from DA (Figure 2.3) (Grünblatt *et al.*, 2004).

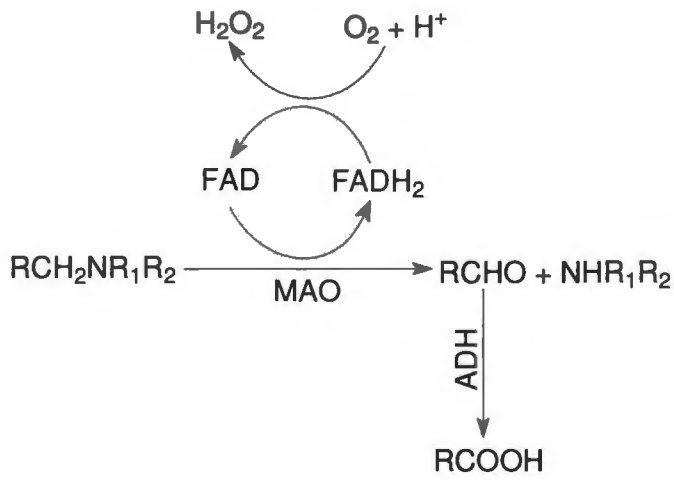


Figure 2.3: The reaction pathway of monoamine metabolism by MAO.

Metabolic products generated by MAO may react with iron in the brain (Symes *et al.*, 1969). H_2O_2 produced with the metabolism of monoamines, can either be converted into the hydroxyl radical (via reaction with iron) or be inactivated by glutathione peroxidase (GPO), which uses glutathione (GSH) as cofactor (Youdim & Bakhle, 2006). The hydoxyl radical formed in the Fenton reaction (Figure 2.4), diminishes cellular anti-oxidants and reacts with, and damages lipids, proteins and DNA. MAO-B activity increases with age, leading to increased concentrations of the components (H_2O_2) of the Fenton reaction and subsequently increased hydroxyl radical formation.

This analysis suggests that MAO-B inhibitors may be able to protect neuronal tissue against metabolic injury by reducing the formation of the toxic products produced by the MAO-B catalytic cycle. This reduces oxidative stress and slows neuronal cell death (Youdim *et al.*, 2006). Therefore, MAO inhibitors may likely be neuroprotective (Edmondson *et al.*, 2009). By acting as neuroprotective agents, MAO-B inhibitors may thus have further relevance in the treatment of Parkinson's disease.

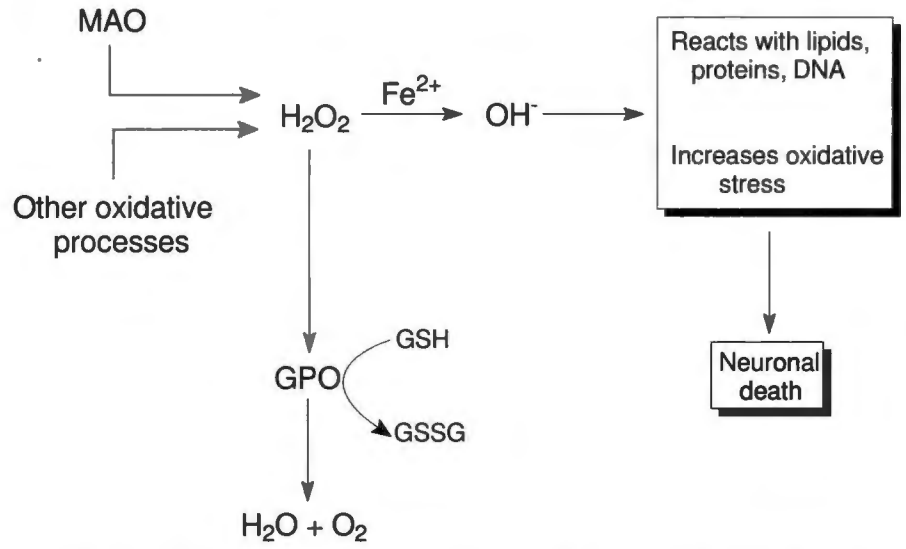


Figure 2.4: The mechanism of neurotoxicity by hydrogen peroxide (H_2O_2), induced by the Fenton reaction.

MAO inhibitors as antidepressants: As mentioned, MAO inhibitors were first marketed as antidepressants, with iproniazid being the first MAO inhibitor to be discovered. Initially used for the treatment of tuberculosis, it was subsequently discovered that iproniazid produced antidepressant effects in patients. Both noradrenaline and serotonin are substrates of MAO-A.

MAO-A is responsible for metabolising serotonin in the central nervous system (CNS) and deactivating circulatory catecholamines as well as vasopressors, such as tyramine in the intestine (Fernandez & Chen, 2007). Inhibition of MAO-A in the CNS, leads to elevated DA, NA and serotonin levels (Pletscher, 1991). MAO-A inhibitors thus demonstrate remarkable antidepressant action, but their clinical value is compromised by serious side effects, including liver toxicity, the 'cheese reaction' and the serotonin syndrome (Youdim *et al.*, 1988). The occurrence of the 'cheese reaction' prompted researchers to develop reversible MAO-A inhibitors such as moclobemide, which is an example of an active antidepressant (Youdim & Bakhle, 2006).

2.1.3 Adverse effects of MAO-A and MAO-B inhibition.

The 'cheese reaction' is induced by tyramine and other dietary amines usually present in foods (most commonly in cheeses). Under normal circumstances, this dietary amine is metabolised by MAO-A in the gut wall and liver, preventing it from entering the systemic circulation. With the use of MAO-A inhibitors, tyramine and other dietary amines are free to enter the systemic circulation. Here they release NA from peripheral adrenergic neurons, leading to a hypertensive response which can be fatal (Figure 2.5) (Youdim & Bakhle, 2006; Youdim *et al.*, 2006; Youdim & Weinstock, 2004; Hasan *et al.*, 1988; Finberg & Tenne, 1982; Chen & Swope, 2005). Reversible MAO-A inhibitors do not cause the 'cheese reaction' because dietary amines can displace the reversible inhibitor from MAO-A and thus be normally metabolised in the intestine (Haefely *et al.*, 1992).

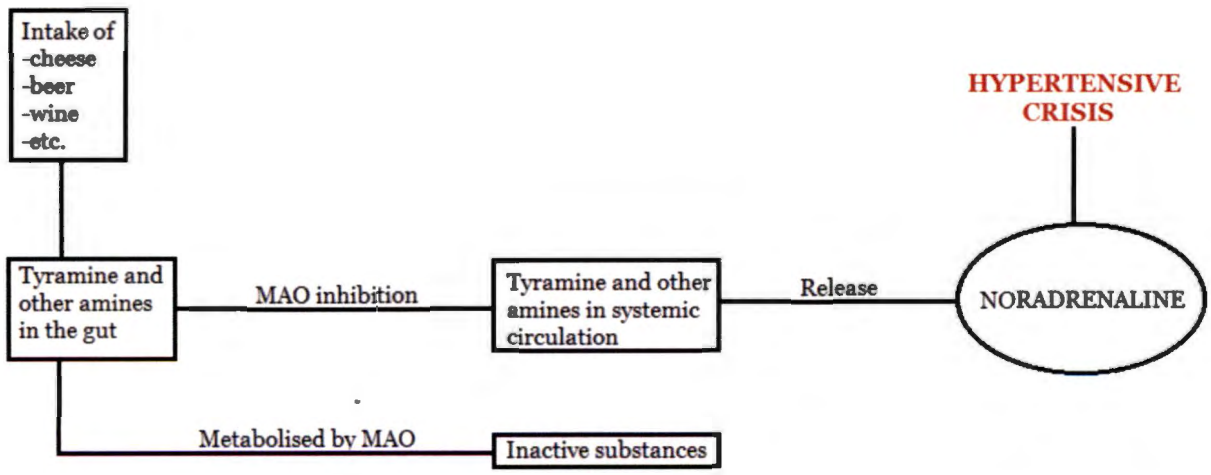


Figure 2.5: The mechanism of the 'cheese reaction'.

The serotonin syndrome occurs with an over activity of serotonin in the CNS. Due to the role that MAO-A plays in metabolising serotonin, the use of MAO-A inhibitors in combination with other serotonin enhancing drugs such as selective serotonin reuptake inhibitors and tricyclic antidepressants, can lead to the serotonin syndrome. This is a serious condition which may be life-threatening (Boyer & Shannon, 2005). The serotonin syndrome is characterised by the following symptoms: restlessness, hallucination, rapid heartbeat, sudden blood pressure changes, overactive reflexes, elevated body temperature, nausea, vomiting and diarrhoea (Fernandez & Chen, 2007; Chen & Swope, 2005).

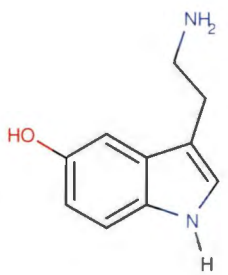


Figure 2.6: The structure of serotonin.

2.1.4 Catalytic mechanism of MAO-A and MAO-B

The exact mechanism of MAO catalysis is still unknown, but possible mechanisms proposed, include the single electron transfer mechanism suggested by Silverman and co-workers (1995), the polar nucleophilic mechanism and the hydride transfer mechanism.

The single electron transfer mechanism involves firstly the oxidation of the amine by one electron, producing an aminium cation radical and flavin radical intermediate. This results in the intermediary product with an acidic α-proton, allowing proton abstraction by a basic amino acid residue in the active site (Figure 2.7). A second single electron transfer yields the fully reduced flavin and the imine product. However, the MAO-A and MAO-B active sites do not contain basic amino acid residues and there exists no direct evidence to support the formation of a flavin radical intermediate (Silverman *et al.,* 1980).

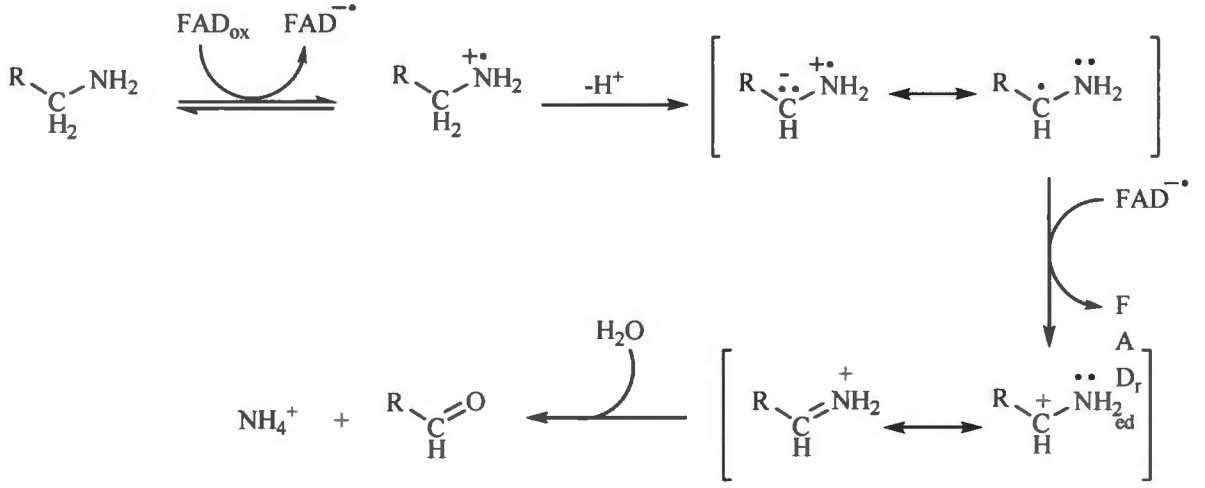


Figure 2.7: Single electron transfer (SET) mechanism of MAO catalysis (Edmondson *et al.*, 2007).

The polar nucleophilic mechanism first proposed by Miller & Edmondson (1999), is a more likely mechanism. The deprotonated amine substrate covalently binds to the 4a position of the flavin, in a nucleophilic manner. This results in a flavin-substrate adduct which enables position N5 to act as a strong active site base. Position N5 exhibits a pKa in the range of 25-27, which is sufficient for α-pro-R-H abstraction from the substrate (Edmondson *et al.*, 2004b). With formation of the reduced flavin, the imine product is released (Figure 2.8) (Binda *et al.*, 2002a; Edmondson *et al.*, 2004).

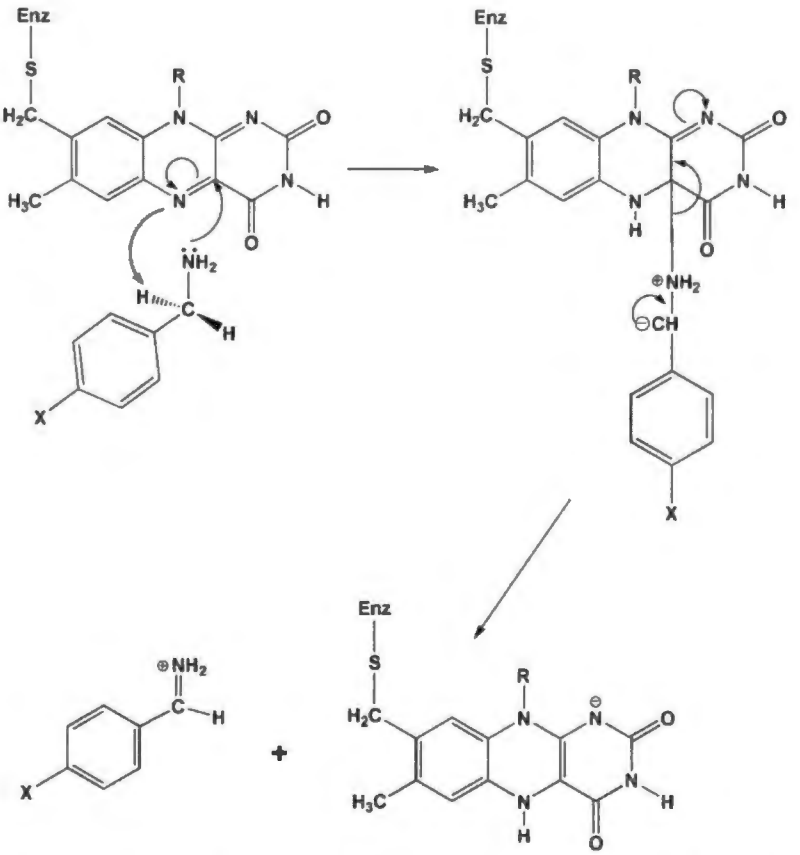


Figure 2.8: Polar nucleophilic mechanism of MAO catalysis. The products of amine oxidation are the protonated imine and the flavin hydroquinone (Edmondson *et al.*, 2007).

The hydride transfer mechanism was recently suggested since there is no direct evidence against this mechanism. Other amine oxidases such as the D-amino acid oxidases employ a single hydride transfer mechanism (Figure 2.9) (Fitzpatrick, 2010). In this mechanism a hydride is simply transferred from the α-carbon of the substrate to the N5 of the flavin to yield the reduced flavin and the imine product.

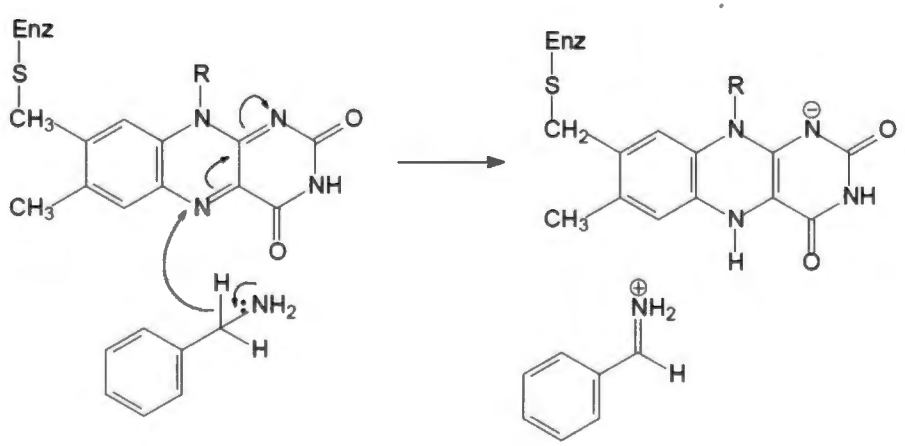


Figure 2.9: The hydride transfer mechanism of MAO catalysis (Fitzpatrick, 2010).

2.1.5 The three dimensional structure of MAO

MAO-B:

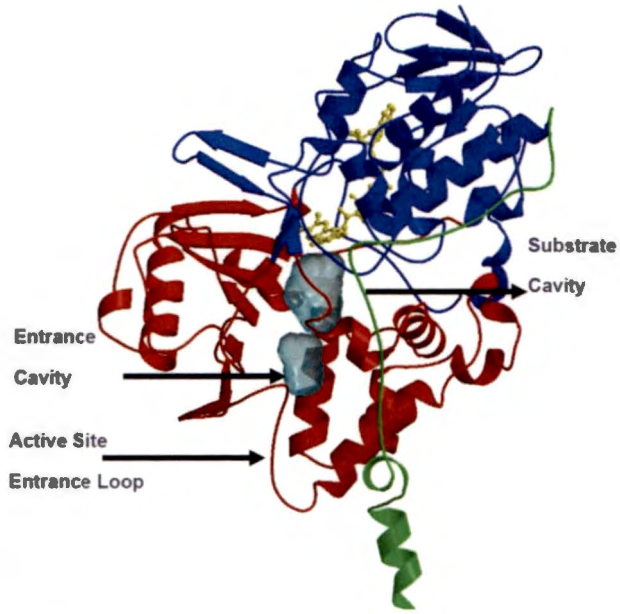


Figure 2.10: The three dimensional structure of human MAO-B. The ball and stick model in yellow represents the covalent flavin moiety. The blue represents the flavin binding domain. The red is the substrate domain. The green represents the membrane binding domain (Edmondson *et al.*, 2007).

An FAD cofactor is covalently bound to the MAO protein via an 8α-thioether linkage to Cys397. Human MAO-B crystallises as a dimer and is tightly bound at the C-terminal of MAO, through a polypeptide segment (α-helix) to the mitochondrial outer membrane. The hydrophobic C-terminal consists of 32 amino acids. It protrudes perpendicularly into the phospholipid bilayer of the mitochondrial membrane, anchoring the enzyme (Binda *et al.*, 2002b; Edmondson *et al.*, 2004).

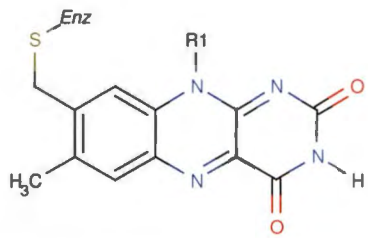


Figure 2.11: The partial structure of the FAD cofactor.

Two theories exist concerning the conformation of the C-terminal within the mitochondrial membrane. The first theory proposes that the C-terminal traverses the membrane with 5 amino acids protruding on the other side.

The second theory suggests that the C-terminal is folded within the membrane allowing the end of the helix to protrude on the same side as the enzyme (Figure 2.12) (Binda *et al.*, 2004). The C-terminal anchoring into the mitochondrial membrane might be crucial for MAO catalytic activity.

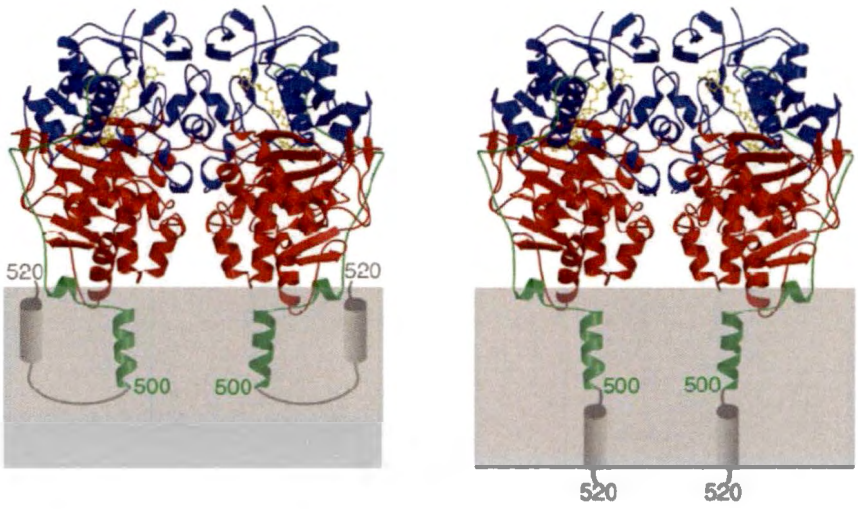


Figure 2.12: The two suggested conformations of C-terminal anchoring of MAO-B within the mitochondrial outer membrane. The first panel shows the C-terminal protruding on the same side of the enzyme and the second panel shows the C-terminal exiting the membrane on the other side (Binda *et al*, 2004).

MAO-B has apolar groups at various positions in the sequence and consists of 520 amino acids (Fraaije & Mattevi, 2000; Binda *et al,* 2002a, 2007). To reach the flavin coenzyme, a substrate molecule must negotiate a protein loop at the entrance to one of two cavities. These cavities include the "entrance cavity" and the "substrate cavity", separated by the Ile199 side chain. This residue thus serves as a "gate" between the two cavities.

The active site of MAO-B consists of a 420 $Å^3$ hydrophobic cavity and is connected to an entrance cavity of 290 $Å^3$. An aromatic cage formed by residues Tyr 398 and Tyr 435 is the recognition site for the substrate amino group. These residues are orientated perpendicular to the plane of the flavin ring (Binda *et al,* 2002a; 2003). The entrance cavity connects the surface of the protein to the substrate cavity in MAO-B. Besides Ile199, residues Leu171, Phe168 and Tyr326 also defines the border between the two cavities. The two cavities may fuse, forming a single larger cavity able to accommodate larger substrates and inhibitors (Binda *et al,* 2002a; Edmondson *et al.*, 2007). This occurs when the side chain of Ile199 rotates from the cavity.

MAO-A:

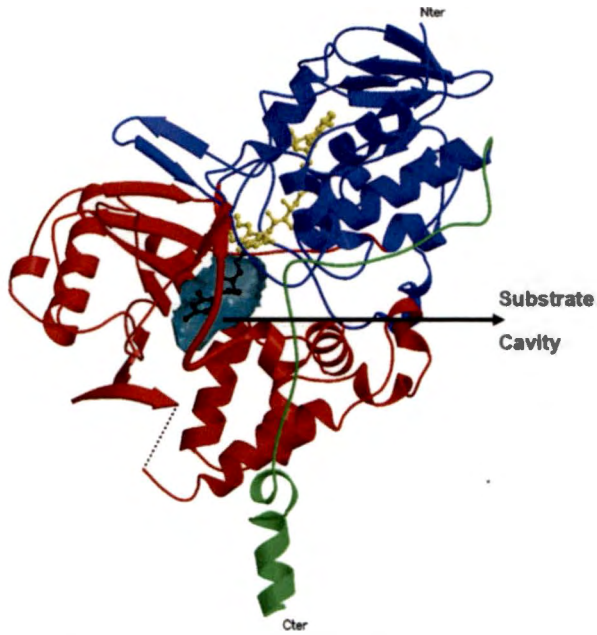


Figure 2.13: The three dimensional structure of human MAO-A. The colouring is similar to those in figure 2.10 (Edmondson *et al.*, 2007).

Human MAO-A crystallises as a monomer. The structure of MAO-A can be divided into two domains, the extra-membrane domain and the membrane binding domain. The extra-membrane domain is further divided into two regions, the FAD binding region and substrate/inhibitor binding region (Son *et al.*, 2008).

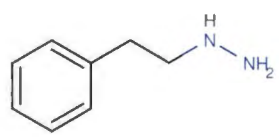
In contrast to human MAO-B, both human and rat MAO-A have single substrate binding cavities with protein loops at the entrances of the active site cavities. Compared to MAO-B, the active site cavity of MAO-A is much smaller, shorter and wider. Similar to MAO-B, human MAO-A is also hydrophobic, with a volume of 550 $Å^3$. The covalent attachment of the FAD cofactor in MAO-A and the tyrosine residues which form the "aromatic cage" in the active sites, are identical to those of MAO-B (Edmondson *et al.*, 2007; De Colibus *et al.*, 2005).

The difference in active site residues between MAO-A and MAO-B may contribute to the different substrate and inhibitor specificities of the two isoforms. For example, in MAO-A the Phe208 residue is in the homologues position to the MAO-B Ile199 residue. Ile 199 in MAO-B is able to rotate from the active site to allow larger inhibitors, including safinamide, to bind to the fused entrance and substrate cavities.

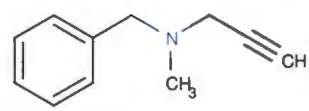
Phe208 in MAO-A on the other hand, is not able to rotate from the active site in order to accommodate larger inhibitors. This is due to the fact that the phenyl side chain of Phe208 is much larger than the side chain of Ile199. Also, in the position of Tyr326 in MAO-B, MAO-A has residue Ile335. Because of its larger size, Tyr326 in MAO-B would restrict the binding of certain inhibitors such as clorgyline, where Ile335 would allow such binding within MAO-A (De Colibus *et al.*, 2005; Son *et al.*, 2008).

2.1.6 Inhibitors of MAO

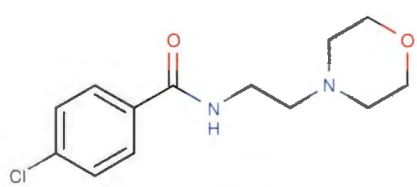
MAO inhibitors were used as antidepressants in the 1950s, with the first inhibitor being iproniazid, initially developed as tuberculosis treatment. Since iproniazid was found ineffective for tuberculosis treatment, other hydrazine derivatives such as phenelzine were developed. The severe liver toxicity caused by iproniazid, was overcome by the development of non-hydrazine derivatives, including pargyline and tranylcypromine. Hypertensive crises were still a major side effect of MAO-A inhibitors (Youdim *et al.*, 2006). However, selective MAO-B inhibitors and reversible MAO-A inhibitors, including moclobemide and lazabemide, did not lead to hypertensive crisis (Da Prada *et al.*, 1990).



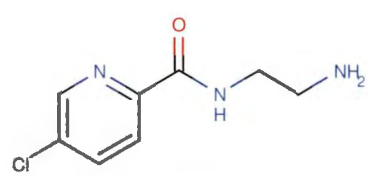
Phenelzine



Pargyline



Moclobemide



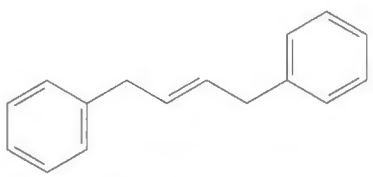
Lazabemide

Figure 2.14: The structures of phenelzine, pargyline, moclobemide and lazabemide.

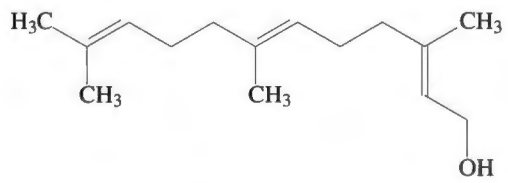
Inhibitors of MAO-A and MAO-B can be divided into two classes, namely reversible and irreversible inhibitors. Within these classes selective and non-selective inhibitors may be found.

Reversible inhibitors bind non-covalently to the active sites of MAO-A and MAO-B and an increase of the substrate concentration can overcome inhibition. Irreversible inhibitors bind in an irreversible manner. The irreversible inhibitor is oxidised to the active inhibitor which covalently binds, via interaction with the FAD cofactor, to the active site of the enzymes. Irreversible inhibition can only be overcome by *de novo* enzyme synthesis and do not allow competition with other substrates, hampering normal metabolism of certain substrates (Lees, 2005; Abeles & Maycock, 1976; Binda *et al.*, 2003).

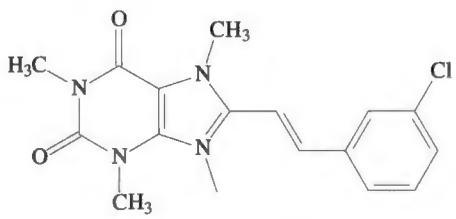
Examples of reversible MAO-B inhibitors include 1,4-diphenyl-2-butene, discovered in MAO-B crystallisation experiments (Binda *et al.*, 2003); trans,trans-farnesol a component of tobacco smoke (Hubálek *et al.*, 2005); 8-(3-chlorostyryl)caffeine (CSC) also an A_{2A} adenosine receptor antagonist (Chen *et al.*, 2002) and safinamide, a DA modulator (Caccia *et al.*, 2006; Fernandez & Chen, 2007).



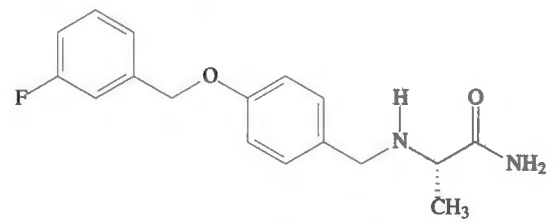
1,4-Diphenyl-2-butene



Trans,trans-farnesol



8-(3-Chlorostyryl)caffeine



Safinamide

Figure 2.15: The structures of reversible MAO-B inhibitors, 1,4-diphenyl-2-butene, trans,trans-farnesol, 8-(3-chlorostyryl)caffeine and safinamide.

Examples of irreversible MAO-B inhibitors are the propargylamines, selegiline (Knoll & Magyar, 1972), rasagiline (Finberg *et al.*, 1981) and the experimental inhibitor PF-9601 N (Perez *et al.*, 2003). Selegiline undergoes first pass hepatic metabolism and forms three metabolites, desmethylselegiline, l-methamphetamine and l-amphetamine.

The amphetamine metabolites are potentially neurotoxic and are associated with cardiovascular and psychiatric adverse effects (Churchyard *et al.*, 1997).

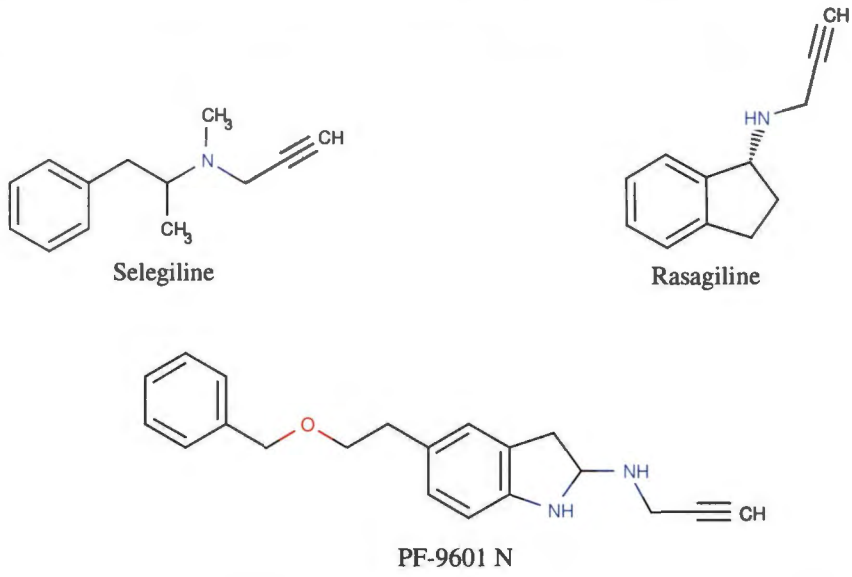
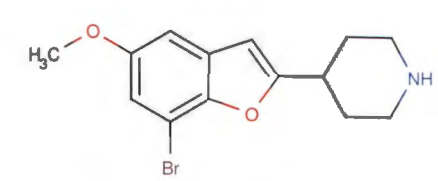
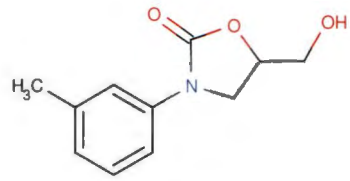


Figure 2.16: The structures of irreversible MAO-B inhibitors, selegiline, rasagiline and PF-9601 N.

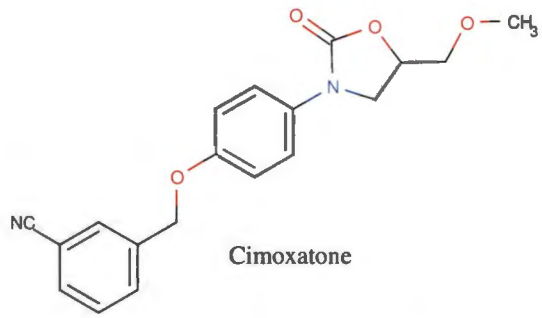
Reversible selective MAO-A inhibitors are clinically used in the treatment of various depression states and include brofaromine, toloxatone, cimoxatone and befloxatone (Bortolato *et al.*, 2008).



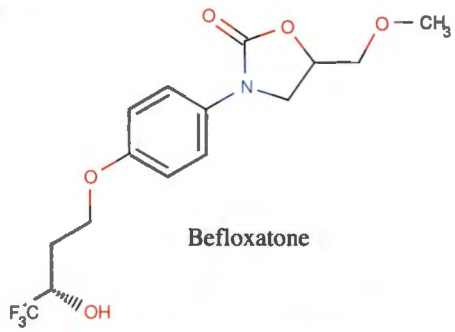
Brofaromine



Toloxatone



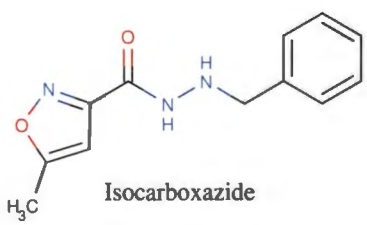
Cimoxatone



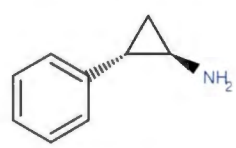
Befloxatone

Figure 2.17: The structures of reversible MAO-A inhibitors, brofaromine, toloxatone, cimoxatone and befloxatone.

An example of a selective, irreversible MAO-A inhibitor is clorgyline, and examples of nonselective irreversible MAO inhibitors include isocarboxazide and tranylcypromine (Riederer *et al.,* 2004). Although clorgyline and isocarbaxazide are effective in the treatment of depressive disorders, their clinical use as antidepressants is prevented by the 'cheese reaction'.



Isocarboxazide



Tranylcypromine

Figure 2.18: The structures of isocarboxazide and tranylcypromine.

2.1.7 Conclusion

This chapter discussed the general background of MAO, including the therapeutic applications of both MAO-A and MAO-B inhibitors. Furthermore, the three dimensional structures of MAO-A and MAO-B, as well as mechanistic proposals for MAO catalysis were discussed. MAO inhibitors were also briefly discussed. The main focus for this study is to synthesise new inhibitors, with specificity for MAO-B. The next chapter wil provide additional background to this thesis in the form of a review of the pharmacological properties of 3,4-dihydro-2(1*H*)-quinolinones, one of the series of compounds studied in this thesis.

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CHAPTER 3

MANUSCRIPT A

Article submitted to Mini-Reviews in Medicinal Chemistry:

A review of the pharmacological properties of 3,4-dihydro-2(1*H*)-quinolinones

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

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

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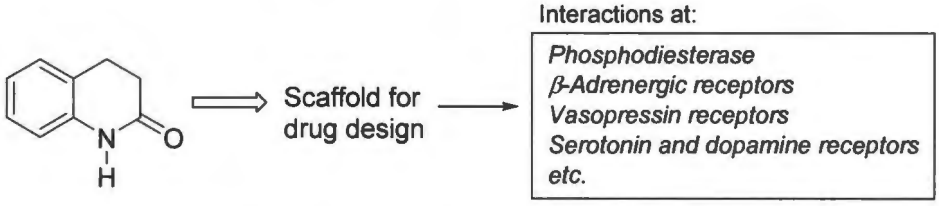
Running title: 3,4-Dihydro-2(1*H*)-quinolinones

Keywords: aripiprazole; carteolol; cilostazol; 3,4-dihydro-2(1*H*)-quinolinone; pharmacology; drug design

Graphical abstract:



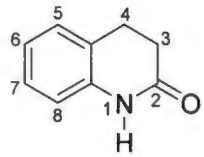
A review of the pharmacological actions of compounds containing the 3,4-dihydro-2(1*H*)-quinolinone scaffold concludes that the 3,4-dihydro-2(1*H*)-quinolinone moiety is a versatile and potentially useful scaffold in medicinal chemistry.

ABSTRACT

The 3,4-dihydro-2(1*H*)-quinolinone moiety is present in a number of pharmacologically active compounds. These include registered drugs such as cilostazol, carteolol and aripiprazole as well as numerous experimental compounds. Compounds containing the 3,4-dihydro-2(1*H*)-quinolinone moiety also exhibit a variety of activities in both the peripheral and central tissues, which includes phosphodiesterase inhibition, blocking of β-adrenergic receptors, antagonism of vasopressin receptors and interaction with serotonin and dopamine receptors. Based on its versatility in drug design and action, this paper reviews the pharmacological actions of compounds containing the 3,4-dihydro-2(1*H*)-quinolinone scaffold with emphasis being placed on the most important and significant members of each activity class.

1. INTRODUCTION

Small organic molecules are powerful tools in the medicinal sciences and function as therapeutic and diagnostic agents, as well as probes to elucidate biological processes. In modern medicine, small molecule therapeutics most often exerts pharmacological actions by binding to protein receptors and enzymes. It is therefore not surprising that compounds that bind to a specific protein target bear close structural resemblance and often share a common molecular scaffold. Molecular scaffolds are thus an important concept in medicinal chemistry and are ordinarily the starting point for the design and development of new therapeutics. Since compounds that share a specific molecular scaffold may possess dissimilar pharmacological activities, a specific scaffold may serve as point of departure for different drug design projects. The 3,4-dihydro-2(1*H*)-quinolinone moiety (**1**) is an example of this concept and is present in a variety of compounds exhibiting different biological activities (Fig. 1). Examples of well-known 3,4-dihydro-2(1*H*)-quinolinones are cilostazol (**2**), carteolol (**4**) and aripiprazole (**5**), compounds that have been registered for therapeutic use in humans. A number of experimental compounds also are 3,4-dihydro-2(1*H*)-quinolinones. Based on our interest in the design of quinolinone-derived monoamine oxidase (MAO) inhibitors, we review in this paper the pharmacological properties of compounds containing the 3,4-dihydro-2(1*H*)-quinolinone scaffold [1]. A Pubmed search with the term "3,4-dihydro-2(1*H*)-quinolinone" yields 97 results, which were for the most part considered in compiling this review. This paper will demonstrate the versatility of the 3,4-dihydro-2(1*H*)-quinolinone moiety as useful scaffold for future medicinal chemistry projects.



3,4-Dihydro-2(1*H*)-quinolinone

Fig. 1. The structure of 3,4-dihydro-2(1*H*)-quinolinone (**1**).

2. CHEMISTRY OF 3,4-DIHYDRO-2(1*H*)-QUINOLINONE

3,4-Dihydro-2(1*H*)-quinolinone(**1**) may be considered to be a bicyclic system of a δ-lactam fused to a phenyl ring. 3,4-Dihydro-2(1*H*)-quinolinone exhibits moderate lipophilicity with a calculated logP of 1.34 (ChemSketch) and a pKa of ~14, which makes it unionised at physiological pH. The amide may act as both hydrogen donor and acceptor with protein residues. Due to presence of two sp^3 hybridised carbons, the 3,4-dihydro-2(1*H*)-quinolinone moiety is nonplanar (Fig. 2). The amide function of 3,4-dihydro-2(1*H*)-quinolinone is stable towards acid and base catalysed hydrolysis.

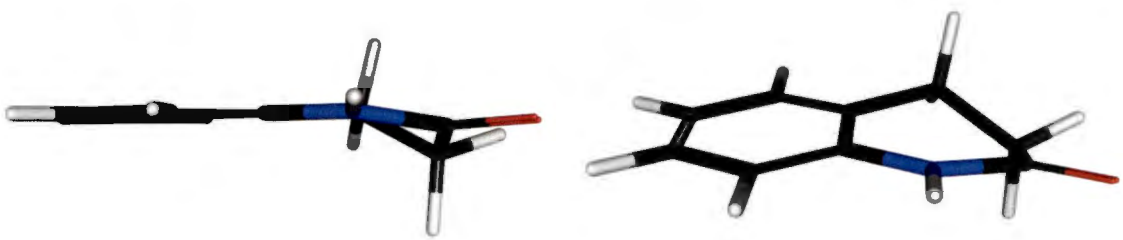


Fig. 2.Two perspectives of a low energy conformer of 3,4-dihydro-2(1*H*)-quinolinone (**1**), calculated by the MMFF94 force field.

3. THE PHARMACOLOGY OF 3,4-DIHYDRO-2(1*H*)-QUINOLINONES

3.1. Phosphodiesterase inhibition

Cilostazol [6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-3,4-dihydro-2(1*H*)-quinolinone, OPC-13013, **2**] is a medication approved for the alleviation of intermittent claudication (pain in the legs that occurs with walking and disappears with rest) in individuals with peripheral vascular disease (Fig. 3). In this respect cilostazol inhibits platelet activation but also induces vasodilation [2]. Although it is generally believed that cilostazol may mediate these effects by acting as a specific inhibitor of phosphodiesterase type 3 (PDE3) to elevate intracellular cAMP, the exact mechanism of action is not fully understood [3]. Based on its unique mechanisms of action cilostazol is under investigation as treatment for a variety of indications. For example cilostazol may protect the heart against ischemia/reperfusion injury [4] and exert neuroprotective effects against cerebral ischemia [5].

Cilostazol derivatives have been synthesised as potential inhibitors of 12(S)-hydroxyeicosatetraenoic acid (12-HETE) release from platelets, a process which plays an important role in the pathogenesis of various circulatory disorders and arteriosclerosis. 3,4-Dihydro-6-[3-(1-o-tolylimidazol-2-yl)sulfinylpropoxy]-2(1*H*)-quinolinone (**3**) was found to be one of the most potent inhibitors of 12-HETE release, and showed in vivo inhibitory activity on platelet adhesion in rats[6]. The S-(+)-enantiomer of this compound exhibited a superior potency and in vivo activity compared to the R-(–)-enantiomer.

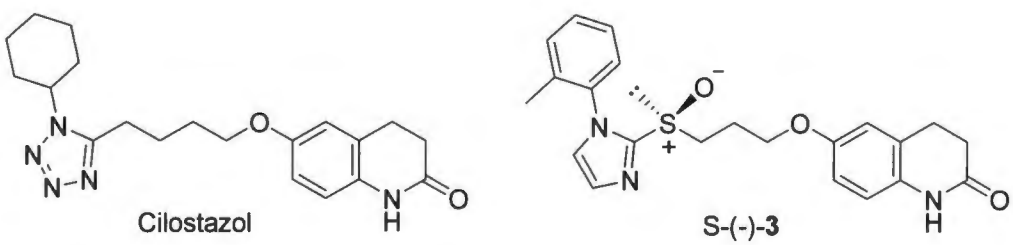
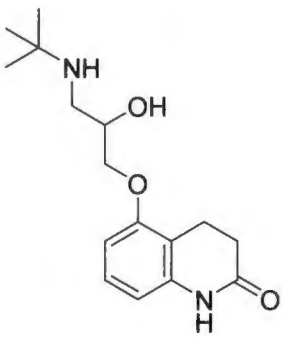


Fig. 3.The structure of cilostazol (**2**) and **3**.

3.2. Blocking of β-adrenergic receptors

3,4-Dihydro-2(1*H*)-quinolinones also may act as antagonists of β-adrenergic receptors. This is exemplified by carteolol [5-(3-tert-butylamino-2-hydroxy-propoxy)-3,4-dihydro-2(1*H*)-quinolinone, **4**], a non-selective β1- and β2-adrenergic receptor-blocking agent registered for the ophthalmic treatment of glaucoma (Fig. 4).Carteolol is active via the oral and intravenous routes and produces potent, long lasting adrenergic receptor blocking [7]. Carteolol has strong intrinsic sympathomimetic activity (ISA), up to 30-fold more powerful than that of propranolol.



Carteolol

Fig. 4.The structure of carteolol (**4**).

3.3. Interaction with serotonin and dopamine receptors

Aripiprazole [7-(4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butyloxy-3,4-dihydro-2-(1*H*)-quinolinone, OPC-14597, **5**] is an antipsychotic with a mechanism of action that differs from typical and atypical antipsychotics (Fig. 5). Aripiprazole interacts with a range of receptors, including serotonin (5-HT) and dopamine receptors [8]. In this respect, aripiprazole possesses partial agonist activity at D_2 and 5-HT_{1A} receptors, antagonist activity at 5-HT_{2A} receptors and agonist activity at dopamine autoreceptors [8-10]. Aripiprazole is currently registered for use in schizophrenia and bipolar disorder.

A related compound, OPC-14523 [1-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl]-5-methoxy-3,4-dihydro-2(1*H*)-quinolinone, **6**] is an experimental antidepressant whose mechanism of action involves potent partial agonist activity at 5-HT_{1A} receptors [11], agonist activity at sigma receptors and possibly inhibition of serotonin reuptake [12,13].

In another study of interest, 3-piperazinyl-3,4-dihydro-2(1*H*)-quinolinone derivatives were designed to act as mixed dopamine D_2/D_4receptor antagonists, an activity profile that is similar to that of clozapine, a clinically used antipsychotic agent [14]. Examples of mixed antagonists are compounds **7** and **8** (Fig. 5). These have the advantage of possessing weak α_1-affinities, which would minimise undesirable cardiovascular effects. 3,4-Dihydro-2(1*H*)-quinolinone derivatives have also been synthesised and shown to possess high 5-HT_{1A} and 5-HT_{1D} antagonist potencies, with some derivatives also possessing affinities for 5-HT_{1B} and the serotonin transporter (SerT). By acting at 5-HT_1 autoreceptors and the SerT, such compounds, exemplified by structure **9**, control synaptic 5-HT levels and thus represent useful pharmacological tools for animal behavioural and disease models [15].

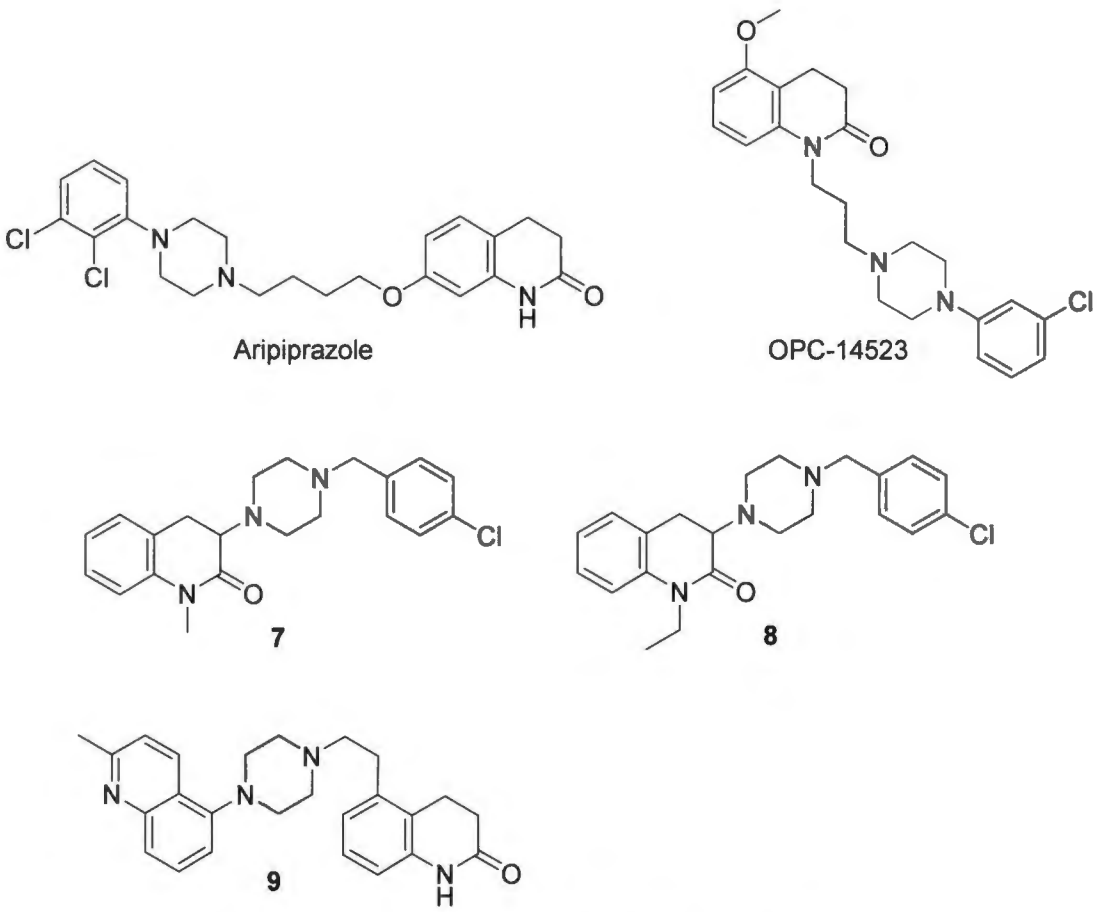
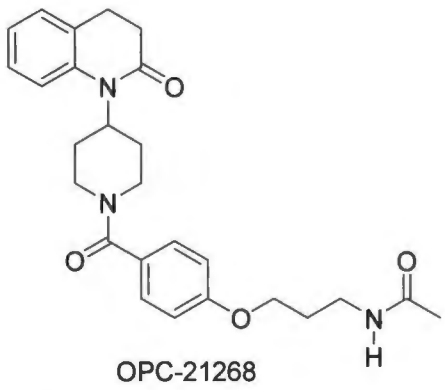


Fig. 5.The structures of aripiprazole (**5**), OPC-14523(**6**) and compounds **7–9**.

3.4. Antagonism of vasopressin receptors

The arginine vasopressin receptor 1A (V1A receptor or AVPR1A) is one of the three major receptor types for arginine vasopressin (AVP). This receptor is found in the brain as well as in the periphery in the liver, kidney and vasculature [16]. OPC-21268 [1-[1-[4-(3-acetylaminopropoxy)benzoyl]-4-piperidyl]-3,4-dihydro-2(1*H*)-quinolinone, **10**] is a 3,4-dihydro-2(1*H*)-quinolinone compound that acts as a vasopressin V1A receptor-selective antagonist (Fig. 6). In vivo, this compound antagonises AVP-induced vasoconstriction and pressor responses. It has thus been suggested that OPC-21268 may be useful for the treatment of hypertension and congestive heart failure [17].

OPC-21268 also serves as a pharmacological tool to probe the physiological roles of V1A receptors and has entered clinical trials for a number of indications, for example for the treatment of albuminuria in patients with non-insulin dependent diabetes mellitus [18].

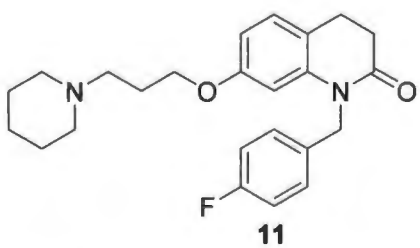


OPC-21268

Fig. 6.The structure of OPC-21268 (**10**).

3.5. Sigma receptor antagonism and agonism

Sigma-1 (σ1) receptor antagonists possess analgesic properties and may be useful for the management of pain. 3,4-Dihydro-2(1*H*)-quinolinone derivatives have been reported to antagonise σ1 receptors with compound **11** exhibiting a K_i of 1.22 nM (Fig. 7) [19]. Sigma receptor agonists, in turn, exert antidepressant effects [20]. In this respect, a series of 3,4-dihydro-2(1*H*)-quinolinones have been shown to act as sigma receptor agonists with antidepressant-like activity in animal models [21]. This behaviour is exemplified by OPC-14523 (**6**) which reduces immobility time in the forced-swimming test in mice.

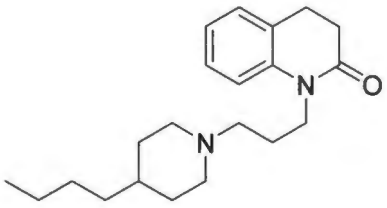


11

Fig. 7.The structure of compound **11**.

3.6. Muscarinic acetylcholine receptor agonism

77-LH-28-1 [1-[3-(4-butyl-1-piperidinyl)propyl]-3,4-dihydro-2(1*H*)-quinolinone, **12**] is a 3,4-dihydro-2(1*H*)-quinolinone derivative that acts as an allosteric agonist of M1 muscarinic acetylcholine receptors (Fig. 8). Experimental evidence has shown that 77-LH-28-1is bioavailable and penetrates the blood-brain barrier, making it a suitable tool to study M1 receptor activation in vivo [22]. Selective M1 agonists may represent useful agents for a number of clinical conditions, including improving cognitive deficits and behavioural disturbances in Alzheimer's disease patients [23].



77-LH-28-1

Fig. 8.The structure of 77-LH-28-1 (**12**).

3.7. N-Methyl-D-aspartate (NMDA) antagonism

NMDA receptors are glutamate-gated ion channel proteins that play important roles in many neuronal processes including learning, memory and pain transmission. NMDA receptors are heteromers, consisting of NR1, NR2A, NR2B, NR2C and NR2D subunits. Among these, NMDA receptors with the NR2B-subunits are involved in pain transmission and have thus been targeted for the treatment of pain. Compound **13** [(-)-6-[2-[4-(3-fluorophenyl)-4-hydroxy-1-piperidinyl]-1-hydroxyethyl]-3,4-dihydro-2(1*H*)-quinolinone], a 3,4-dihydro-2(1*H*)-quinolinone derivative, as an orally active and selective antagonist of the NR2B-subunit of NMDA receptors and a potential development candidate for the management of pain (Fig. 9) [24]. This compound exhibits an IC_{50} value of 5 nM for the NR2B-subunit.

3,4-Dihydro-2(1*H*)-quinolinone derivatives have also been designed for combined antagonist activity at the glycine site of the NMDA receptors as well as at AMPA receptors. An example of such a compound is L-698,544 (**14**), which has been found to be active in an anticonvulsant animal model [25].

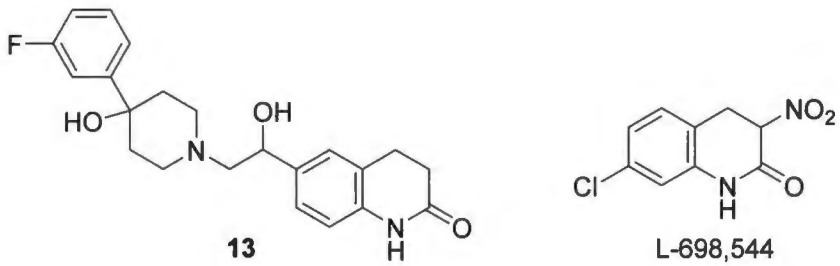
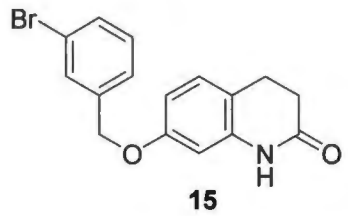


Fig. 9.The structures of compound **13** and L-698,544 (**14**).

3.8. Monoamine oxidase inhibition

The MAO enzymes consist of two genetically distinct isoforms, MAO-A and MAO-B, and are key role-players in the catabolism of neurotransmitters and dietary amines. Inhibitors of the MAOs are of considerable pharmacological importance with MAO-A inhibitors being used as antidepressant agents while MAO-B inhibitors are established therapy for Parkinson's disease [26]. Literature reports that a series of 3,4-dihydro-2(1*H*)-quinolinones acts as specific inhibitors of the MAO-B isoform with the most potent homologue, compound **15**, exhibiting an IC_{50} value of 0.0029 μM (Fig. 10) [1]. This compound is much less potent as a MAO-A inhibitor (IC_{50} = 7.98 μM). Interestingly, substitution on C7 of the 3,4-dihydro-2(1*H*)-quinolinone moiety yields higher potency inhibition compared to analogous substitution on C6.

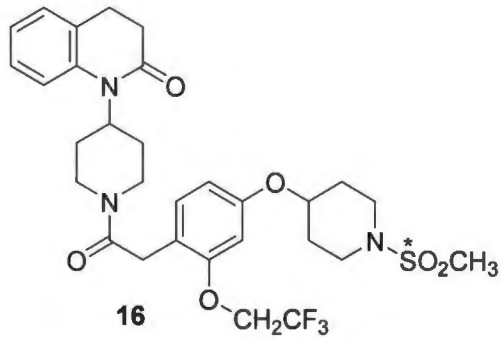


15

Fig. 10.The structure of compound **15**.

3.9. Oxytocin receptor antagonism

3,4-Dihydro-2(1*H*)-quinolinones have also been considered as antagonists of human oxytocin receptors. Derivative **16** was labeled with [^{35}S] and shown to exhibit sub-nanomolar affinities for human oxytocin receptors, with good selectivity over vasopressin receptors (Fig. 11). Compound **16** therefore represents a potential tool for studying the pharmacology of oxytocin receptors in human and non human primate tissue [27].



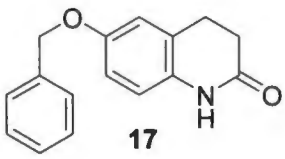
16

Fig. 11.The structure of compound **16**.

3.10. Anticonvulsant activities

As mentioned above, by acting as combined antagonists of NMDA and AMPA receptors, simple 3,4-dihydro-2(1*H*)-quinolinones (e.g. L-698,544) exert anticonvulsant activity in animal models [25].

Another study reports that simple alkyloxy substitution on C6 of 3,4-dihydro-2(1*H*)-quinolinone also yields compounds with anticonvulsant activity in animals, although the mechanism of action is not clear [28]. This is exemplified by 6-benzyloxy-3,4-dihydro-2(1*H*)-quinolinone (**17**) (Fig. 12).

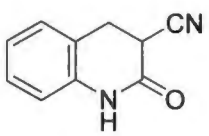


17

Fig. 12.The structure of compound **17**.

3.11. 3,4-Dihydro-2(1*H*)-quinolinones as contraceptives

A 3,4-dihydro-2(1*H*)-quinolinone compound, 84-182 (3-cyano-3,4-dihydro-2(1*H*)-quinolinone,**18**), has been shown to possess contraceptive activity in female hamsters, guinea pig and rhesus monkeys when administered during the peri-implantation period (the period that extends from the time the blastocyst is free in the uterus, through the processes of attachment, to the start of trophoblast differentiation) (Fig. 13). The compound, however, does not prevent implantation and does not interrupt an established pregnancy [29].



84-182

Fig. 13.The structure of 84-182 (**18**).

3.12. Positive inotropic action

Positive inotropic agents increase myocardial contractility, and are used to support cardiac function in various conditions including congestive heart failure, myocardial infarction and cardiomyopathy. Cilostazol (**2**), by acting as a specific inhibitor of PDE3, is a positive inotropic agent, useful for the treatment of congestive heart failure. Y-20487 [6-(3,6-dihydro-2-oxo-2*H*-1,3,4-thiadiazin-5-yl)-3,4-dihydro-2(1*H*)-quinolinone, **19**] is another example of such a cardiotonic agent and acts by inhibition of both PDE3 and PDE4 isoenzymes (Fig. 14) [30]. In this regard, PDE4 inhibition may reduce the breakdown of cAMP generated by β-adrenergic receptor stimulation while inhibition of PDE3 may result in cAMP accumulation and thus a direct inotropic effect. Another positive inotropic agent, OPC-8212 [3,4-dihydro-6-[4-(3,4-dimethoxybenzoyl)-1-piperazinyl]-2(1*H*)-quinolinone, **20**], is a compound that produces bradycardia and antitachycardiac effects in animals and humans [31]. This compound may act by elevating intracellular cAMP levels, leading to activation of calcium currents. This compound, also known as vesnarinone, possesses unique and complex mechanisms of action and inhibits PDE3, which increases intracellular calcium, and also affects numerous myocardial ion channels. Vesnarinone has been used for the treatment of advanced congestive heart failure, but has been withdrawn from the market due to a narrow therapeutic window [32-34].

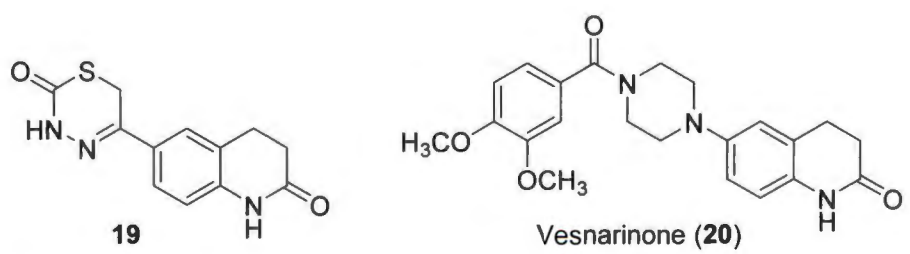


Fig. 14.The structures of Y-20487 (**19**) and OPC-8212 (vesnarinone, **20**).

4. NATURAL PRODUCTS CONTAINING 3,4-DIHYDRO-2(1*H*)-QUINOLINONES

It is interesting to note that 3,4-dihydro-2(1*H*)-quinolinones have been found as secondary metabolites in natural products. Microbial fungi isolated from marine sources have been considered for their potential of providing new natural products that are structurally unusual and biologically highly active. In this respect 3,4-dihydro-2(1*H*)-quinolinones **21–24** have been isolated from *Penicilliumjanczewskii* cultures and found to possess some degree of cytotoxicity towards mammalian tissue cultures (Fig. 15) [35].

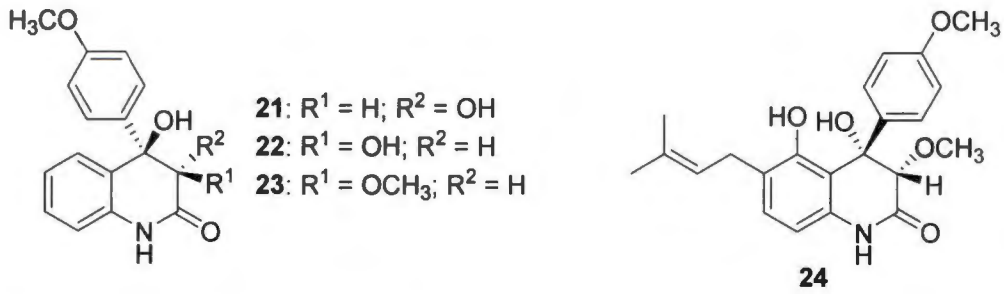


Fig. 15.The structures of compounds **21–24**.

5. METABOLISM OF 3,4-DIHYDRO-2(1*H*)-QUINOLINONES

In drug design, metabolism is an important consideration since this may, to a large extent, determine the bioavailability and toxicity profiles of a compound. Some knowledge of the metabolic transformation of 3,4-dihydro-2(1*H*)-quinolinone may be obtained from studies with drugs containing this moiety. For example, carteolol is converted, with species-specific differences, almost exclusively and to a relatively small extent into 8-hydroxycarteolol, a reaction catalysed by cytochrome P450 (CYP) 2D6 (Fig. 16) [7,36]. Glucuronides and oxidative O-dealkylation of carteolol and 8-hydroxycarteolol are also possible [37,38]. Cilostazol, in turn, undergoes hydroxylation to yield OPC-13326 as predominant metabolite and OPC-13217 as second most predominant metabolite (Fig. 17). These transformations are catalysed by CYP3A4 and CYP3A5, respectively [39].

Metabolism of aripiprazole occurs via CYP3A4 and CYP2D6 to yield dehydroaripiprazole (OPC-14857) as principal metabolite (Fig. 18) [40,41]. This transformation possibly occurs via dehydration of the 4-hydroxy metabolite. Vesnarinone, in turn, is metabolised by CYP2E1 and CYP3A4 to its primary metabolite OPC-18692 (Fig. 19) [42]. In this instance, metabolism occurs in the side chain and not the 3,4-dihydro-2(1*H*)-quinolinone moiety of vesnarinone[43]. From these examples, it may be concluded that the principal metabolic reaction of the 3,4-dihydro-2(1*H*)-quinolinone moiety is aliphatic hydroxylation on the C4 position, which may be followed by dehydration to yield the 2-oxoquinoline metabolite. Aromatic hydroxylation of the phenyl ring also is a possibility, which may be followed by phase 2 reactions such as glucuronidation.

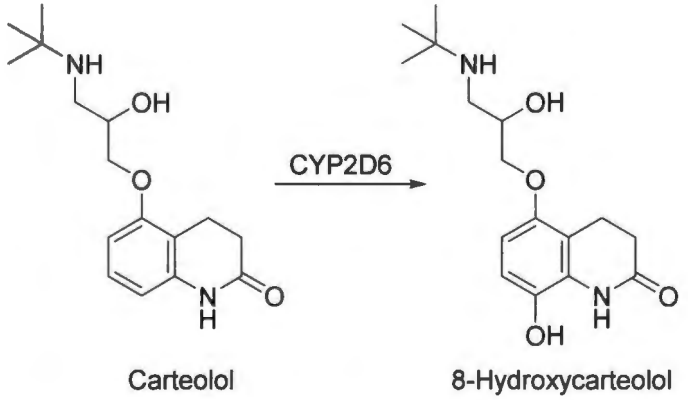


Fig. 16.The metabolism of carteolol.

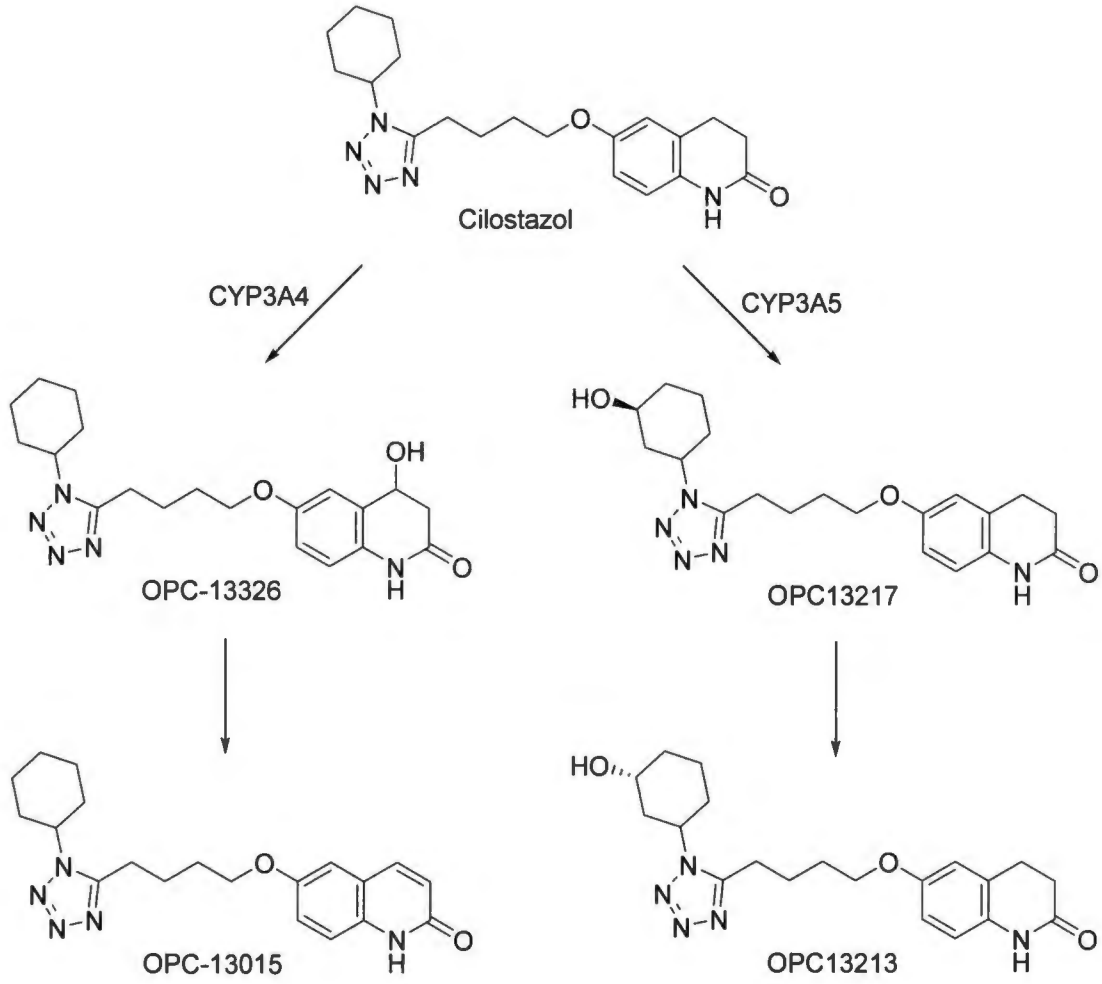


Fig. 17.The metabolism of cilostazol.

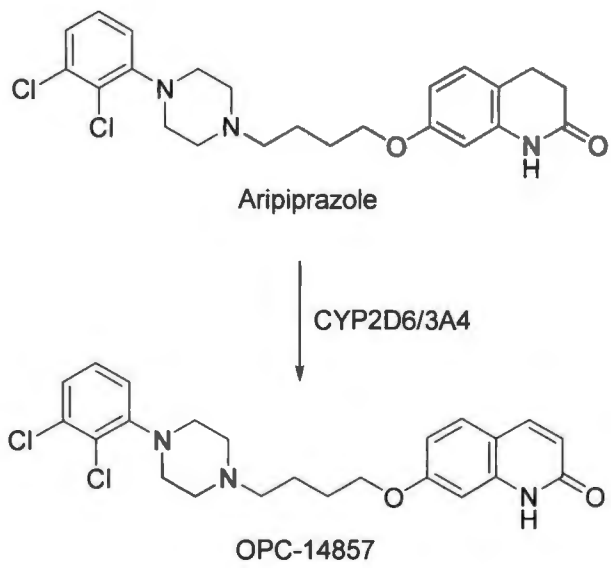


Fig. 18.The metabolism of aripiprazole to its major metabolite, dehydroaripiprazole (OPC-14857).

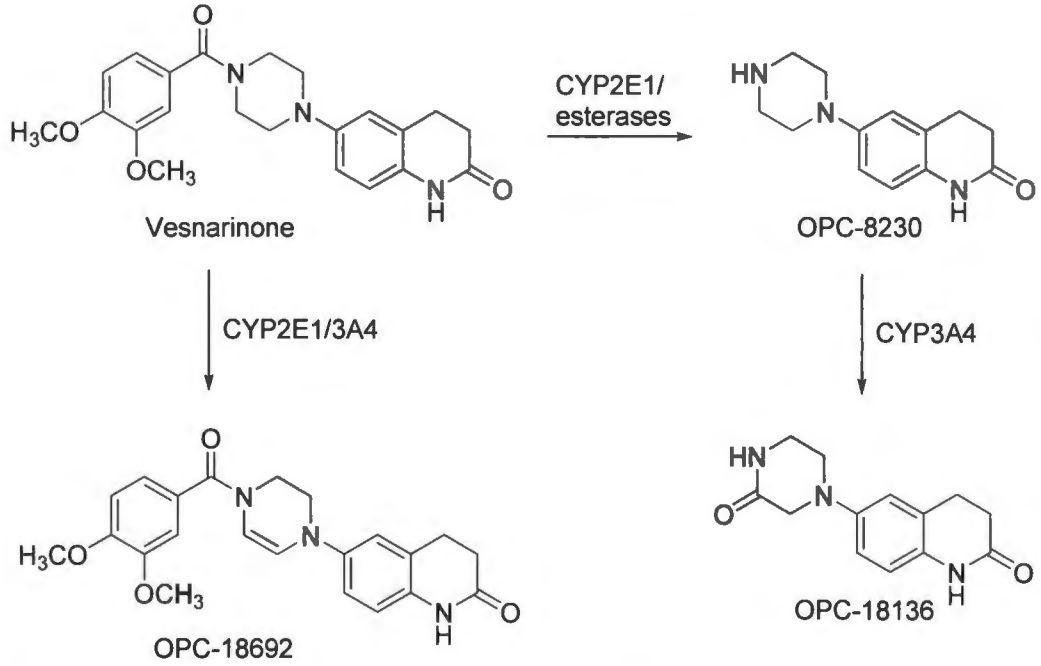


Fig. 19.The metabolism of vesnarinone to its major metabolite, OPC-18692.

6. CONCLUSION

This review shows that the 3,4-dihydro-2(1*H*)-quinolinone moiety is present in a number of pharmacologically active compounds, acting in the peripheral tissues as well as the central nervous system. Although it is not nearly as abundant in drug structures as many other scaffolds, 3,4-dihydro-2(1*H*)-quinolinone most likely would be an appropriate choice for many drug design projects. In this respect, an analysis of the metabolic routes of selected 3,4-dihydro-2(1*H*)-quinolinones does not alert to any potential liabilities. The 3,4-dihydro-2(1*H*)-quinolinone moiety has the distinct advantage of many points of attachment for functional groups and side chains, and with substitution on C3 and C4 chiral compounds can be generated. It may thus be concluded that the 3,4-dihydro-2(1*H*)-quinolinone moiety is a versatile and potentially useful scaffold in the medicinal chemist's toolkit.

CONFLICT OF INTEREST

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

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CHAPTER 4

MANUSCRIPT B

Article submitted to Drug Research:

C6- and C7-Substituted 3,4-dihydro-2(1*H*)-quinolinones as inhibitors of monoamine oxidase.

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 L. Meiring.
- 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 L. Meiring's Ph.D thesis.

C6- and C7-Substituted 3,4-dihydro-2(1*H*)-quinolinones as inhibitors of monoamine oxidase

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Running title: Quinolinones as inhibitors of MAO

Abstract

Purpose: Monoamine oxidase (MAO) inhibitors are considered to be useful therapeutic agents and isoform specific inhibitors are employed for the treatment of depression and Parkinson's disease. MAO inhibitors are also under investigation for the treatment of disorders ranging from Alzheimer's disease, prostate cancer and certain cardiomyopathies. While a number of irreversible MAO inhibitors are available in the clinic, reversible inhibitors, particularly of the MAO-B isoform are still being developed. Based on our interest in discovering reversible inhibitors with specificity for MAO-B, we have recently reported that, among a series of ten 3,4-dihydro-2(1*H*)-quinolinone derivatives, are high potency MAO-B inhibitors, with a number of homologues displaying good selectivities for MAO-B over the MAO-A isoform.

Methods and findings: To expand on these promising findings and to derive structure-activity relationships, the current study synthesizes a series of fourteen 3,4-dihydro-2(1*H*)-quinolinone derivatives. An evaluation of their MAO inhibition properties shows that all derivatives are MAO-B specific with the most potent inhibitor (**3a**) displaying an IC_{50} value of 0.0014 μM. Selectivities for MAO-B ranged from 99 to 40,000-fold.

Conclusions: It may thus be concluded that substitution of 3,4-dihydro-2(1*H*)-quinolinone on C6 and C7 with a variety of side chains yields highly potent and selective MAO-B inhibitors, compounds with existing and prospective therapeutic applications.

Keywords: monoamine oxidase, MAO, reversible inhibition, 3,4-dihydro-2(1*H*)-quinolinone, structure-activity relationship.

Introduction

The monoamine oxidases (MAOs) are key metabolic enzymes that are expressed in most mammalian tissues. The two isoforms, MAO-A and MAO-B, metabolize neurotransmitter amines such as serotonin, noradrenaline, adrenaline and dopamine, thereby terminating their physiological actions in the peripheral tissues and central nervous system [1]. Since the MAOs modulate the levels of key neurotransmitters, inhibitors of these enzymes have been used in the clinic for the treatment of neuropsychiatric and neurodegenerative disorders such as depression and Parkinson's disease [1]. In the central nervous system, serotonin is mostly metabolized by the MAO-A isoform, and MAO-A inhibitors are considered effective treatment of major depression [2,3]. MAO-B inhibitors are used for the treatment of Parkinson's disease where they prevent the metabolic degradation (by MAO-B) of dopamine in the brain. In this regard, MAO-B inhibitors are often combined with L-dopa, the direct metabolic precursor of dopamine and treatment of choice [4–6]. MAO-B inhibitors thus enhance the therapeutic efficacy of L-dopa and allow for a reduction of the effective L-dopa dosage.

The MAOs also are metabolic barriers, preventing dietary amines from entering the systemic circulation and brain. Thus intestinal (and peripheral) MAO-A metabolizes the sympathomimetic amine, tyramine (present in cheese, wine etc.), and prevents it from entering the systemic circulation in excessive amounts, which could lead to a potentially severe rise in blood pressure. This response, termed the "cheese effect", is often observed when irreversible MAO-A inhibitors are combined with tyramine-containing food, and imposes the restrictive use of these drugs in the clinic [7–9]. MAO-B in the brain microvasculature, in turn, represents a metabolic barrier for β-phenethylamine entry into the brain.

β-Phenethylamine is a false neurotransmitter which releases neuronal dopamine and inhibits its active uptake [10,11]. MAO-B inhibitors drastically increase the brain levels of β-phenethylamine and the resulting enhancement of extracellular dopamine concentrations may, at least in part, be responsible for the symptomatic benefit of these drugs in Parkinson's disease [12].

Paradoxically, the MAOs may also activate xenobiotics to yield metabolites which may be extremely harmful, as exemplified by the activation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) by MAO-B to yield 1-methyl-4-phenylpyridinium (MPP^+), a compound that induces a parkinsonian syndrome in experimental animals and humans [13].

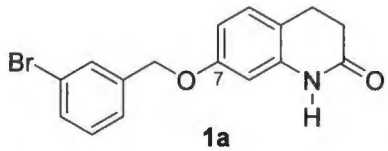
The by-products of the MAO catalytic cycle also is of pharmacological and toxicological interest. Hydrogen peroxide and aldehyde intermediates formed by the MAOs may be injurious if not effectively cleared. In the brain these species may damage neuronal cells and contribute to neurodegeneration in disorders such as Parkinson's disease [14,15]. The MAO-B isoform seems to be of relevance in this regard since MAO-B activity increases as the human brain ages, while MAO-A activity remains largely unchanged [16]. Based on these considerations it has been suggested that inhibitors of MAO-B may be neuroprotective in age-related diseases such as Parkinson's disease. Hydrogen peroxide formed by MAO-A in the heart, in turn, has been implicated in age-related cardiac cellular degeneration in rats [17], thus establishing a rationale for MAO-A inhibitors as treatment for certain cardiomyopathies. Recently the role of MAO-A inhibitors in cancer treatment has been investigated. MAO-A levels are found to be elevated in certain types of cancer tissue such as prostate cancer, and MAO-A inhibition may, in synergism with survivin suppressants, inhibit cancer cell growth, migration and invasion [18,19].

Based on the pharmacological importance of the MAOs, the discovery of potent and isoform specific inhibitors are pursued by a number of research groups [20–22]. As discussed above, inhibitors specific for the MAO-B isoform are appropriate for Parkinson's disease therapy for the following reasons: (a) MAO-B inhibition conserves central dopamine and enhance dopamine levels after L-dopa therapy, (b) MAO-B inhibition increases the brain levels of β-phenethylamine, which leads to the indirect enhancement of extracellular dopamine concentrations, (c) MAO-B inhibition reduces harmful metabolic by-products of MAO-B in the brain, thereby protecting against neurodegeneration and (d) MAO-B inhibition may prevent the activation of proneurotoxins such as MPTP.

We have recently reported that a series of 3,4-dihydro-2(1*H*)-quinolinone derivatives are high potency MAO-B inhibitors with certain homologues displaying good specificity [23]. For example, the most potent MAO-B inhibitor, 7-(3-bromobenzyloxy)-3,4-dihydro-2(1*H*)-quinolinone (**1a**), exhibits an IC_{50} value of 0.0029 μM with a 2751-fold selectivity for MAO-B over the MAO-A isoform (Fig. 1). Another compound, 7-(3-chlorobenzyloxy)-3,4-dihydro-2(1*H*)-quinolinone (**1b**) inhibits MAO-B with an IC_{50} of 0.0062 μM, while displaying an IC_{50} of >100 μM for MAO-A. Importantly, it was found that substitution on the C7 position of the 3,4-dihydro-2(1*H*)-quinolinone moiety leads to significantly more potent inhibition compared to substitution on C6, and that a benzyloxy substituent on C7 is more favorable than 2-phenylethoxy and 3-phenylpropoxy substitution. Based on the promising potencies and specificities displayed by some of these 3,4-dihydro-2(1*H*)-quinolinones, the present study aims to discover additional homologues with high MAO-B inhibition potencies and specificities, and to further derive structure-activity relationships (SARs).

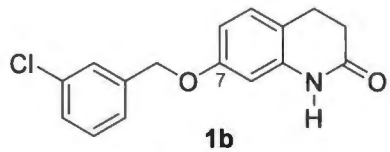
For this purpose, the current study synthesizes a series of fourteen 3,4-dihydro-2(1*H*)-quinolinone derivatives (Table 1).

As shown, substitution with the benzyloxy, phenylethoxy and 2-phenoxyethoxy moieties on both C6 (compounds **2**) and C7 (compounds **3**) were considered.



1a

IC_{50}(MAO-A) = 7.98 μM
IC_{50}(MAO-B) = 0.0029 μM



1b

IC_{50}(MAO-A) >100 μM
IC_{50}(MAO-B) = 0.0062 μM

Fig. 1. The structures of 7-(3-bromobenzyloxy)-3,4-dihydro-2(1*H*)-quinolinone (**1a**) and 7-(3-chlorobenzyloxy)-3,4-dihydro-2(1*H*)-quinolinone (**1b**).

Materials and methods

Chemicals and instrumentation

Reagents and solvents required for the chemistry and biology were obtained from Sigma-Aldrich (St. Louis, MO, USA) and were used without further purification.

Kynuraminedihydrobromide and insect cell microsomes containing recombinant human MAO-A and MAO-B (5 mg protein/mL) were also obtained from Sigma-Aldrich. ^{1}H NMR and ^{13}C NMR spectra were recorded with a BrukerAvance III 600 spectrometer (Karlsruhe, Germany) employing DMSO-d_6 as solvent. The chemical shifts are given in parts per million (δ) and were referenced to the residual solvent signal. The spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets), t (triplet) or m (multiplet). High resolution mass spectra (HRMS) were recorded with a BrukermicrOTOF-Q II mass spectrometer functioning in atmospheric-pressure chemical ionization (APCI) mode (positive mode). Melting points (mp) were measured with a Buchi B-545 melting point apparatus (BüchiLabortechnik, Flawil, Switzerland) and are uncorrected.

The progress of reactions was monitored with silica gel 60 aluminum coated TLC sheets (Merck, Darmstadt, Germany).

For this purpose the mobile phase consisted of ethyl acetate, and the developed sheets were visualized under an UV-lamp at a wavelength of 254 nm. Fluorescence spectrophotometry was carried out with a Varian Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, Santa Clara, USA) equipped with a microplate reader.

Chemistry

*Procedure for the synthesis of 3,4-dihydro-2(1H)-quinolinone derivatives **2a–g** and **3a–g***

The 3,4-dihydro-2(1*H*)-quinolinone derivatives were prepared according to a previously reported protocol from the key reagents, 6-hydroxy-3,4-dihydro-2(1*H*)-quinolinone (**4**) and 7-hydroxy-3,4-dihydro-2(1*H*)-quinolinone (**5**), which are commercially available (Sigma-Aldrich) [23]. Reagents **4** or **5** (1.50 mmol) was suspended in ethanol (6 mL) containing KOH (1.66 mmol), and the required alkyl bromide (1.50 mmol) was added. The reaction was heated at reflux for 5 h and, upon completion (as judged by TLC), was poured into aqueous NaOH (1%). The precipitate that formed was collected by filtration and crystallized from ethanol.

*6-(4-Chlorobenzyloxy)-3,4-dihydro-2(1H)-quinolinone (**2a**)*

The title compound was prepared in a yield of 6%: mp 194.3 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.92 (s, 1H), 7.42 (s, 4H), 6.84 (d, J = 2.5 Hz, 1H), 6.80 – 6.72 (m, 2H), 5.01 (s, 2H), 2.80 (t, J = 7.6 Hz, 2H), 2.38 (t, J = 7.6 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.93, 153.37, 136.39, 132.37, 132.08, 129.47, 128.47, 124.99, 115.85, 114.52, 113.37, 68.67, 30.37, 25.14.APCI-HRMS *m/z*: calcd for $C_{16}H_{15}Cl35NO_2(MH^+)$, 288.0786, found 288.0776.

*6-(4-Bromobenzyloxy)-3,4-dihydro-2(1H)-quinolinone (**2b**)*

The title compound was prepared in a yield of 4%: mp 185.2 – 212.7 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.92 (s, 1H), 7.56 (d, J = 8.3 Hz, 2H), 7.37 (d, J = 8.6 Hz, 2H), 6.84 (d, J = 2.5 Hz, 1H), 6.80 – 6.72 (m, 2H), 5.00 (s, 2H), 2.80 (t, J = 7.5 Hz, 2H), 2.38 (t, J = 7.5 Hz, 2H).^{13}C NMR (151 MHz, DMSO-d_6) δ 169.91, 153.34, 136.82, 132.08, 131.38, 129.77, 124.98, 120.89, 115.84, 114.51, 113.37, 68.68, 30.36, 25.13.APCI-HRMS *m/z*: calcd for $C_{16}H_{15}BrNO_2$ (MH^+), 332.0281, found 332.0260.

*6-(3-Methylbenzyloxy)-3,4-dihydro-2(1H)-quinolinone (**2c**)*

The title compound was prepared in a yield of 44%: mp 163.3 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.92 (s, 1H), 7.25 (t, J = 7.5 Hz, 1H), 7.22 (s, 1H), 7.19 (d, J = 7.6 Hz, 1H), 7.11 (d, J = 7.4 Hz, 1H), 6.85 (d, J = 2.5 Hz, 1H), 6.80 – 6.73 (m, 2H), 4.97 (s, 2H), 2.81 (t, J = 7.5 Hz, 2H), 2.38 (t, J = 7.4 Hz, 2H), 2.30 (s, 3H).^{13}C NMR (151 MHz, DMSO-d_6) δ 169.79, 153.58, 137.55, 137.20, 131.92, 128.39, 128.31, 128.18, 124.88, 124.72, 115.76, 114.38, 113.24, 69.50, 30.35, 25.11, 21.02.APCI-HRMS *m/z*: calcd for $C_{17}H_{18}NO_2$ (MH^+), 268.1332, found 268.1357.

*6-(4-Methylbenzyloxy)-3,4-dihydro-2(1H)-quinolinone (**2d**)*

The title compound was prepared in a yield of 37%: mp 173.3 – 177 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.91 (s, 1H), 7.29 (d, J = 7.9 Hz, 2H), 7.17 (d, J = 7.8 Hz, 2H), 6.84 (d, J = 2.5 Hz, 1H), 6.79 – 6.72 (m, 2H), 4.96 (s, 2H), 2.80 (t, J = 7.5 Hz, 2H), 2.38 (t, J = 7.4 Hz, 2H), 2.28 (s, 3H).^{13}C NMR (151 MHz, DMSO-d_6) δ 169.79, 153.55, 136.97, 134.23, 131.88, 128.95, 127.72, 124.85, 115.74, 114.41, 113.29, 69.36, 30.35, 25.11, 20.79.APCI-HRMS *m/z*: calcd for $C_{17}H_{18}NO_2$ (MH^+), 268.1332, found 268.1324.

*6-[2-(3-Methylphenyl)ethoxy]-3,4-dihydro-2(1H)-quinolinone (**2e**)*

The title compound was prepared in a yield of 6%: mp 135.6 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.90 (s, 1H), 7.17 (t, J = 7.5 Hz, 1H), 7.10 (s, 1H), 7.07 (d, J = 7.7 Hz, 1H), 7.01 (d, J = 7.5 Hz, 1H), 6.79 – 6.72 (m, 2H), 6.70 (dd, J = 8.6, 2.7 Hz, 1H), 4.08 (t, J = 6.9 Hz, 2H), 2.94 (t, J = 6.9 Hz, 2H), 2.80 (t, J = 7.6 Hz, 2H), 2.37 (t, J = 7.6 Hz, 2H), 2.27 (s, 3H).^{13}C NMR (151 MHz, DMSO-d_6) δ 169.84, 153.63, 138.31, 137.36, 131.76, 129.61, 128.23, 126.92, 126.01, 124.91, 115.82, 114.07, 113.01, 68.48, 35.01, 30.38, 25.09, 21.05.APCI-HRMS *m/z*: calcd for $C_{18}H_{20}NO_2$ (MH^+), 282.1489, found 282.1473.

*6-[2-(4-Methylphenyl)ethoxy]-3,4-dihydro-2(1H)-quinolinone (**2f**)*

The title compound was prepared in a yield of 19%: mp 152.1 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.90 (s, 1H), 7.17 (d, J = 7.8 Hz, 2H), 7.09 (d, J = 7.7 Hz, 2H), 6.77 – 6.71 (m, 2H), 6.69 (dd, J = 8.6, 2.7 Hz, 1H), 4.06 (t, J = 6.9 Hz, 2H), 2.92 (t, J = 6.9 Hz, 2H), 2.79 (t, J = 7.6 Hz, 2H), 2.37 (t, J = 7.4 Hz, 2H), 2.25 (s, 3H).^{13}C NMR (151 MHz, DMSO-d_6) δ 169.90, 153.67, 135.35, 135.25, 131.75, 128.93, 128.86, 124.94, 115.85, 114.08, 113.00, 68.57, 34.66, 30.39, 25.10, 20.71.APCI-HRMS *m/z*: calcd for $C_{18}H_{20}NO_2$ (MH^+), 282.1489, found 282.1484.

*6-[2-Phenoxyethoxy]-3,4-dihydro-2(1H)-quinolinone (**2g**)*

The title compound was prepared in a yield of 6%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.93 (s, 1H), 7.28 (t, J = 7.3 Hz, 2H), 7.01 – 6.88 (m, 3H), 6.82 (s, 1H), 6.79 – 6.72 (m, 2H), 4.29 – 4.24 (m, 2H), 4.24 – 4.18 (m, 2H), 2.81 (t, J = 7.7 Hz, 2H), 2.39 (t, J = 7.7 Hz, 2H).^{13}C NMR (151 MHz, DMSO-d_6) δ 169.85, 158.32, 153.51, 131.97, 129.58, 124.95, 120.76, 115.84, 114.49, 114.13, 113.12, 66.61, 66.24, 30.37, 25.12.APCI-HRMS *m/z*: calcd for $C_{17}H_{18}NO_3$ (MH^+), 284.1281, found 284.1273.

*7-(4-Chlorobenzyloxy)-3,4-dihydro-2(1H)-quinolinone (**3a**)*

The title compound was prepared in a yield of 56%: mp 162.4 – 163.3 °C (ethanol). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.06 (s, 1H), 7.33 (s, 4H), 7.02 (d, *J* = 8.2 Hz, 1H), 6.55 (dd, *J* = 8.3, 2.5 Hz, 1H), 6.45 (d, *J* = 2.4 Hz, 1H), 4.97 (s, 2H), 2.87 (t, *J* = 7.5 Hz, 2H), 2.60 (t, *J* = 7.6 Hz, 2H).^{13}C NMR (151 MHz, $CDCl_3$) δ 172.28, 158.04, 138.27, 135.25, 133.73, 128.73, 128.71, 128.62, 116.21, 108.94, 102.63, 69.30, 30.95, 24.51.APCI-HRMS *m/z*: calcd for $C_{16}H_{15}ClNO_2$ (MH^+), 288.0786, found 288.0790.

*7-(4-Bromobenzyloxy)-3,4-dihydro-2(1H)-quinolinone (**3b**)*

The title compound was prepared in a yield of 25%: mp 193.4 – 194.5 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.01 (s, 1H), 7.56 (d, *J* = 8.2 Hz, 2H), 7.36 (d, *J* = 8.3 Hz, 2H), 7.03 (d, *J* = 8.3 Hz, 1H), 6.53 (dd, *J* = 8.3, 2.5 Hz, 1H), 6.48 (d, *J* = 2.5 Hz, 1H), 5.00 (s, 2H), 2.76 (t, *J* = 7.5 Hz, 2H), 2.39 (t, *J* = 7.5 Hz, 2H).^{13}C NMR (151 MHz, DMSO-d_6) δ 170.39, 157.35, 139.26, 136.58, 131.38, 129.75, 128.48, 120.93, 116.06, 107.85, 102.18, 68.40, 30.73, 24.04.APCI-HRMS *m/z*: calcd for $C_{16}H_{15}BrNO_2$ (MH^+), 332.0281, found 332.0259.

*7-(3-Methylbenzyloxy)-3,4-dihydro-2(1H)-quinolinone (**3c**)*

The title compound was prepared in a yield of 39%: mp 151.3 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.01 (s, 1H), 7.25 (t, *J* = 7.5 Hz, 1H), 7.22 (s, 1H), 7.19 (d, *J* = 7.7 Hz, 1H), 7.12 (d, *J* = 7.4 Hz, 1H), 7.03 (d, *J* = 8.2 Hz, 1H), 6.54 (dd, *J* = 8.2, 2.5 Hz, 1H), 6.50 (d, *J* = 2.5 Hz, 1H), 4.97 (s, 2H), 2.76 (t, *J* = 7.6 Hz, 2H), 2.39 (t, *J* = 7.3 Hz, 2H), 2.30 (s, 3H).^{13}C NMR (151 MHz, DMSO-d_6) δ 170.33, 157.59, 139.22, 137.59, 136.97, 128.45, 128.42, 128.34, 128.20, 124.74, 115.83, 107.80, 102.08, 69.22, 30.73, 24.02, 21.01.APCI-HRMS *m/z*: calcd for $C_{17}H_{18}NO_2$ (MH^+), 268.1332, found 268.1328.

*7-(4-Methylbenzyloxy)-3,4-dihydro-2(1H)-quinolinone (**3d**)*

The title compound was prepared in a yield of 7%: mp 152.1 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.00 (s, 1H), 7.29 (d, J = 8.0 Hz, 2H), 7.17 (d, J = 7.8 Hz, 2H), 7.02 (d, J = 8.2 Hz, 1H), 6.53 (dd, J = 8.3, 2.5 Hz, 1H), 6.49 (d, J = 2.5 Hz, 1H), 4.96 (s, 2H), 2.75 (t, J = 7.5 Hz, 2H), 2.39 (t, J = 7.5 Hz, 2H), 2.28 (s, 3H).^{13}C NMR (151 MHz, DMSO-d_6) δ 170.32, 157.56, 139.21, 137.04, 134.01, 128.97, 128.40, 127.74, 115.79, 107.85, 102.12, 69.08, 30.73, 24.01, 20.78.APCI-HRMS *m/z*: calcd for $C_{17}H_{18}NO_2$ (MH^+), 268.1332, found 268.1318.

*7-[2-(4-Methylphenyl)ethoxy]-3,4-dihydro-2(1H)-quinolinone (**3e**)*

The title compound was prepared in a yield of 9%: mp 160 – 161.8 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.96 (s, 1H), 7.17 (d, J = 7.8 Hz, 2H), 7.10 (d, J = 7.7 Hz, 2H), 7.02 (d, J = 8.3 Hz, 1H), 6.47 (dd, J = 8.3, 2.5 Hz, 1H), 6.41 (d, J = 2.5 Hz, 1H), 4.05 (t, J = 6.9 Hz, 2H), 2.93 (t, J = 6.9 Hz, 2H), 2.75 (t, J = 7.5 Hz, 2H), 2.39 (t, J = 7.5 Hz, 2H), 2.25 (s, 3H).^{13}C NMR (151 MHz, DMSO-d_6) δ 170.34, 157.63, 139.20, 135.26, 135.18, 128.91, 128.82, 128.47, 115.62, 107.62, 101.65, 68.27, 34.47, 30.75, 24.00, 20.68.APCI-HRMS *m/z*: calcd for $C_{18}H_{20}NO_2$ (MH^+), 282.1489, found 282.1480.

*7-[2-Phenoxyethoxy]-3,4-dihydro-2(1H)-quinolinone (**3f**)*

The title compound was prepared in a yield of 5%: mp 180 – 186.5 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.01 (s, 1H), 7.28 (t, J = 7.9 Hz, 2H), 7.05 (d, J = 8.3 Hz, 1H), 7.00 – 6.89 (m, 3H), 6.53 (dd, J = 8.3, 2.5 Hz, 1H), 6.47 (d, J = 2.5 Hz, 1H), 4.31 – 4.24 (m, 2H), 4.24 – 4.16 (m, 2H), 2.77 (t, J = 7.5 Hz, 2H), 2.40 (t, J = 7.6 Hz, 2H).^{13}C NMR (151 MHz, DMSO-d_6) δ 170.41, 158.32, 157.59, 139.28, 129.61, 128.55, 120.82, 115.98, 114.52, 107.55, 101.91, 66.40, 66.17, 30.76, 24.04.APCI-HRMS *m/z*: calcd for $C_{17}H_{18}NO_3$ (MH^+), 284.1281, found 284.1254.

*7-[2-(4-Chlorophenoxy)ethoxy]-3,4-dihydro-2(1H)-quinolinone (**3g**)*

The title compound was prepared in a yield of 25%: mp 153.2 °C (ethanol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.01 (s, 1H), 7.32 (d, J = 9.0 Hz, 2H), 7.05 (d, J = 8.2 Hz, 1H), 7.00 (d, J = 9.0 Hz, 2H), 6.52 (dd, J = 8.3, 2.6 Hz, 1H), 6.46 (d, J = 2.5 Hz, 1H), 4.31 – 4.24 (m, 2H), 4.23 – 4.16 (m, 2H), 2.77 (t, J = 7.5 Hz, 2H), 2.40 (t, J = 7.6 Hz, 2H).^{13}C NMR (151 MHz, DMSO-d_6) δ 170.32, 157.51, 157.18, 139.26, 129.29, 128.49, 124.46, 116.30, 115.97, 107.49, 101.87, 66.67, 66.26, 30.72, 24.01.APCI-HRMS *m/z*: calcd for $C_{17}H_{17}ClNO_3$ (MH^+), 318.0891, found 318.0875.

Enzymology

Procedure for the measurement of IC_{50} values for the inhibition of MAO

The protocol for the measurement of IC_{50} values for the inhibition of the MAOs has been reported in literature [24,25]. In short, the enzyme reactions were carried out to a volume of 200 µL in 96-well microtiter plates and contained kynuramine (50 µM), the test inhibitors (0.003–100 µM), and potassium phosphate buffer (pH 7.4, 100 mM).

Each reaction also contained 4% DMSO as co-solvent, and control reactions, performed in the absence of inhibitor, were included for each inhibitor evaluated. The enzyme reactions were initiated with the addition of recombinant human MAO-A (0.0075 mg protein/mL) or MAO-B (0.015 mg protein/mL) and incubated for 20 min at 37 °C. The reactions were subsequently terminated with the addition of 80 µL sodium hydroxide (2 N) and the oxidation product of kynuramine, 4-hydroxyquinoline, was quantified by fluorescence spectrophotometry (λ_{ex} = 310; λ_{em} = 400 nm) [26].

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

Procedure for examining the reversibility of inhibition by dialysis

The protocol for investigating the reversibility of MAO inhibition by dialysis has been reported in literature [24,25]. Recombinant human MAO-B (0.03 mg protein/mL) was combined with the test inhibitor (**2b**) and the mixture (0.8 mL) was preincubated for 15 min at 37 °C. These preincubations were carried out in potassium phosphate buffer (100 mM, pH 7.4) containing 5% sucrose.

Employing this buffer also as dialysis buffer, the mixtures were subsequently dialyzed at 4 °C. For this purpose 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 used (Thermo Scientific, Waltham, MA, USA). The dialysis buffer was replaced with fresh buffer at 3 h and 7 h after the start of dialysis. After 24 h of dialysis, the reactions were diluted twofold with the addition of kynuramine to give a final substrate concentration of 50 μM and a final inhibitor concentration of 2 × IC_{50}.

The reactions (500 μL) were incubated for 20 min at 37 °C, and were subsequently terminated with the addition of NaOH (400 μL of 2 N) and 1000 μL water. The fluorescence emission of 4-hydroxyquinoline in these reactions was measured as described above, employing a 3.5 mL quartz cuvette (pathlength 10 × 10 mm). As negative control, MAO-B was preincubated and dialyzed in the absence of inhibitor. As positive control, MAO-B was preincubated and dialyzed in the presence of a concentration equal to 4 × IC_{50} of the irreversible inhibitor, (R)-deprenyl (IC_{50} = 0.079 μM) [27]. Also included in the study were undialyzed mixtures of MAO-B and **2b**, which were maintained at 4 °C for 24 h and diluted and assayed as above. All reactions were carried out in triplicate and the residual enzyme rates are given as mean ± SD.

Procedure for the construction of Lineweaver-Burk plots

The protocol for constructing Lineweaver-Burk plots has been reported in literature [24,25]. For the inhibition of MAO-B by **2b**, 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 the following inhibitor concentrations:¼ × IC_{50}, ½ × IC_{50}, ¾ × IC_{50}, 1 × IC_{50} and 1¼ × IC_{50}. For each plot, kynuramine served as substrate at eight different concentrations (15–250 μM).

After addition of the inhibitor and substrate, the enzymatic reactions (500 μL) were initiated with the addition of MAO-B (0.015 mg/mL). The enzyme reactions and activity measurements were subsequently carried out as described above for the dialysis experiments and the K_i value was estimated by global (shared) fitting of the inhibition data to the Michaelis-Menten equation using the Prism 5 software package. The K_i value may also be estimated from a plot of the slopes of the Lineweaver-Burke plots versus inhibitor concentration (x-axis intercept equals $-K_i$).

Computer modeling

Procedure for docking with CDOCKER

The procedure for docking with CDOCKER has been reported in literature [24,28]. For the docking studies, the Windows-based Discovery Studio 3.1 software package (Accelrys, San Diego, CA, USA) was used and the reported X-ray crystal structures of human MAO-A (PDB code 2Z5X) and human MAO-B (PDB code 2V5Z) served as protein models [29,30]. The protein models were prepared by firstly calculating the pKa values and protonation states of the ionizable amino acids and subsequently adding hydrogen atoms to the models at pH 7.4. The FAD was set to the oxidized state, fixed atom constraints were applied to the protein backbones and the models were energy minimized using the Smart Minimizer algorithm. With the exception of HOH 710, 718 and 739 in MAO-A, and HOH 1155, 1170 and 1351 in the A-chain of MAO-B, all waters and the co-crystallized ligands were removed and docking was carried out with the CDOCKER algorithm. Ten random conformations were generated for each ligand, the heating target temperature was set to 700 K and full potential mode was used.

The docking solutions were finally refined using in situ ligand minimization with the Smart Minimizer algorithm. The illustrations were prepared with PyMol (Schrödinger, New York, NY, USA) [31].

Results and Discussion

Chemistry

The 3,4-dihydro-2(1*H*)-quinolinone derivatives **2a–g** and **3a–g** were synthesized according to the reported protocol (Fig.2) [32,23]. 6-Hydroxy-3,4-dihydro-2(1*H*)-quinolinone (**4**) or 7-hydroxy-3,4-dihydro-2(1*H*)-quinolinone (**5**), which are commercially available, were suspended in ethanol and treated with the required arylalkyl bromide.

Potassium hydroxide served as base. The reaction mixture was heated at reflux for 5 h and poured into aqueous sodium hydroxide (1%). A precipitate was obtained which was recrystallized from ethanol to yield the target 3,4-dihydro-2(1*H*)-quinolinone derivatives in yields of 4–56%. The structures of **2a–g** and **3a–g** were characterized by ^{1}H NMR, ^{13}C NMR and mass spectrometry as given in the supplementary material. On the ^{1}H NMR spectra, two triplets at approximately 2.8 and 2.4 ppm represent the methylene protons of the 3,4-dihydro-2(1*H*)-quinolinone moiety, while the NH signals are present at 9–10 ppm. On the ^{13}C NMR spectra, the signal of the carbonyl carbon is found at approximately 170 ppm. For **2a–g** the proton on C5 are represented by doublets at approximately 6.85 ppm, while the proton on C7 are represented by a signal at approximately 6.70 ppm (doublet of doublets). For **3a–g** the proton on C8 are represented by doublets at approximately 6.45 ppm, while the protons on C5 and C6 are represented by signals at approximately 7.02 ppm (doublet) and 6.55 ppm (doublet of doublets).

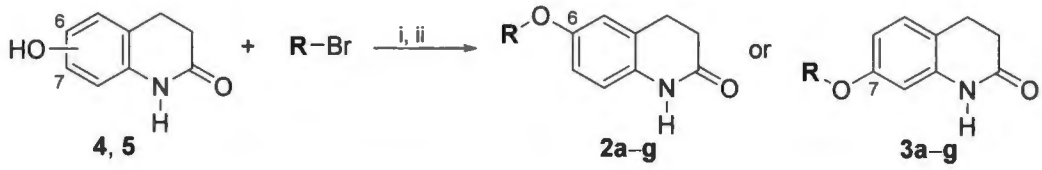


Fig.2. The synthesis of 3,4-dihydro-2(1*H*)-quinolinone derivatives **2a–g** and **3a–g**. Reagents and conditions: (i) KOH, ethanol, reflux; (ii) recrystallization from ethanol.

IC_{50} values and SARs for MAO inhibition

The inhibition of the human MAOs by derivatives **2a–g** and **3a–g** was examined under identical experimental conditions as those of the reported 3,4-dihydro-2(1*H*)-quinolinones [23]. This allows for the direct comparison of IC_{50} values recorded in this study with those cited in the literature report.

IC_{50} values were measured by recording the catalytic rates of human MAO-A and MAO-B in the presence of different concentrations of the test inhibitors spanning at least three orders of magnitude (0.0003–100 μM). Sigmoidal dose–response curves (catalytic rate versus logarithm of inhibitor concentration) were constructed in triplicate from which the IC_{50}values were estimated. The recombinant human MAOs served as enzyme sources and kynuramine was used as substrate for both isoforms. Kynuramine is oxidized by the MAOs to yield 4-hydroxyquinoline, a metabolite which may (after alkalization) be measured at the endpoint of the enzymatic reaction using fluorescence spectrophotometry. Examples of sigmoidal dose–response curves obtained in this study are shown in fig. 3. As shown in Table 1, substitution on both C6 and C7 was considered in order to determine if substitution on C7 of the 3,4-dihydro-2(1*H*)-quinolinone moiety in all instances lead to significantly more potent inhibition compared to substitution on C6, as proposed in a previous study [23].

This study also explores the effect of *meta* and *para* substitution of the benzyloxy side chain with bromine, chlorine and the methyl group for comparison with compounds **1a** and **1b**, previously reported [23]. The effect of the 2-phenoxyethoxy moiety and methyl substituted 2-phenylethoxy side chains were also investigated. The 2-phenoxyethoxy moiety, in particular has been shown to enhance the MAO-B inhibition potency of caffeine derived inhibitors [33].

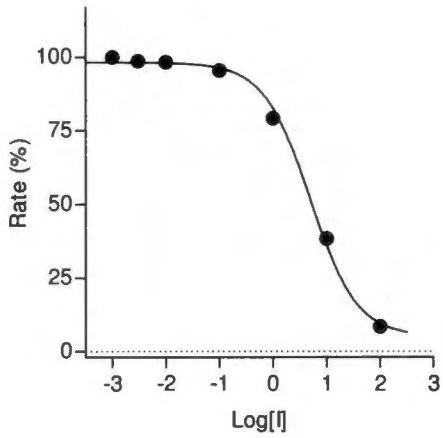
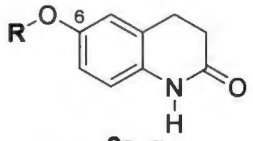
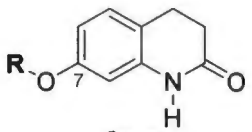


Fig. 3. Sigmoidal dose–response curves for the inhibition of human MAO-B by **2d**.

Table 1. The IC_{50} values for the inhibition of recombinant human MAO-A and MAO-B by compounds **2a–g** and **3a–g**.



2a–g



3a–g

		IC_{50} (μM)[a]		
	R	MAO-A	MAO-B	SI[b]
2a	4-$ClC_6H_4CH_2$–	56.4 ± 20.0	0.017 ± 0.0046	3318
2b	4-$BrC_6H_4CH_2$–	>100[c]	0.0054 ± 0.0011	>18519
2c	3-$CH_3C_6H_4CH_2$–	31.0 ± 2.48	0.313 ± 0.028	99
2d	4-$CH_3C_6H_4CH_2$–	>100[c]	0.098 ± 0.024	>1020
2e	3-$CH_3C_6H_4(CH_2)_2$–	39.3 ± 2.47	0.198 ± 0.110	198
2f	4-$CH_3C_6H_4(CH_2)_2$–	>100[c]	0.025 ± 0.0087	>4000
2g	$C_6H_5O(CH_2)_2$–	>100[c]	0.102 ± 0.0019	>980
3a	4-$ClC_6H_4CH_2$–	28.9 ± 4.22	0.0014 ± 0.0003	20643
3b	4-$BrC_6H_4CH_2$–	>100[c]	0.0025 ± 0.0007	>40000
3c	3-$CH_3C_6H_4CH_2$–	26.4 ± 8.20	0.011 ± 0.00065	2400
3d	4-$CH_3C_6H_4CH_2$–	44.1 ± 5.25	0.018 ± 0.0083	2450
3e	4-$CH_3C_6H_4(CH_2)_2$–	34.5 ± 13.1	0.047 ± 0.021	734
3f	$C_6H_5O(CH_2)_2$–	>100[c]	0.047 ± 0.0080	>2128
3g	4-$ClC_6H_4O(CH_2)_2$–	>100[c]	0.113 ± 0.065	>885

[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 IC_{50}(MAO-A)/IC_{50}(MAO-B).

[c] No inhibition observed at a concentration of 100 μM.

The IC_{50} values show that among both the C6- and C7-substituted 3,4-dihydro-2(1*H*)-quinolinones high potency MAO-B inhibitors exist. Most notably among these are **2b**, **3a** and **3b** which display IC_{50} values of <0.006 μM. The MAO-B inhibition potencies of these compounds are comparable to those of **1a** (IC_{50} = 0.0029) and **1b** (IC_{50} = 0.0062), and are significantly more potent than the reference MAO-B inhibitors lazabemide (IC_{50} = 0.091 μM) and safinamide (IC_{50} = 0.048 μM), evaluated under the same experimental conditions [34]. These compounds also display high specificities for the MAO-B enzyme with selectivity index (SI) values >18,519. In this regard, **3b** is particularly isoform specific since it possesses an IC_{50} for MAO-A inhibition of >100 μM. Similar to the 3,4-dihydro-2(1*H*)-quinolinones studied before, **2a–g** and **3a–g** are relatively weak MAO-A inhibitors with IC_{50}>26.4 μM. From the inhibition data, the following SARs may be derived: (a) Similar to the reported study, C7 substitution yields compounds with higher MAO-B inhibition potencies than C6 substitution. For each pair of homologues, the corresponding C7-substituted compounds display more potent inhibition although differences in inhibition potencies are in many instances very small (compare **2b** vs. **3b**; **2d** vs. **3d**; **2g** vs. **3f**). (b) Chlorine and bromine substitution on the *para* position of the benzyloxy ring yields similar MAO-B inhibition than *meta* substitution (compare **1b** vs. **3a**; **1a** vs. **3b**). Methyl substitution on either the *meta* or *para* positions of the benzyloxy ring is, however, less suitable for MAO-B inhibition compared to the halogens (compare **2c/2d** with **2a/2b**; **3c/3d** with **3a/3b**). (c) Substitution with the 2-phenoxyethoxy moiety on both the C6 (**2g**, IC_{50} = 0.102 μM) and C7 (**3f**, IC_{50} = 0.047 μM) positions of the 3,4-dihydro-2(1*H*)-quinolinone yields good potency MAO-B inhibition. These compounds are particularly specific inhibitors since they display IC_{50} values for MAO-A inhibition of >100 μM. Interestingly, chlorine substitution (**3g**, IC_{50} = 0.113 μM) of the phenoxy ring significantly reduces MAO-B inhibition potency compared to the unsubstituted homologue **3f**.

(d) Among the 2-phenylethoxy substituted compounds (**2e**, **2f**, **3e**), **2f** (IC_{50} = 0.025 μM) is the most potent MAO-B inhibitor. The good potency of this compound demonstrates that, with the appropriate substitution pattern, 2-phenylethoxy substitution may yield similar potency MAO-B inhibitors compared to benzyloxy substitution (compare **2f** vs. **2d**).

Reversibility of MAO-B inhibition

Using compound **1a** as model inhibitor, it has been established that 3,4-dihydro-2(1*H*)-quinolinones inhibits MAO-B with a reversible mode of action, although some tight-binding of the inhibitor to the enzyme may occur [23]. Since **1a** is a C7-substituted homologue, the present study investigates the reversibility of MAO-B inhibition by a C6-substituted 3,4-dihydro-2(1*H*)-quinolinone, compound **2b**. Reversibility of inhibition was investigated by dialysis. Compound **2b** (at a concentration of 4 × IC_{50}) was preincubated with MAO-B for 15 min and subsequently dialyzed for 24 h. The incubation mixtures were diluted twofold with the addition of kynuramine and the formation of 4-hydroxyquinoline was measured at the endpoint of the enzyme reaction. From the 4-hydroxyquinoline concentrations the residual activities were calculated. Similar dialysis experiments were carried out in the absence of inhibitor (negative control) and presence of the irreversible MAO-B inhibitor, (R)-deprenyl (positive control). The activity (rate of 4-hydroxyquinoline formation) of the negative control represents 100% residual activity. As third control, the residual MAO-B activity of undialyzed mixtures of MAO-B and **2b** was also recorded. As shown in Fig. 4, following inhibition with **2b**, dialysis restores enzyme activity to 100% of the negative control (100%). Following inhibition with (R)-deprenyl, enzyme activity is not restored by dialysis with the residual activity at only 2.4%. In undialyzed mixtures of MAO-B and **2b**, inhibition persists and the activity is recorded at 67%.

Since dialysis is expected to restore enzyme activity to 100% for reversible inhibitors, it may be concluded that **2b** is a reversible MAO-B inhibitor.

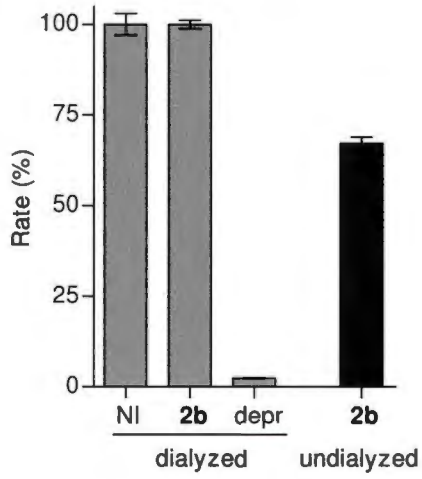


Fig. 4. Compound **2b** inhibits MAO-B with a reversible mode. MAO-B and **2b** (at 4 × IC_{50}) was preincubated for 15 min, dialyzed for 24 h and the residual enzyme activity was measured (**2b**–dialyzed). Similar incubation and dialysis of MAO-B in the absence inhibitor (NI dialyzed) and presence of the irreversible inhibitor, (R)-deprenyl (depr dialyzed), were also carried out. The residual activity of undialyzed mixtures of MAO-B with **2b** was also recorded (**2b**–undialyzed).

Lineweaver-Burk plots and competitive inhibition

This study also set out to measure the enzyme-inhibitor dissociation constant (K_i value) for the reversible interaction between **2b** and MAO-B. To measure the K_i value a set of six Lineweaver-Burk plots was constructed using various inhibitor concentrations (0 μM, ¼ × IC_{50}, ½ × IC_{50}, ¾ × IC_{50}, 1 × IC_{50} and 1¼ × IC_{50}) and eight concentrations of kynyramine (15–250 μM) for each plot. The set of Lineweaver-Burk plots is shown in Fig. 5. Since the lines are linear and intersect on the y-axis it may be concluded that **2b** is a competitive inhibitor of MAO-B. Global (shared) fitting of the inhibition data directly to the Michaelis-Menten equation yields a K_i value of 0.0026± 0.00024μM (R^2 = 0.98).

From a plot of the slopes of the Lineweaver-Burk plots versus inhibitor concentration a similar value (0.0028μM) is obtained (x-axis intercept equals $-K_i$).

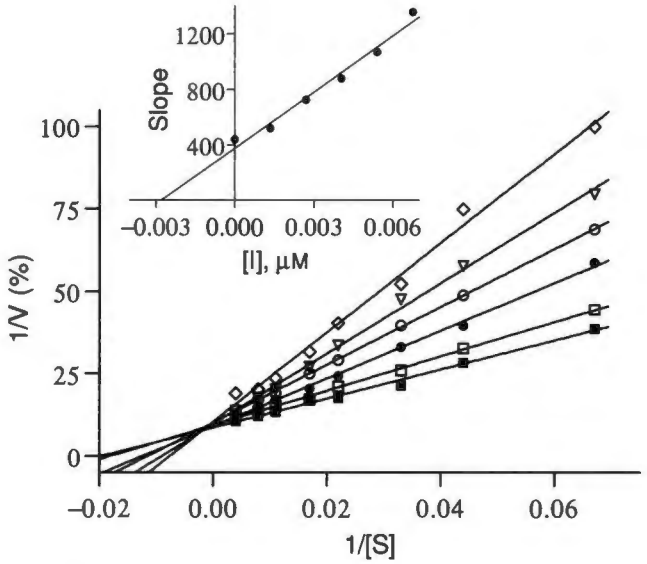


Fig. 5.Lineweaver-Burk plots for the inhibition of MAO-B by **2b**. The inset is a graph of the slopes of the Lineweaver-Burk plots versus inhibitor concentration.

Docking studies

An interesting SAR of MAO-B inhibition by 3,4-dihydro-2(1*H*)-quinolinones is that C7 substitution yields higher potency MAO-B inhibition compared to C6 substitution. In certain instances the differences in MAO-B inhibition are small (e.g. **2b/3b**) while for other homologues (e.g. **2c/3c**) the C7-substituted homologue is significantly more potent than the corresponding C6-substituted homologue. To determine if differing binding modes to MAO-B may be responsible for this SAR, **2c** and **3c** were docked into the MAO-B active site using the CDOCKER docking algorithm of Discovery Studio 3.1 [28]. To propose possible reasons for the weaker MAO-A inhibition of 3,4-dihydro-2(1*H*)-quinolinones, these compounds were also docked into the MAO-A active site. The docking simulations were carried out according to the literature procedure and the reported crystal structures of human MAO-A (PDB code: 2Z5X) [29] and MAO-B (PDB code: 2V5Z) [30] served as protein models [24].

As shown in Fig. 6, **2c** and **3c** exhibit differing binding modes to MAO-B, particularly with respect to the orientation of the 3,4-dihydro-2(1*H*)-quinolinone moiety. Compared to **2c**, the 3,4-dihydro-2(1*H*)-quinolinone moiety of **3c** is rotated by approximately 180 °.

This inversion of orientation allows for the benzyloxy side chain of **3c** to extend into the entrance cavity of the enzyme, similar to **2c**. For **2c**, polar interactions (H-bonding and π–π interaction with Tyr-398) are established in the enzyme's substrate cavity, which are not possible for **3c**. Since **3c** is a more potent MAO-B inhibitor than **2c**, these polar interactions play a lesser role in inhibitor stabilization, and Van der Waals interactions between the benzyloxy side chain and the entrance cavity most likely contribute to a higher degree. This is supported by the observation that **4** and **5**, compounds lacking a C6 or C7 side chain, are very weak MAO-B inhibitors (IC_{50}> 201 μM) [23]. It may thus be concluded that, although the placements and orientations of these side chains are very similar, for **3c** more productive Van der Waals interactions are possible, perhaps because this compound extends slightly deeper into the entrance cavity enabling it to establish interactions with key residues.

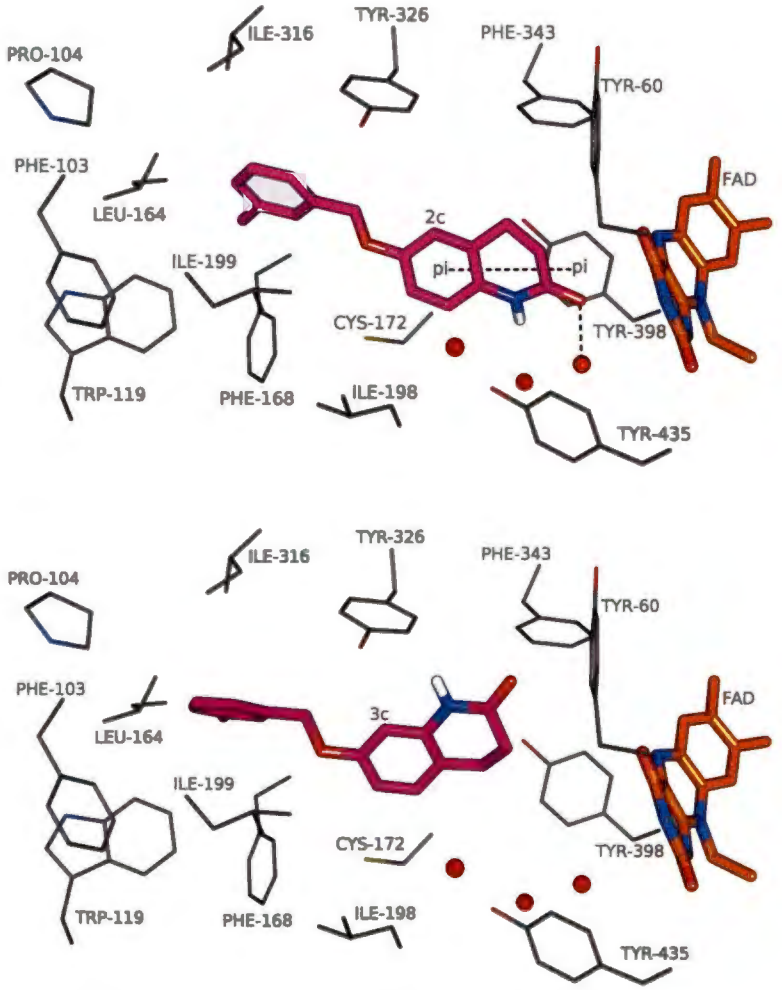


Fig. 6.The proposed binding modes of **2c** and **3c** to MAO-B.

In contrast to MAO-B, compounds **2c** and **3c** exhibit similar binding modes to MAO-A with respect to the orientation of the 3,4-dihydro-2(1*H*)-quinolinone moiety as well as the placement of the benzyloxy phenyl ring (Fig. 7). Both compounds establish polar interactions (H-bonding and π–π interactions) with MAO. The similar binding modes to MAO-A explain their comparable MAO-A inhibition activities. It is noteworthy that **2c** and **3c** bind in folded conformations in MAO-A, while exhibiting extended conformations in MAO-B. In MAO-A restrictions imposed by the side chain of Phe-208 are most likely responsible for the folded conformation. In MAO-B the residue that corresponds to Phe-208 in MAO-A, is Ile-199. For larger inhibitors, the side chain of Ile-199 rotates from the active site cavity, allowing for extension into the entrance cavity.

Larger inhibitors are thus, in general, better accommodated in MAO-B than MAO-A, hence more potent inhibition of MAO-B by the C6- and C7-substituted 3,4-dihydro-2(1*H*)-quinolinones.

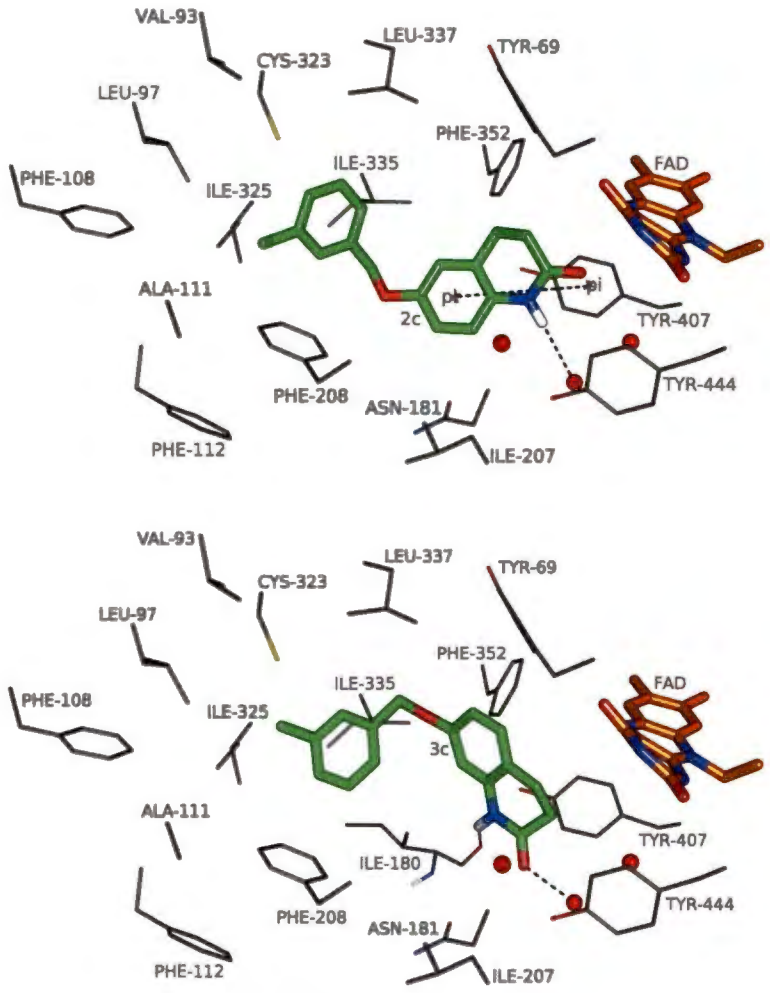


Fig. 7.The proposed binding modes of **2c** and **3c** to MAO-A.

Conclusion

In conclusion, the current study shows that 3,4-dihydro-2(1*H*)-quinolinones, in general, are MAO-B specific inhibitors. Three compounds, **2b**, **3a** and **3b**, were identified with high potency MAO-B inhibition (IC_{50}< 0.006 μM). Specific MAO-B inhibitors such as these represent potential candidate drugs for the treatment of Parkinson's disease, with a low risk of provoking the side effects associated with MAO-A inhibition (e.g. "cheese effect"). As shown with **2b**, this class acts as reversible and competitive MAO-B inhibitors.

Acknowledgements

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Conflict of Interest

The authors declare that they have no conflicts of interest to disclose.

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Supplementary Material

C6- and C7-substituted 3,4-dihydro-2(1*H*)-quinolinones as inhibitors of monoamine oxidase

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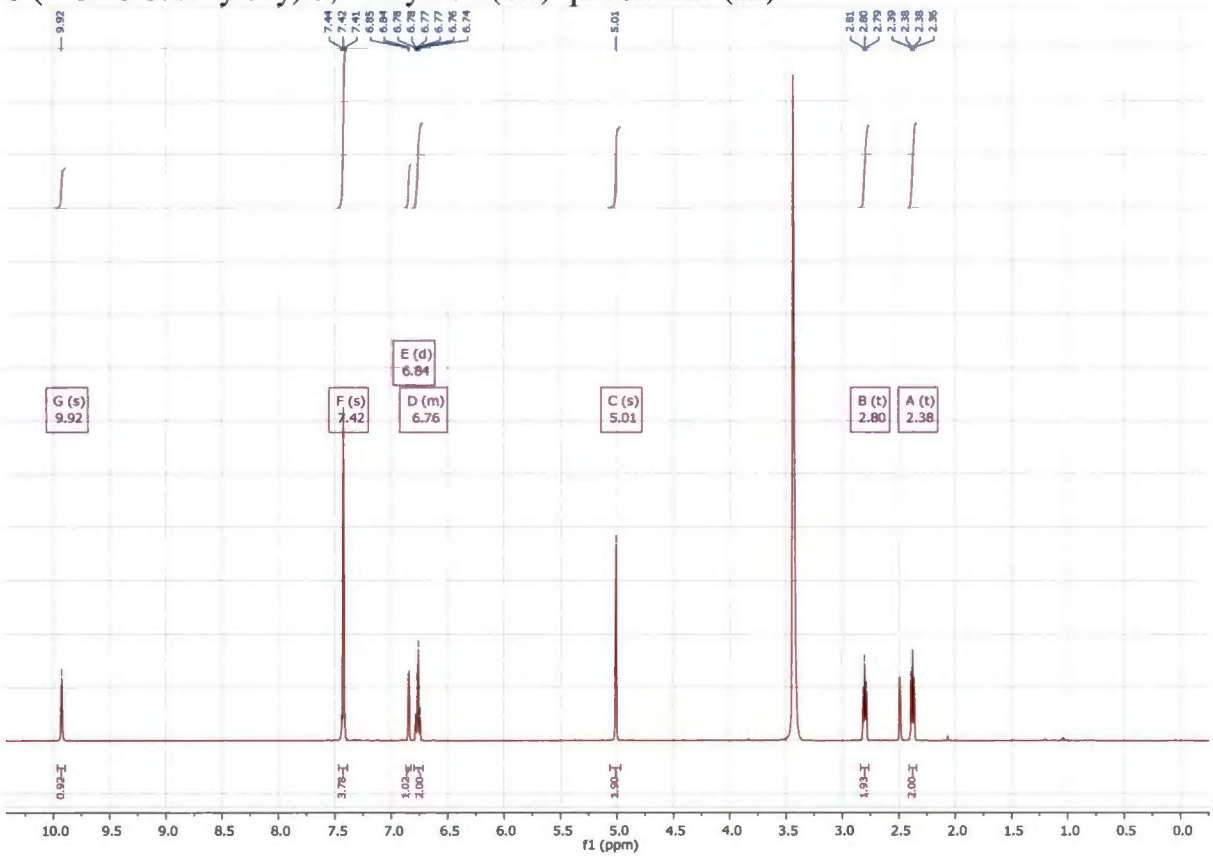
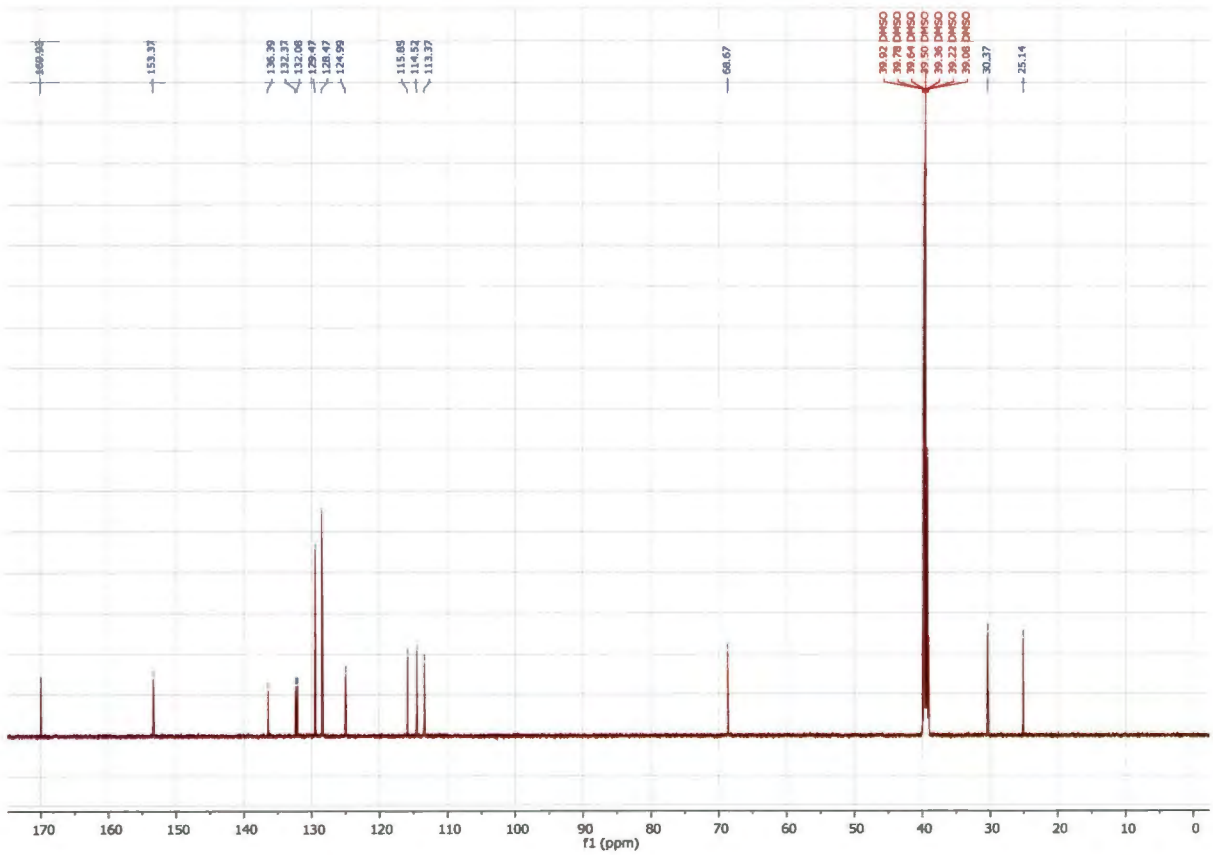
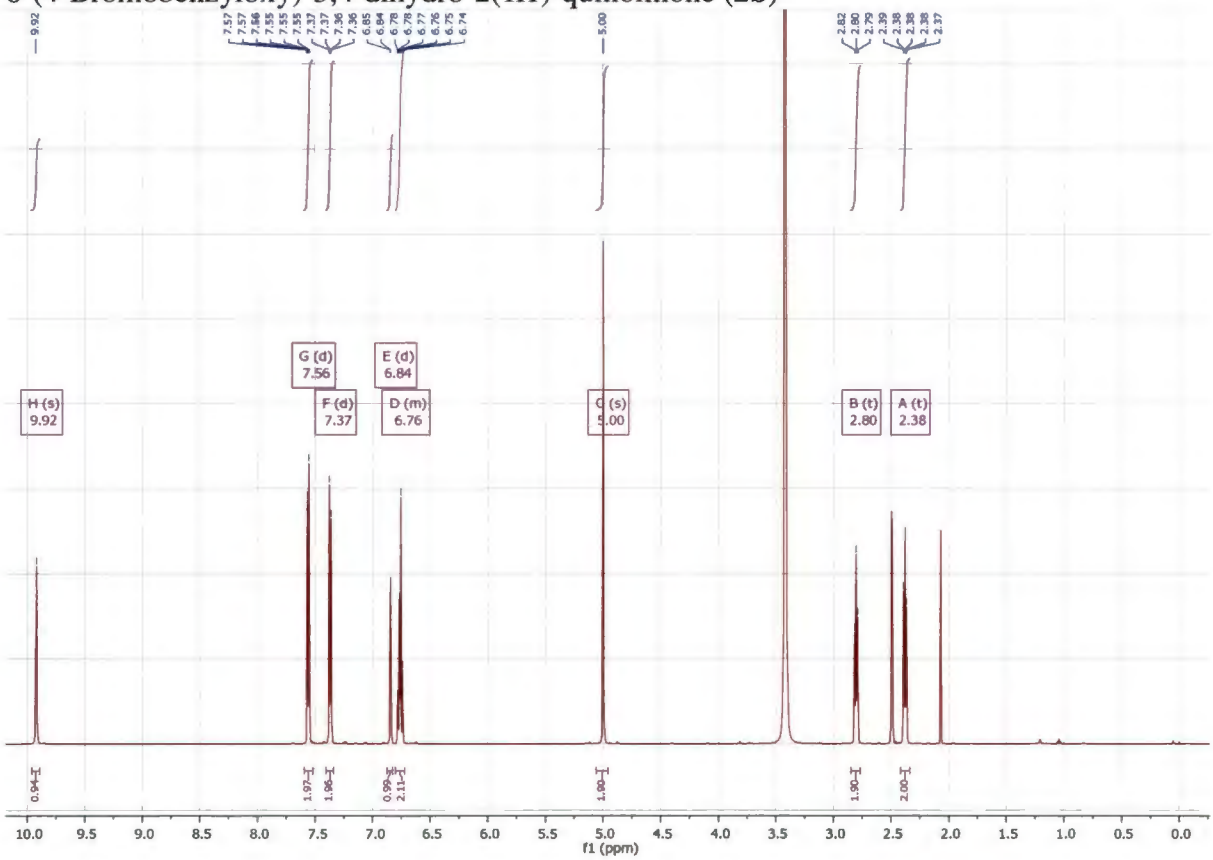
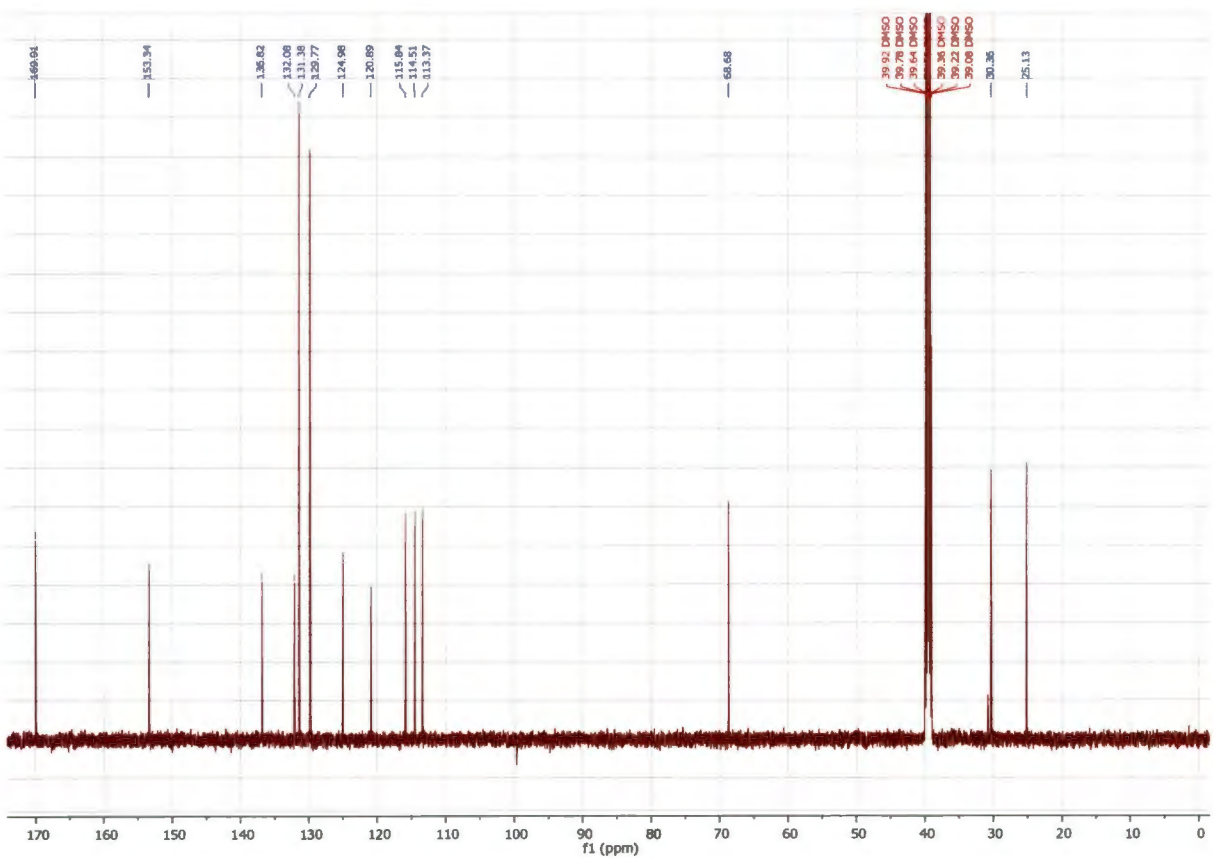
6-(4-Chlorobenzyloxy)-3,4-dihydro-2(1*H*)-quinolinone (**2a**)



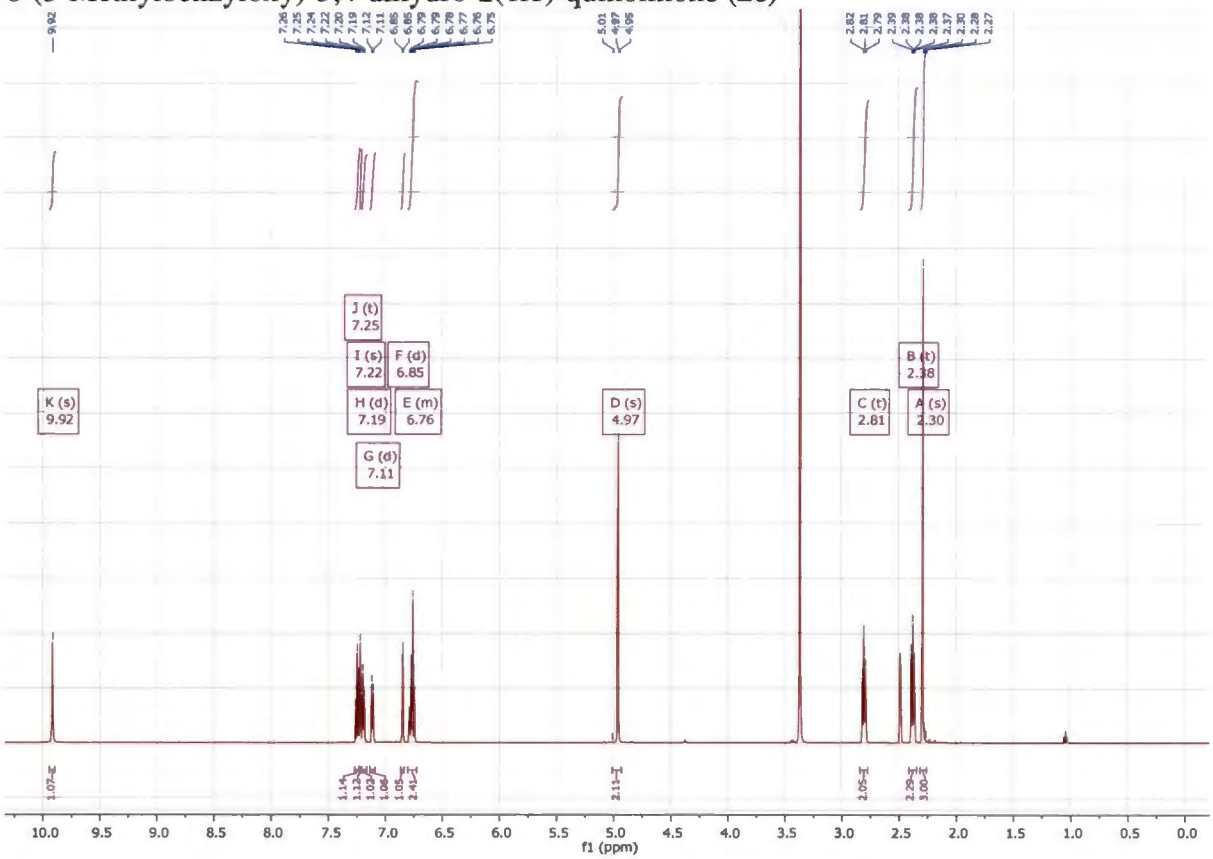


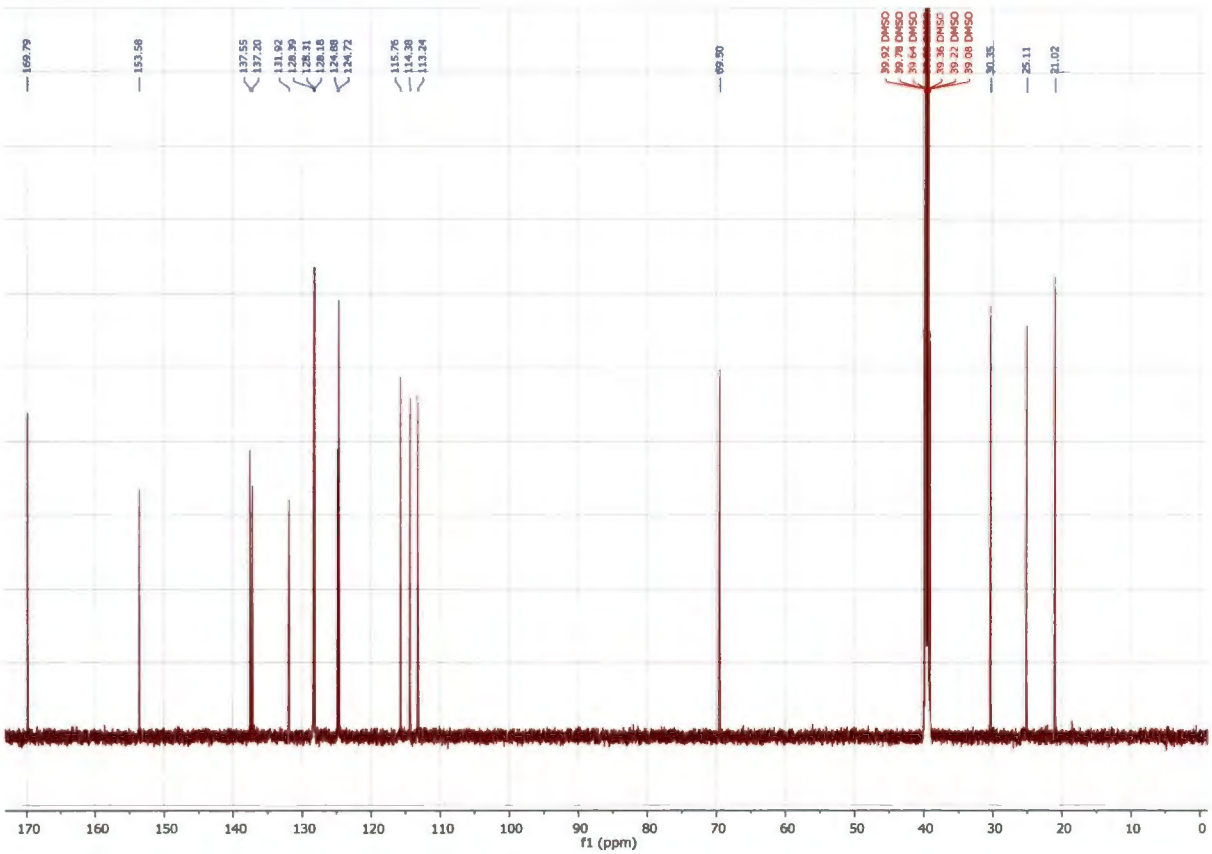
6-(4-Bromobenzyloxy)-3,4-dihydro-2(1*H*)-quinolinone (**2b**)





6-(3-Methylbenzyloxy)-3,4-dihydro-2(1*H*)-quinolinone (**2c**)





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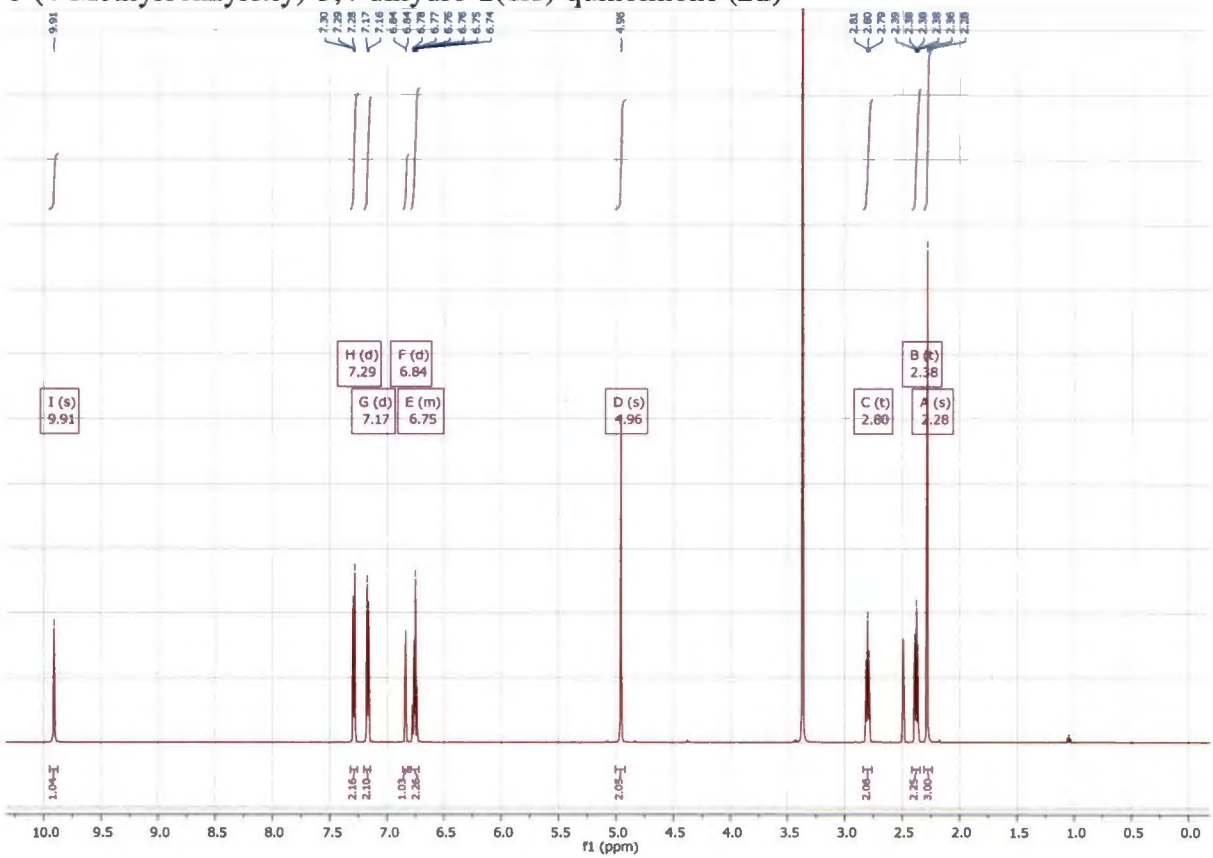
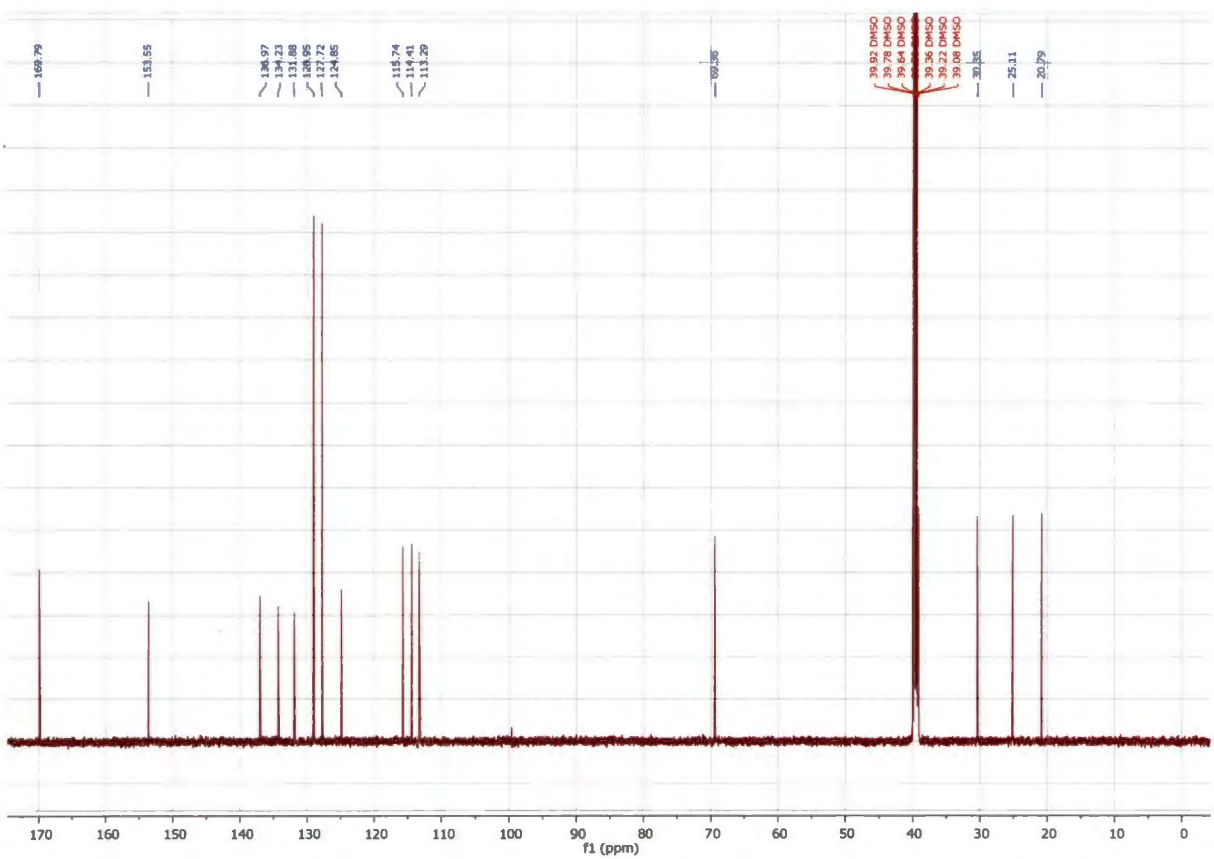
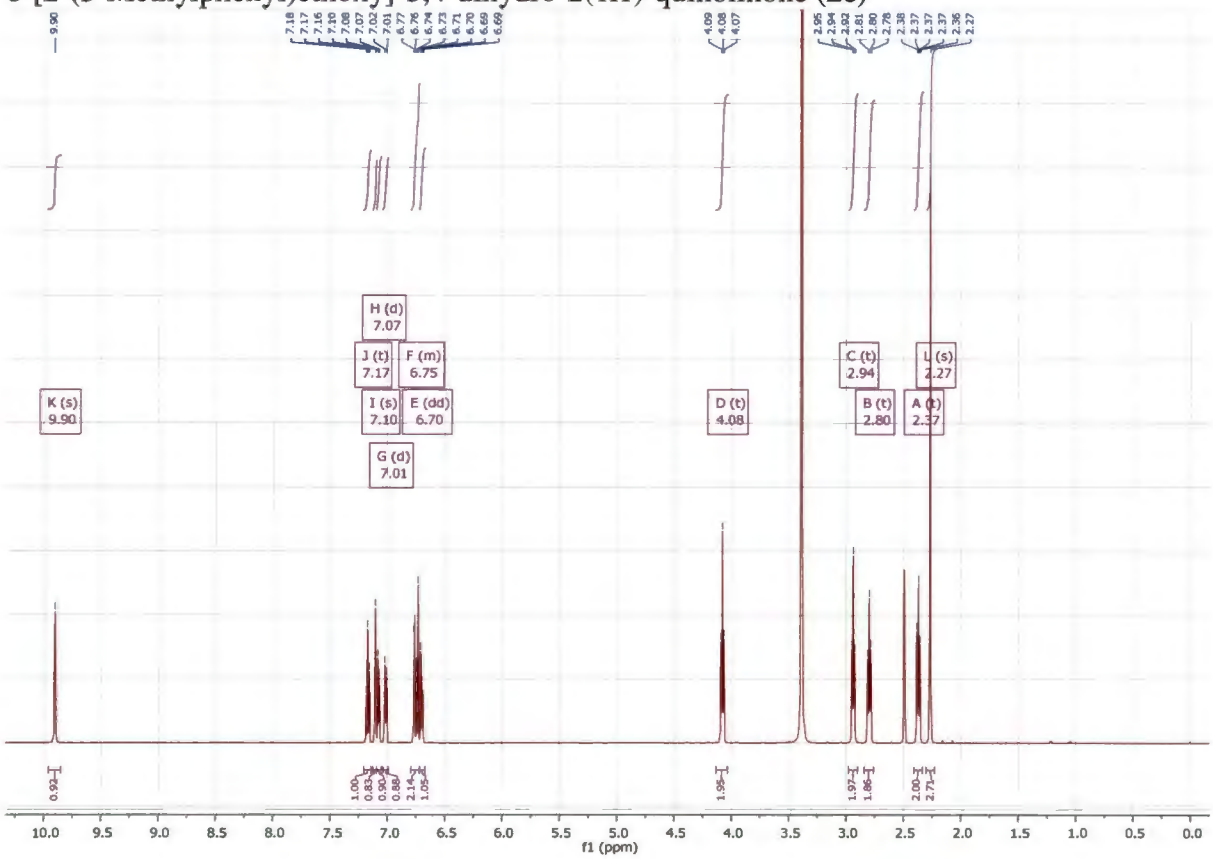
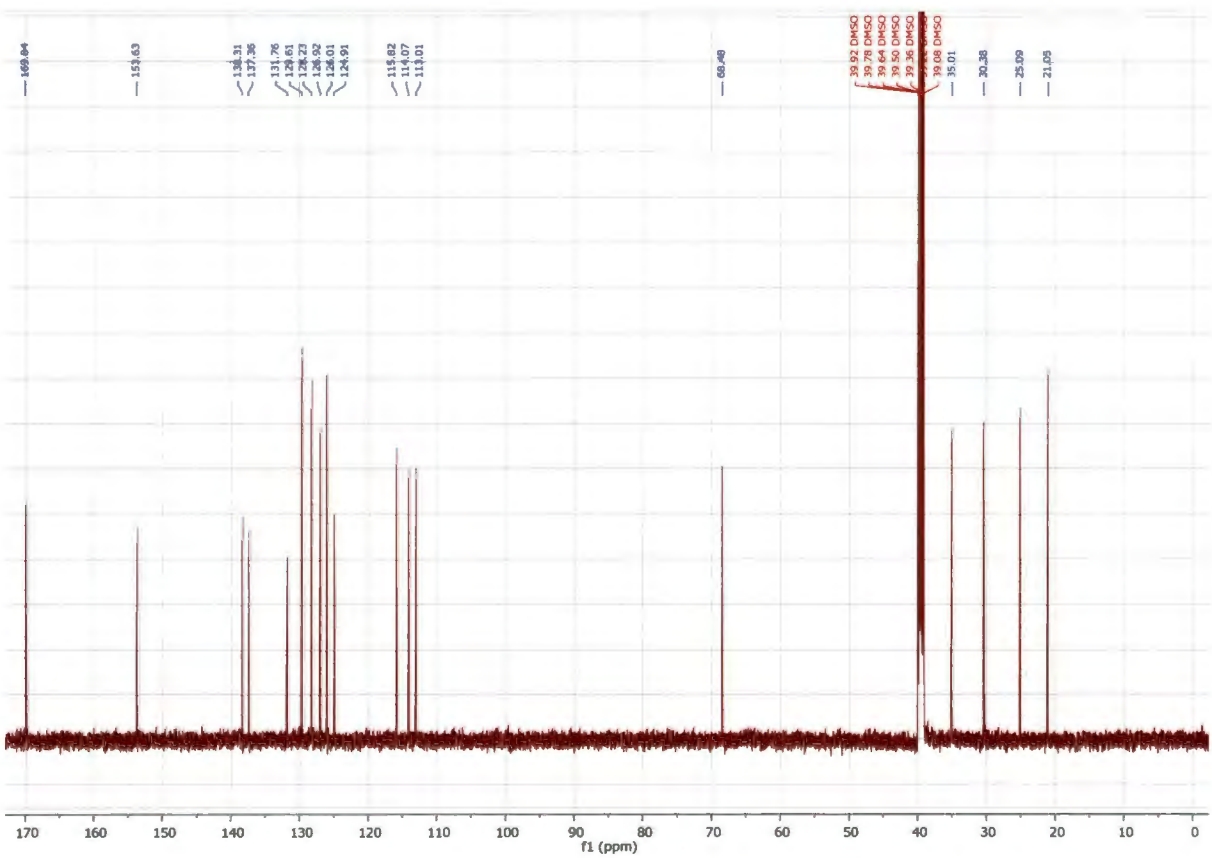


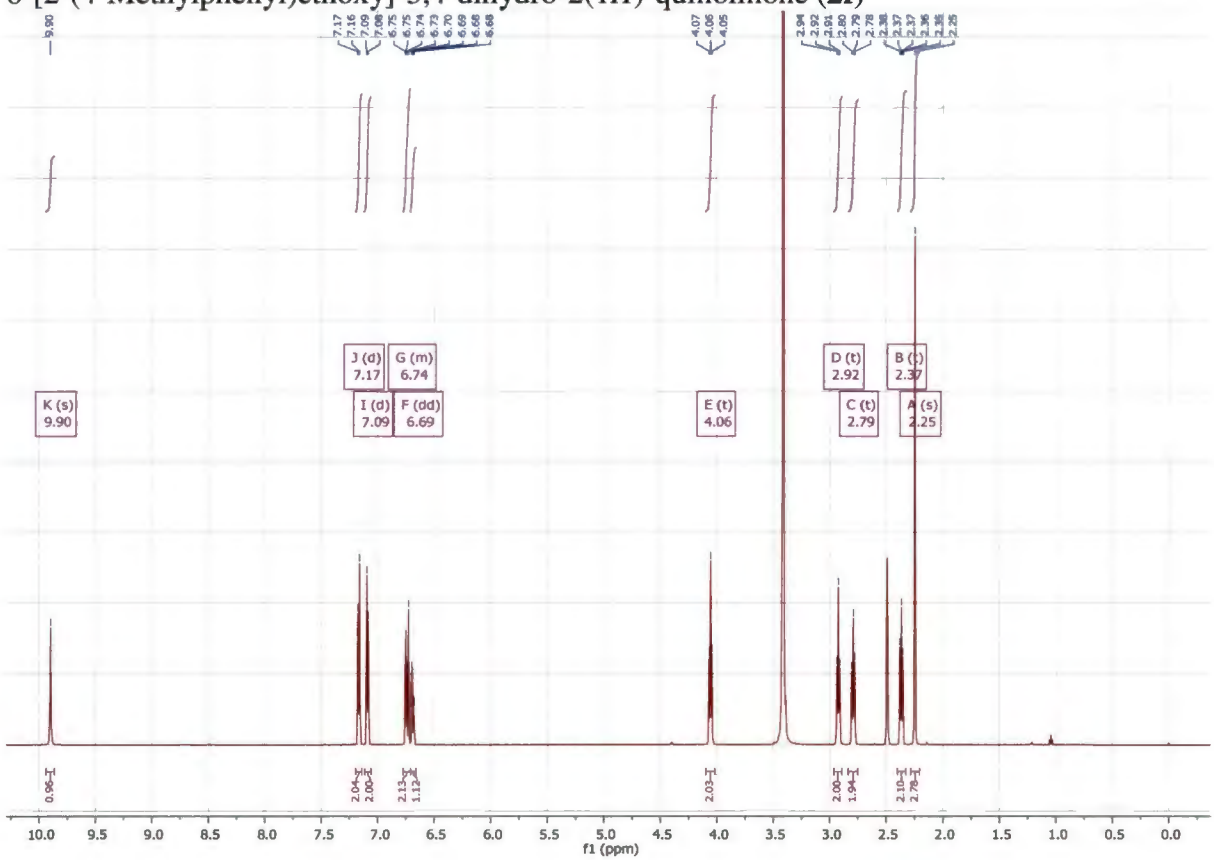


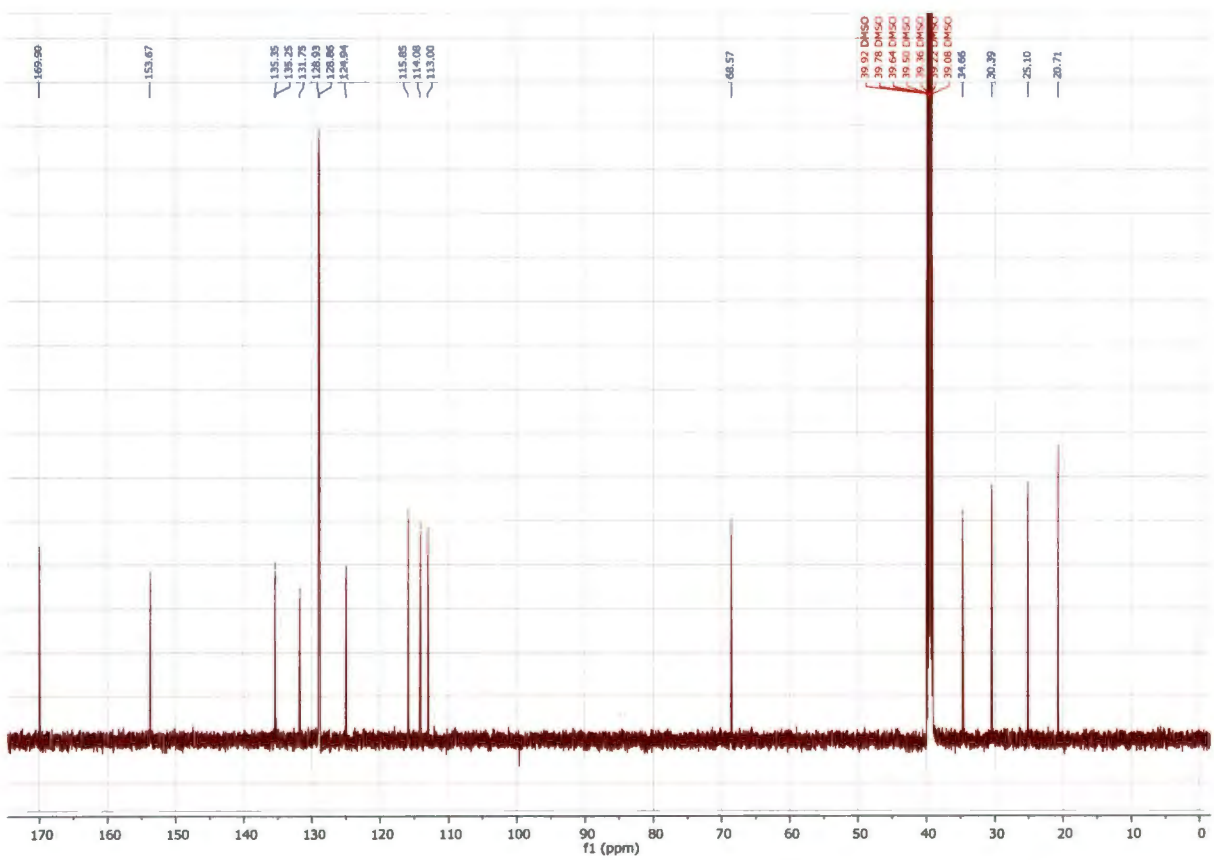
6-[2-(3-Methylphenyl)ethoxy]-3,4-dihydro-2(1*H*)-quinolinone (**2e**)



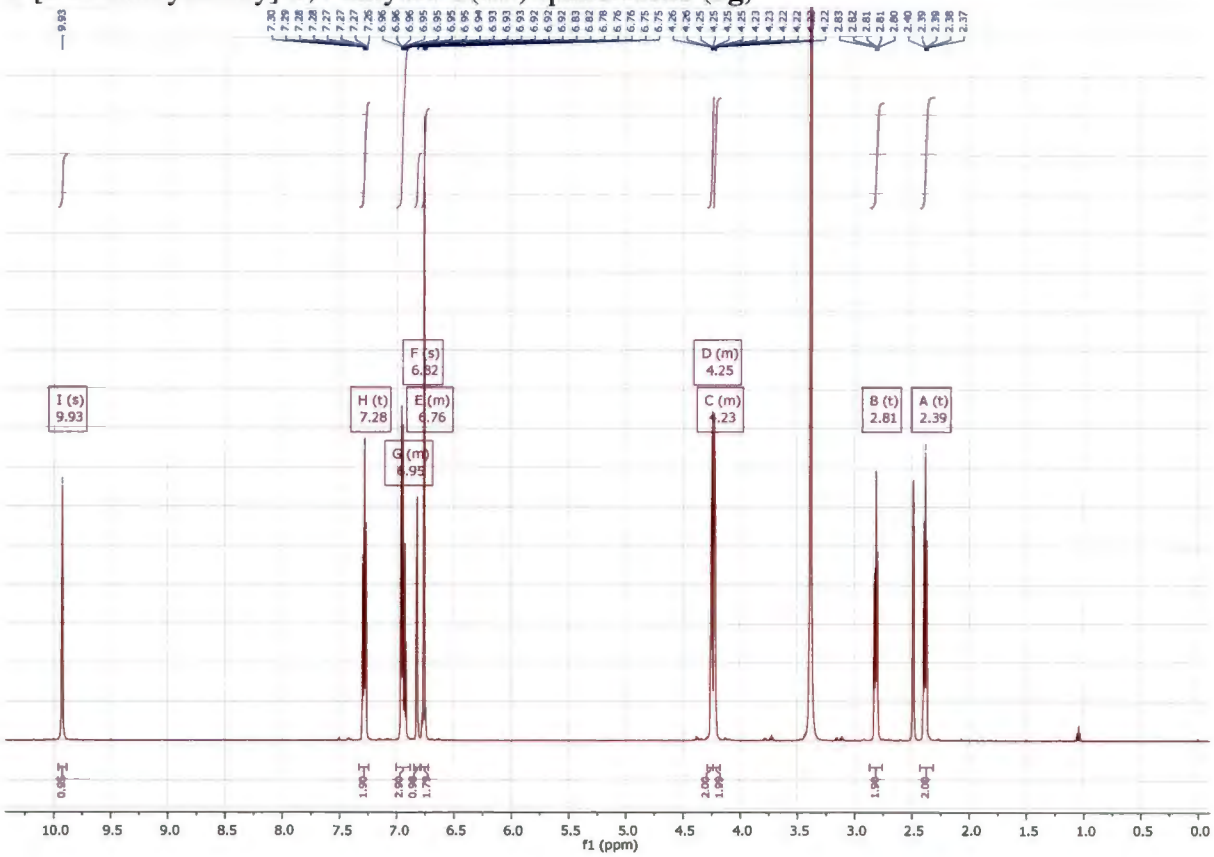


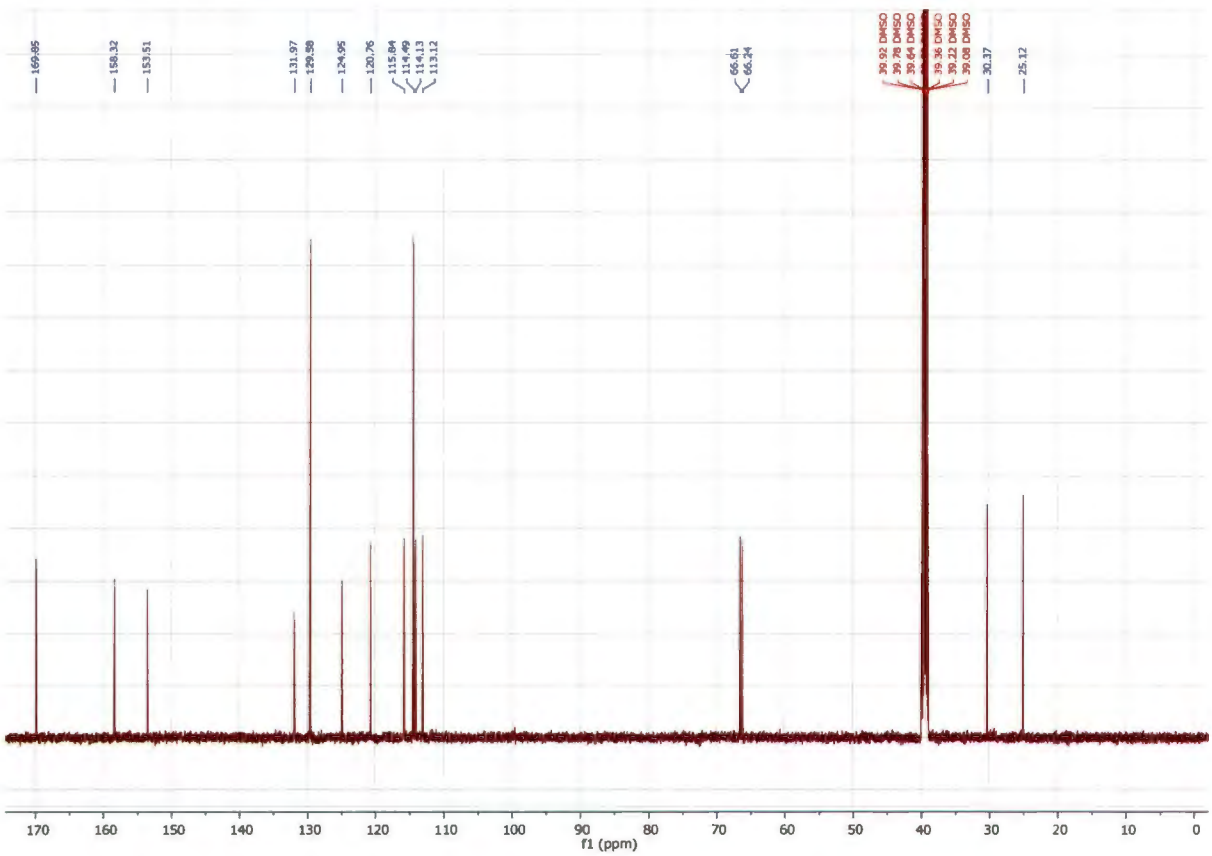
6-[2-(4-Methylphenyl)ethoxy]-3,4-dihydro-2(1*H*)-quinolinone (**2f**)



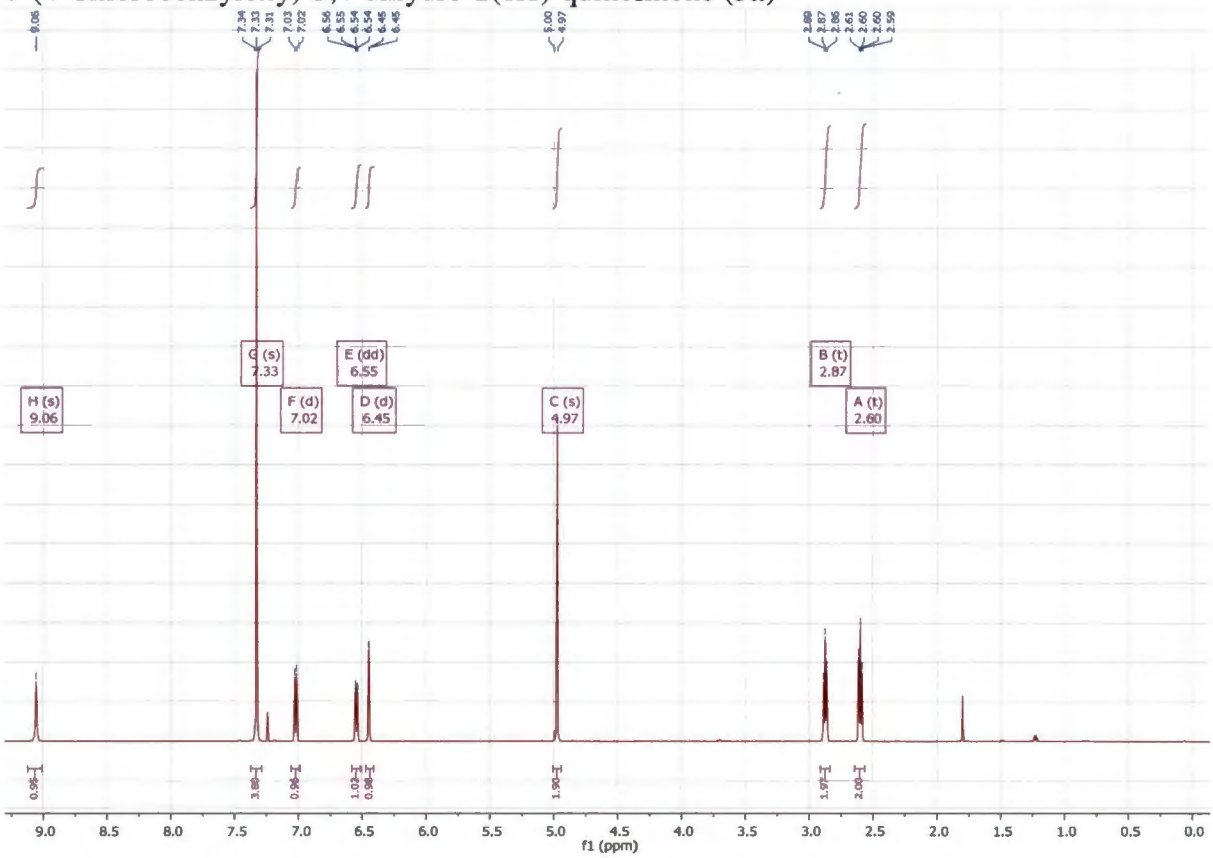


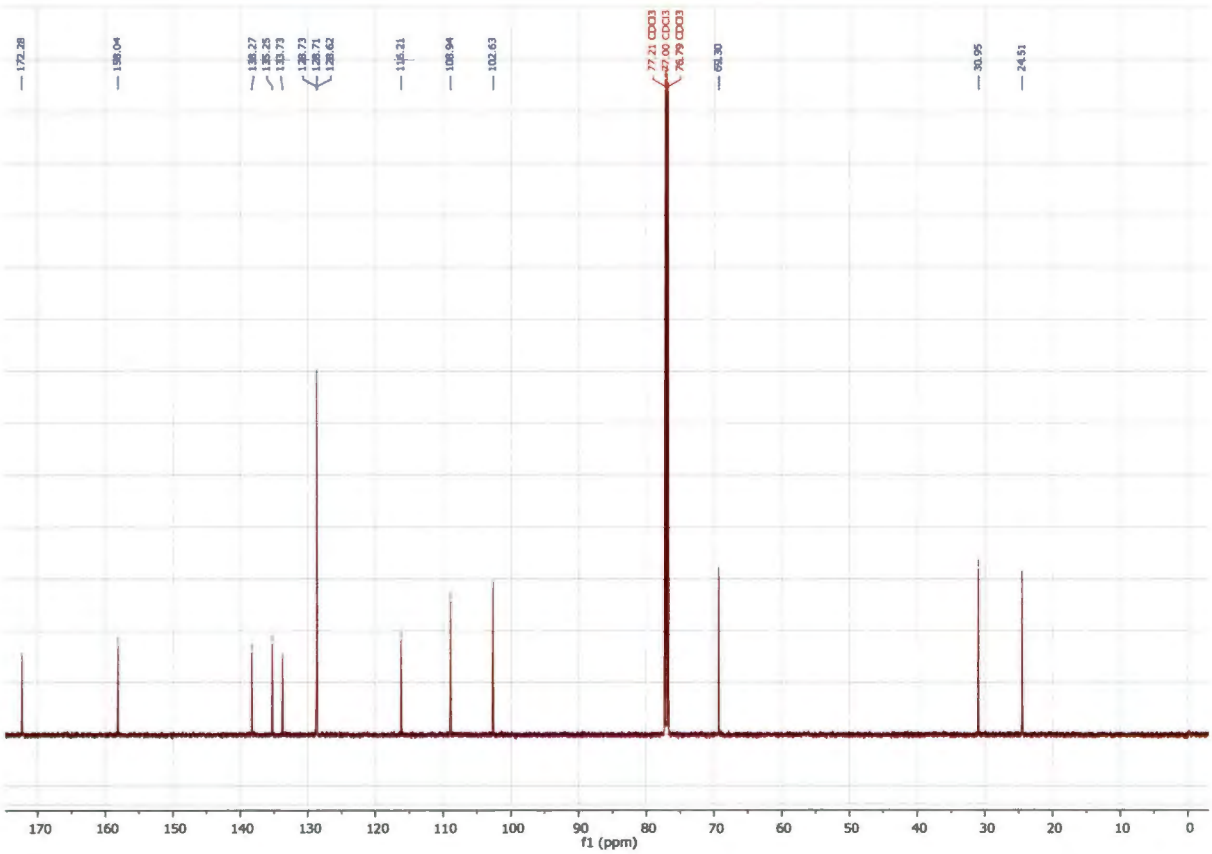
6-[2-Phenoxyethoxy]-3,4-dihydro-2(1*H*)-quinolinone (**2g**)



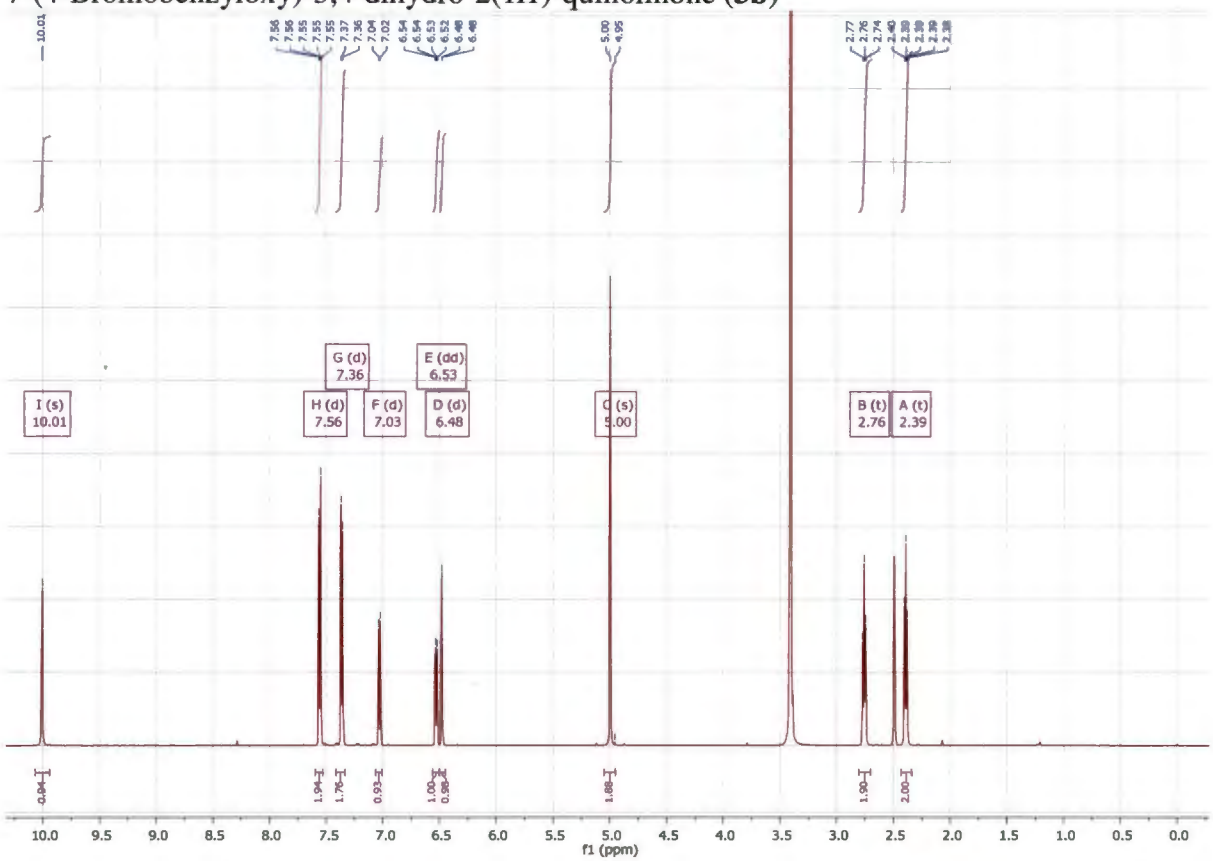


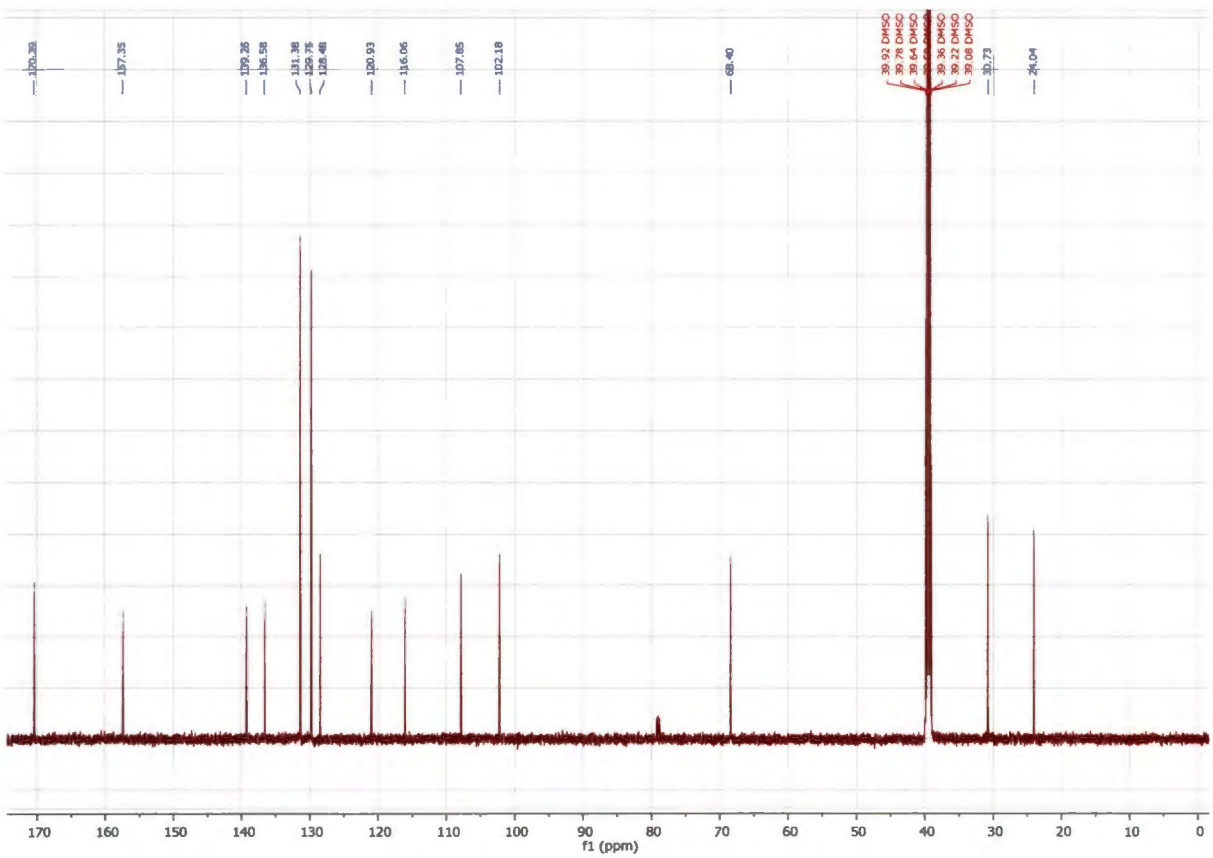
7-(4-Chlorobenzyloxy)-3,4-dihydro-2(1*H*)-quinolinone (**3a**)



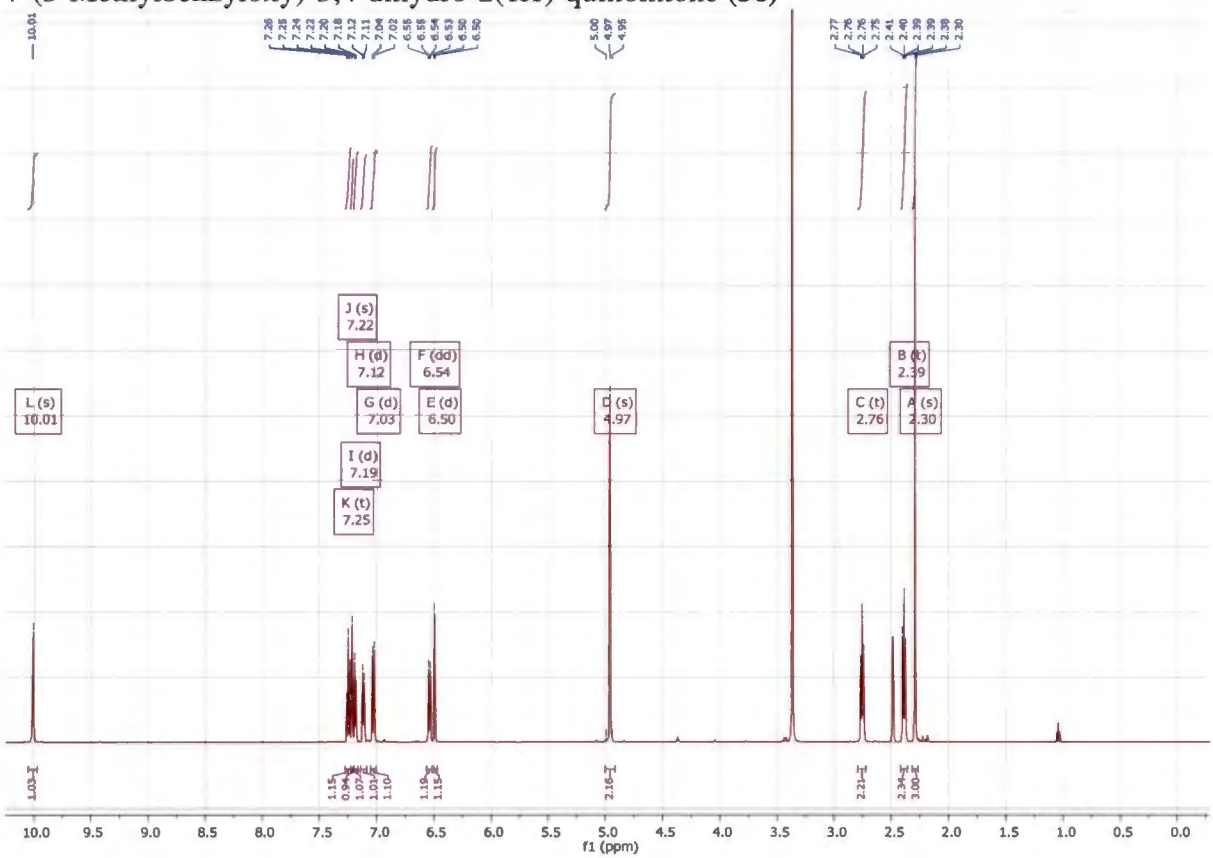


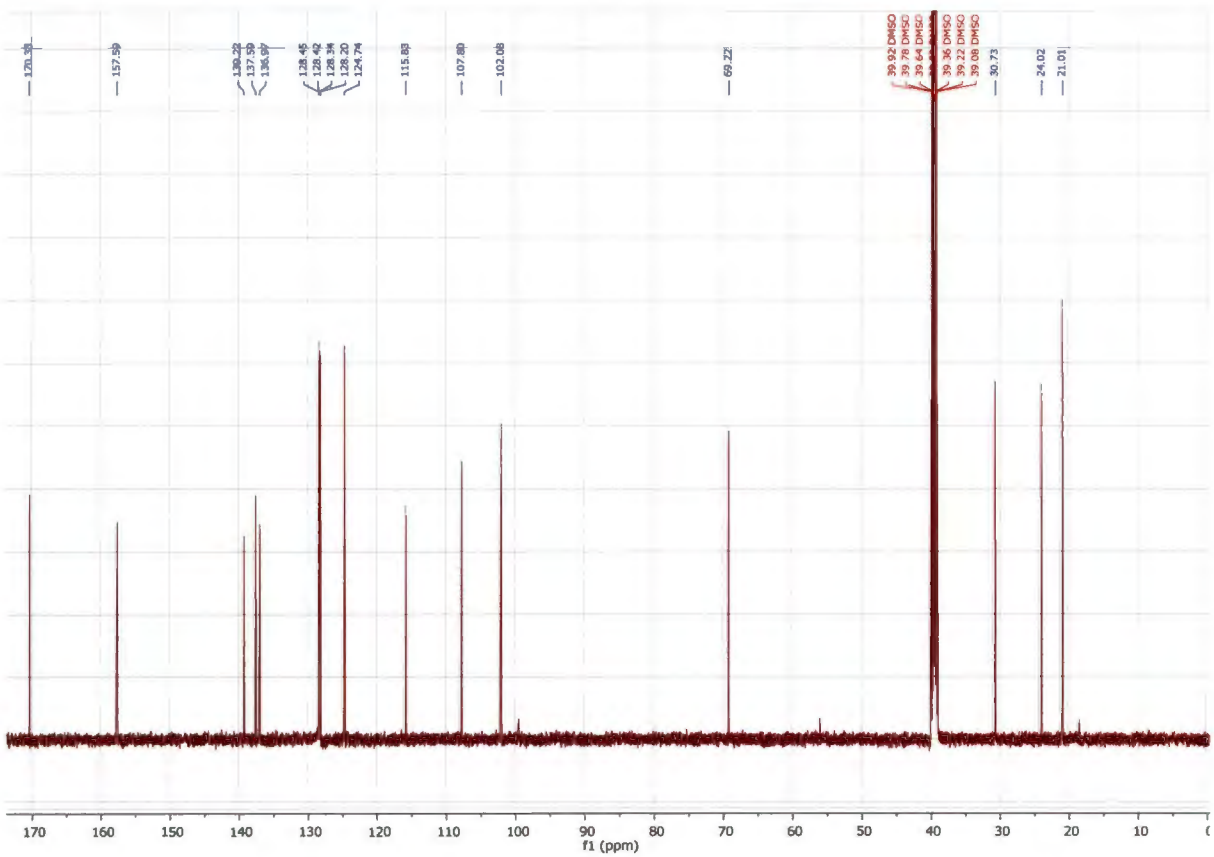
7-(4-Bromobenzyloxy)-3,4-dihydro-2(1*H*)-quinolinone (**3b**)



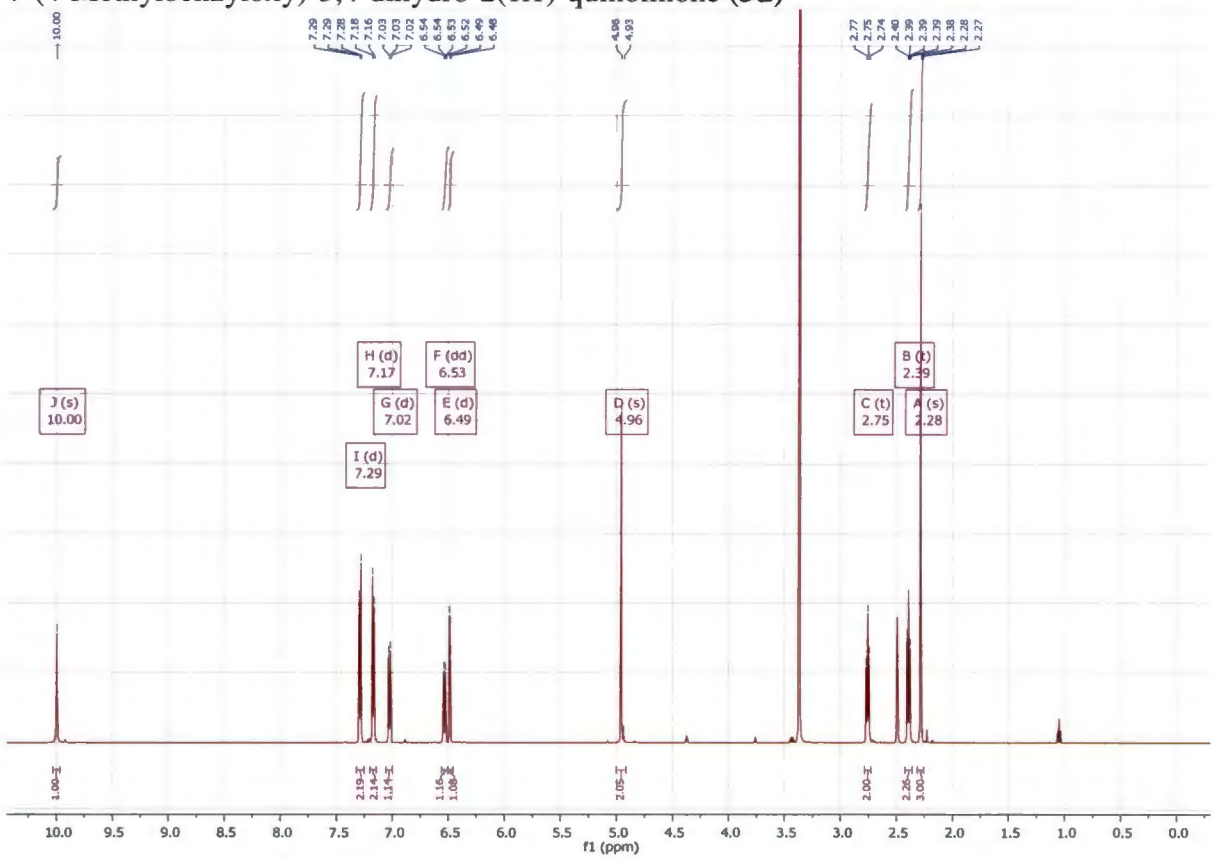


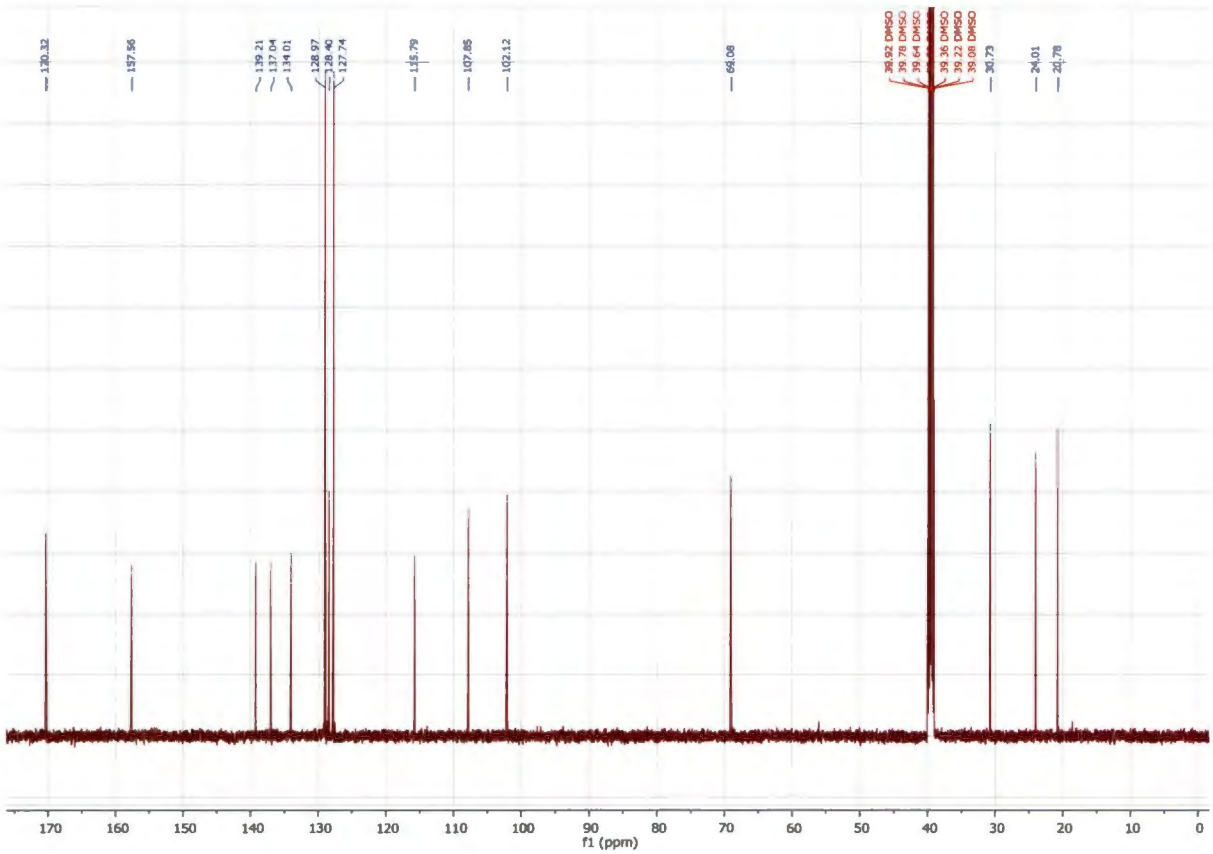
7-(3-Methylbenzyloxy)-3,4-dihydro-2(1*H*)-quinolinone (**3c**)



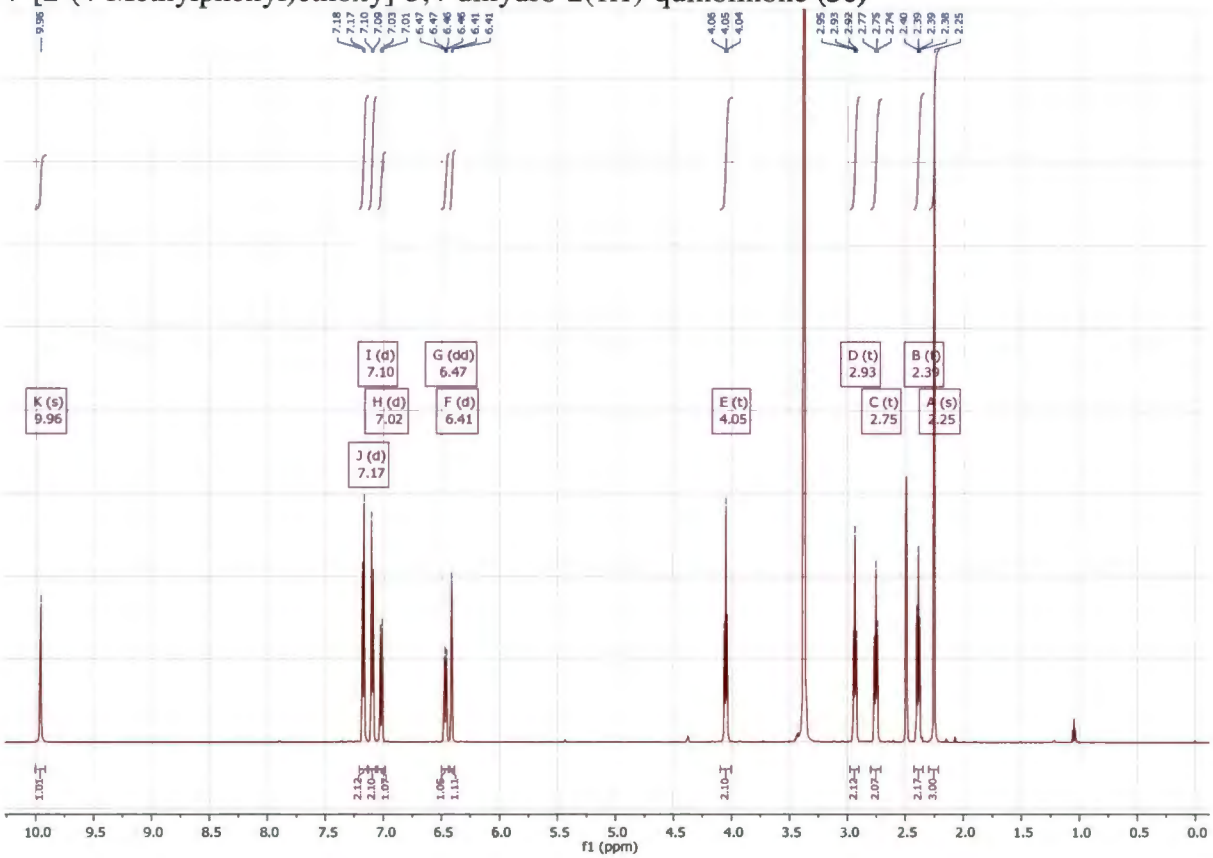


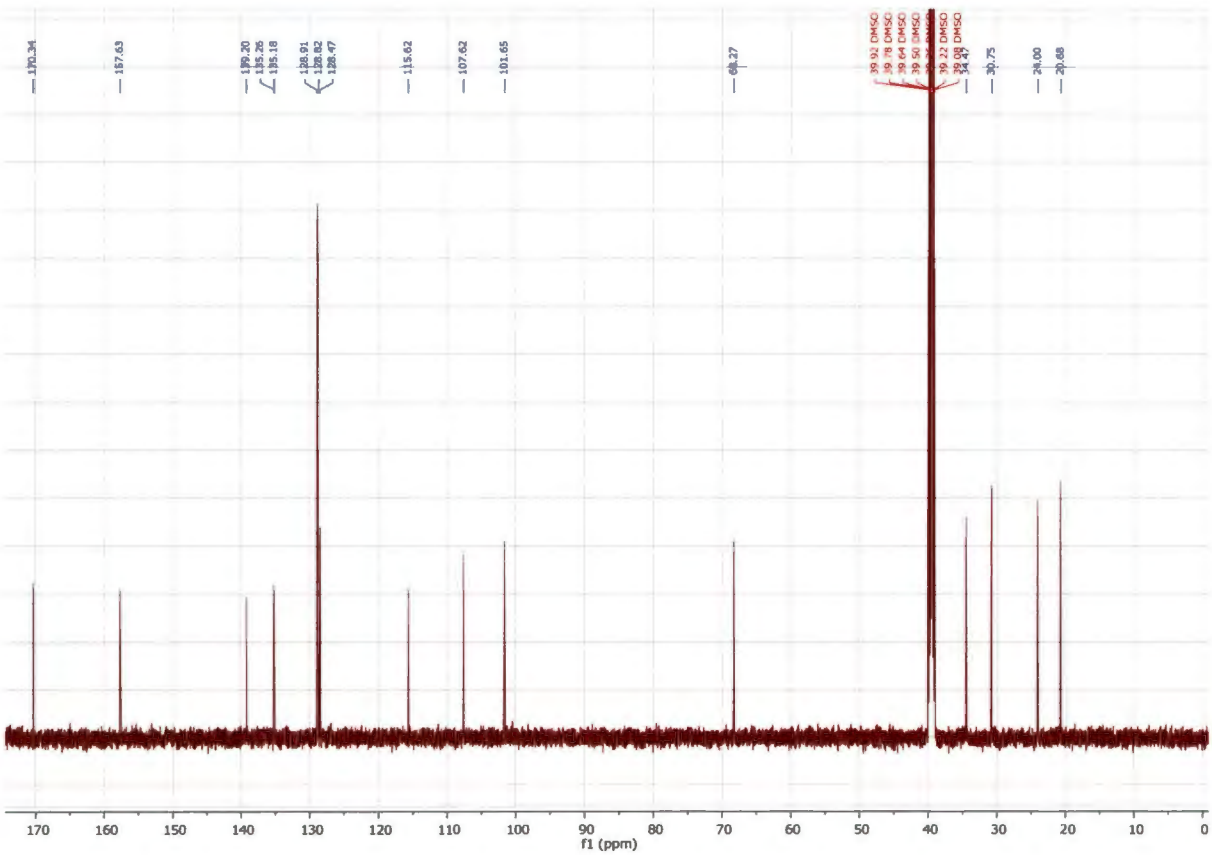
7-(4-Methylbenzyloxy)-3,4-dihydro-2(1*H*)-quinolinone (**3d**)



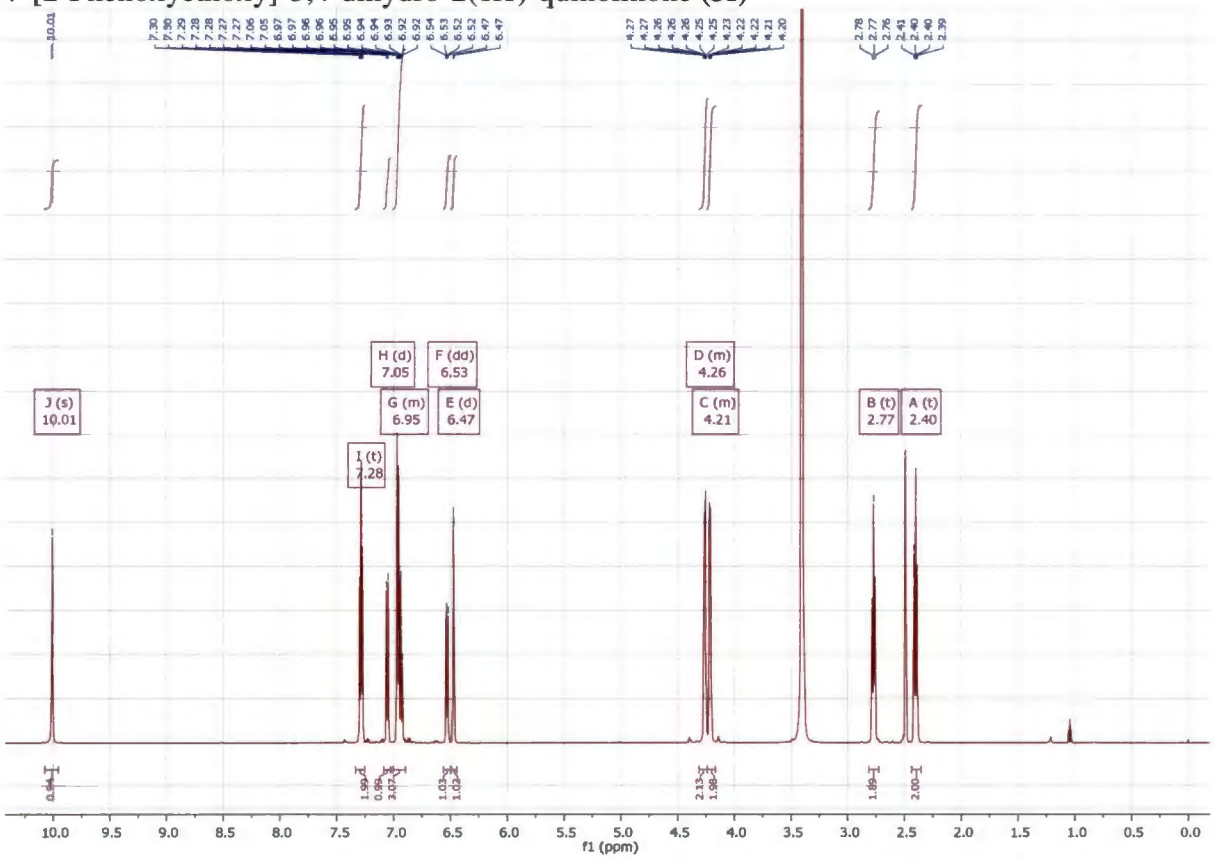


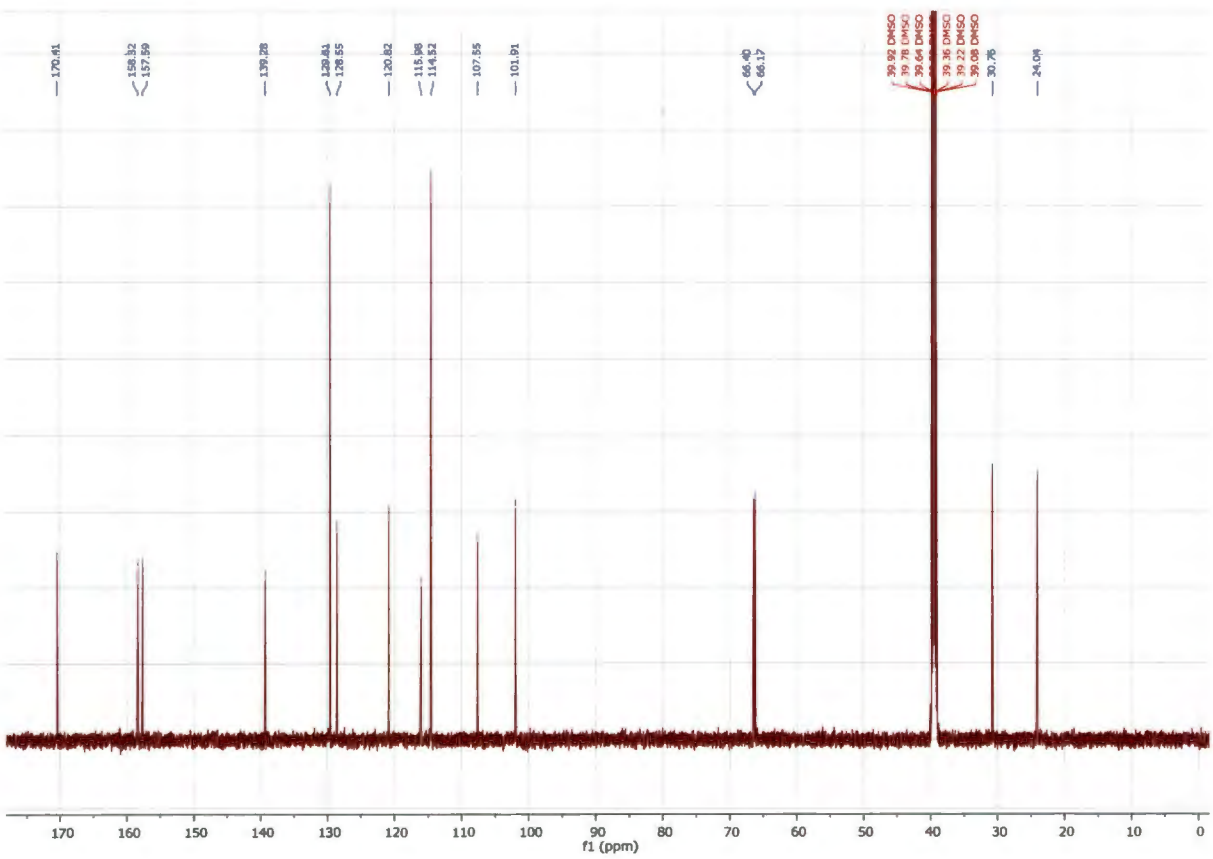
7-[2-(4-Methylphenyl)ethoxy]-3,4-dihydro-2(1*H*)-quinolinone (**3e**)



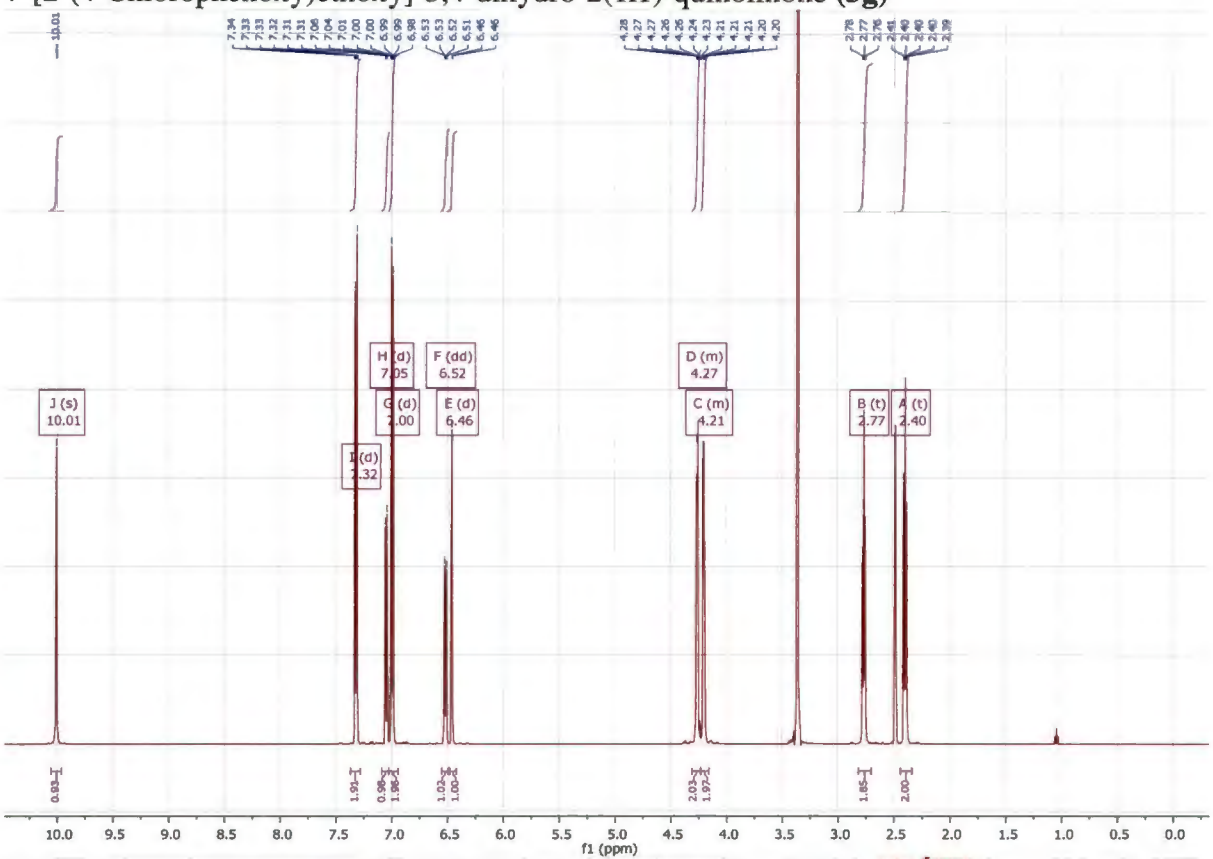


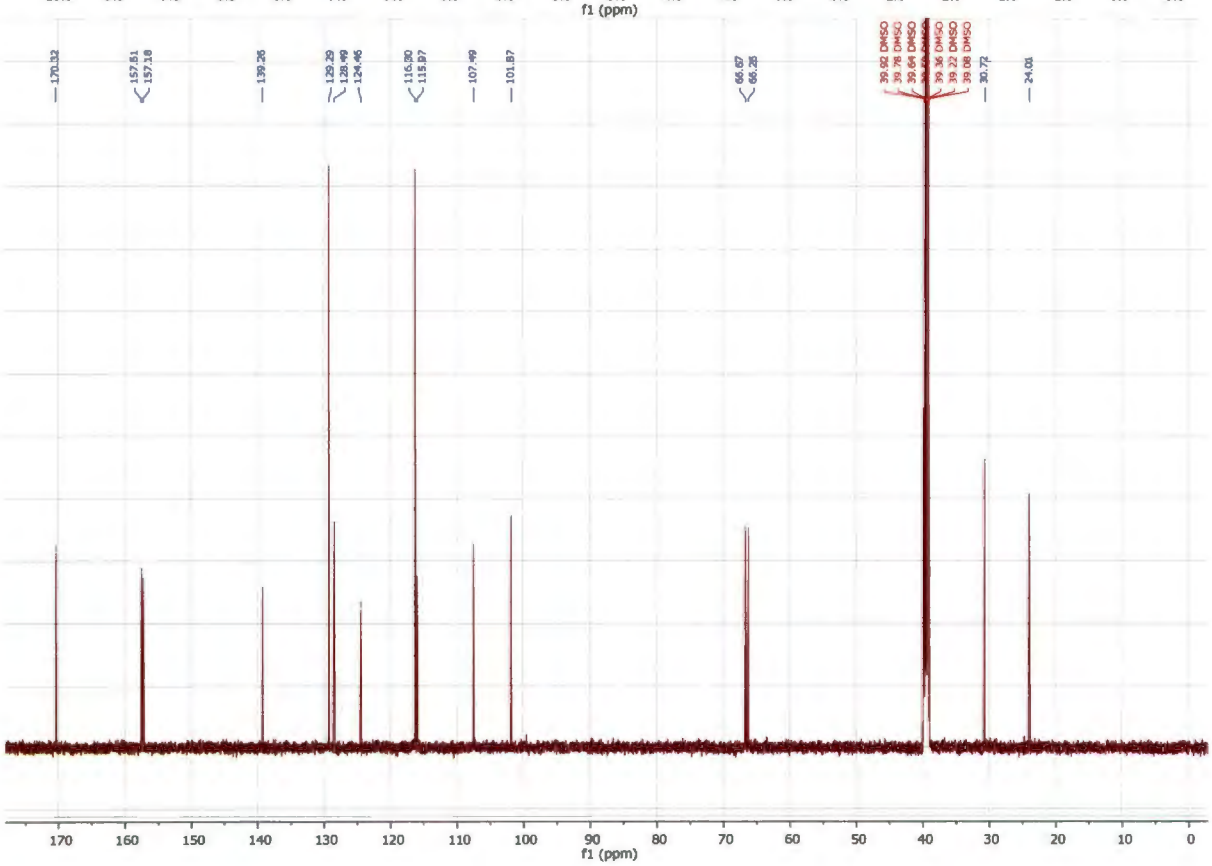
7-[2-Phenoxyethoxy]-3,4-dihydro-2(1*H*)-quinolinone (**3f**)





7-[2-(4-Chlorophenoxy)ethoxy]-3,4-dihydro-2(1*H*)-quinolinone (**3g**)





CHAPTER 5

MANUSCRIPT C

The evaluation of 2-phenoxyethoxy-substituted tetralones as inhibitors of monoamine oxidase

Authors' contributions:

- The experimental work, interpretation of results and documentation of this study was carried out by L. Meiring.
- 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 L. Meiring's Ph.D thesis.

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Running title: β-Tetralones as MAO inhibitors

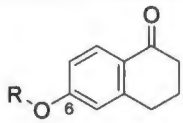
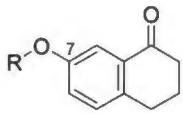
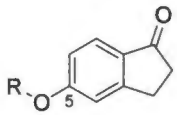
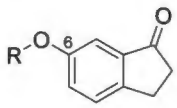
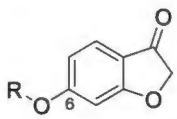
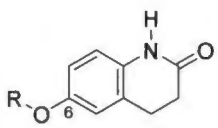
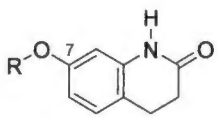
Keywords: monoamine oxidase, MAO, inhibition, reversible, tetralone.

Abstract: Previous studies have shown that α-tetralone (3,4-dihydro-2*H*-naphthalen-1-one) derivatives are highly potent monoamine oxidase (MAO) inhibitors with substitution on both the C6- and C7-positions yielding active compounds. Although α-tetralones are selective for the MAO-B isoform, this class of compounds also inhibits MAO-A with IC_{50} values <2.25 μM. The present study investigates the possibility that the regioisomer, β-tetralone (3,4-dihydro-1*H*-naphthalen-2-one), may also act as a suitable scaffold for MAO inhibitor design. β-Tetralone derivatives, however, proved to be difficult to synthesise and purify, and after numerous experiments one derivative, 7-(2-phenoxyethoxy)-3,4-dihydronaphthalen-2(1*H*)-one (**17**), was obtained. For comparison, the C6- and C7-substituted α-tetralone homologues of this compound (**18** and **19**, respectively) were also synthesised. Interestingly **17** was found to be a weak inhibitor of human MAO-A (IC_{50} = 56.2 μM) compared to the reported α-tetralones as well as to compounds **18** (IC_{50} = 1.96 μM) and **19** (IC_{50} = 1.81 μM). Compound **17**, however, was a potent human MAO-B inhibitor (IC_{50} = 0.033 μM). It was also established that **17** is a reversible and competitive MAO-B inhibitor (K_i = 0.128 μM). It may thus be concluded that, in contrast to the α-tetralones reported to date, **17** is a more selective MAO-B inhibitor. Selective MAO-B inhibitors are considered useful agents for the treatment of Parkinson's disease and are being investigated for slowing the progression of Alzheimer's disease.

The monoamine oxidase (MAO) enzymes consist of two distinctive isoforms, MAO-A and MAO-B (Youdim & Bahkle, 2006). These enzymes are present in all mammals and metabolise amine-containing neurotransmitters and compounds derived from the diet. Because of the key roles that they play in neurotransmitter metabolism, the MAOs are targets for several disease states, both in the central nervous system and in the peripheral tissues (Ramsay *et al.*, 2012; Ramsay *et al.*, 2013). Although similar in their amino acid sequences and structures of their active sites, MAO-A and MAO-B have different substrate specificities and also display different tissue distribution (Shih *et al.*, 1999; Son *et al.*, 2008). As a result, inhibitors of the two isoforms are employed for treatment of different disorders. MAO-A inhibitors are, for example, established therapy for depressive illness and act by blocking central serotonin metabolism (Lum and Stahl, 2012). MAO-B inhibitors are used in the treatment of Parkinson's disease where they reduce the MAO-B-catalysed metabolism of dopamine (Youdim *et al.*, 2006; Finberg, 2014). MAO-B inhibitors are also under investigation for Alzheimer's disease therapy and, in this regard, may act by a number of mechanisms (Naoi and Maruyama, 2010; Cai, 2014). Evidence suggests that MAO-A inhibitors may represent potential therapy for age-dependent cardiac cellular degeneration (Maurel *et al.*, 2003) and advanced prostate cancer (Flamand *et al.*, 2010). MAO-B inhibitors in particular have been advocated as potential neuroprotective agents by reducing MAO-generated hydrogen peroxide formation in the brain (Youdim & Bahkle, 2006). For neuroprotection in disorders associated with ageing such as Parkinson's disease, MAO-B is the relevant isoform since MAO-B activity increases in the brain with age, while MAO-A activity remains unchanged (Fowler *et al.*, 1997).

Interest in the pharmacology and potential new clinical applications of MAO inhibitors are thus substantial and a number of research efforts are aimed at discovering new chemical classes of MAO inhibitors, and deriving structure-activity relationships of known classes in inhibitors (Carradori and Silvestri, 2015; Carradori and Petzer, 2015). Tetralones play an important role in synthetic organic chemistry and are often used as starting materials for the synthesis of heterocyclic compounds, active pharmaceutical ingredients and natural products and their derivatives (Carreño *et al.*, 2006). The tetralone moiety is also frequently encountered in compounds displaying pharmacological activities (Manvar *et al.*, 2015). In this regard, recent reports that C6- and C7-substituted α-tetralone derivatives (**1** and **2**) are potent inhibitors of human MAO are of particular interest (Legoabe *et al.*, 2014; Legoabe *et al.*, 2015). As shown in table 1, the α-tetralone derivatives inhibit both MAO isoforms with specificity for MAO-B. Other chemical classes closely related to α-tetralone have also been reported to potently inhibit the MAOs. These include 1-indanone (**3** and **4**), 3-coumaranone (**5**) and 3,4-dihydro-2(1*H*)-quinolinone (**6** and **7**) derivatives (Mostert *et al.*, 2015; Van Dyk *et al.*, 2015; Meiring *et al.*, 2013). For all of these derivatives substitution occur on the phenyl ring with an alkyloxy group such as the benzyloxy, 2-phenylethoxy, 3-phenylpropoxy and 2-phenoxyethoxy groups. This substitution is crucial for inhibitory activity as the hydroxy-substituted parent compounds (**8–14**) display weak MAO inhibition (Table 2). In the present study we investigate the possibility that the regioisomer of α-tetralone, β-tetralone (3,4-dihydro-1*H*-naphthalen-2-one), may also represent a useful scaffold for MAO inhibitor design (Fig. 1). Based on the close similarity with other moieties successfully used in experimental MAO inhibitors, such as those in table 1, it is anticipated that β-tetralone-derived compounds would act as MAO inhibitors.

Table 1. The reported IC_{50} values for the inhibition of recombinant human MAO-A and MAO-B by α-tetralone (**1** and **2**), 1-indanone (**3** and **4**), 3-coumaranone (**5**) and 3,4-dihydro-2(1*H*)-quinolinone derivatives (**6** and **7**) derivatives (R = alkyl/arylalkyl substituent).

		IC_{50} range (μM)	
		MAO-A	**MAO-B**
1		0.024–2.25[a]	0.0045–0.078[a]
2		0.010–1.26[b]	0.00089–0.047[b]
3		0.504–10.75[c]	0.015–0.298[c]
4		0.032–1.348[c]	0.001–0.03[c]
5		0.586–(>100)[d]	0.004–1.05[d]
6		12.4–50.6[e]	0.086–4.01[e]
7		7.98–(>100)[e]	0.0029–0.191[e]

[a] Values obtained from reference (Legoabe *et al.*, 2014).

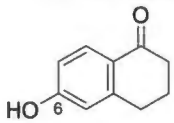
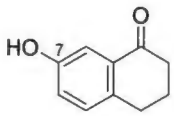
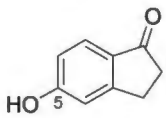
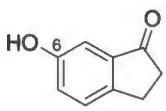
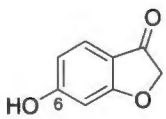
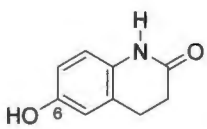
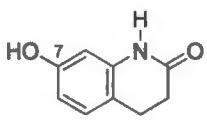
[b] Values obtained from reference (Legoabe *et al.*, 2015).

[c] Values obtained from reference (Mostert *et al.*, 2015).

[d] Values obtained from reference (Van Dyk *et al.*, 2015).

[e] Values obtained from reference (Meiring *et al.*, 2013).

Table 2. The reported IC_{50} values for the inhibition of recombinant human MAO-A and MAO-B by hydroxy-substituted α-tetralones, 1-indanones, 3-coumaranone and 3,4-dihydro-2(1*H*)-quinolinones.

		IC_{50} (μM)	
		MAO-A	**MAO-B**
8		43.8[a] (51.6 ± 0.256)[b]	66.4[a] (108 ± 3.81)[b]
9		39.4[c] (42.0 ± 4.63)[b]	187[c] (85.1 ± 12.3)[b]
10		61.7[d]	>100[d]
11		64.7[d]	85.4[d]
12		>100[e]	12.0[e]
13		161[f]	201[f]
14		183[f]	>100[f]

[a] Values obtained from reference (Legoabe *et al.*, 2014).

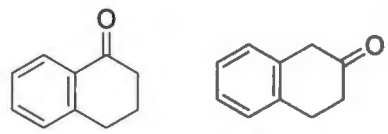
[b] Determined in the current study [mean ± standard deviation (SD)].

[c] Values obtained from reference (Legoabe *et al.*, 2015).

[d] Values obtained from reference (Mostert *et al.*, 2015).

[e] Values obtained from reference (Van Dyk *et al.*, 2015).

[f] Values obtained from reference (Meiring *et al.*, 2013).



α-Tetralone β-Tetralone

Fig. 1. The structures of α-tetralone and β-tetralone.

For this study, β-tetralone will be substituted on the C7-position with alkyloxy groups. This selection was based on the commercial availability of 7-methoxy-2-tetralone (**15**) in sufficient quantities to allow for the synthetic modification thereof.

To synthesise the β-tetralone derivatives a similar approach was followed as that reported for the synthesis of substituted α-tetralone derivatives (Fig. 2) (Legoabe *et al.*, 2014; Legoabe *et al.*, 2015). The key starting material, 7-methoxy-2-tetralone (**15**) was treated with aluminum trichloride ($AlCl_3$, anhydrous) in toluene to yield 7-hydroxy-2-tetralone (**16**). 7-Hydroxy-2-tetralone (**16**), in turn, was reacted with the selected alkyl bromide in acetone, with K_2CO_3 serving as base. These reactions were carried out at reflux temperature for 6 h. The workup consisted of filtration of the reaction through a pad of celite, and purification of the product with silica gel column chromatography (ethyl acetate/petroleum ether 40:60). C7-Substituted ethers of β-tetralone, however, proved to be difficult to synthesise and after reacting 7-hydroxy-2-tetralone (**16**) with benzyl bromide (including 4-chlorobenzyl bromide), 2-phenylethyl bromide, 3-phenylpropyl bromide, cyclohexylmethyl bromide and 2-bromoethyl phenyl ether, only the latter reaction yielded significant amounts (>1%) of product [7-(2-phenoxyethoxy)-3,4-dihydronaphthalen-2(1*H*)-one (**17**)] with acceptable purity (Fig. 3). The reaction of 2-bromoethyl phenyl ether with 6-hydroxy-1-tetralone (**8**) and 7-hydroxy-1-tetralone (**9**), however, proceeded with moderate yields (34–49%), and high purity products were obtained without the requirement of column chromatography. Compounds **18** and **19** thus synthesised were purified by recrystallisation from ethanol.

6-Hydroxy-1-tetralone (**8**) and 7-hydroxy-1-tetralone (**9**) were synthesised from the methoxy homologues, **20** and **21**, as described previously (Legoabe *et al.*, 2014; Legoabe *et al.*, 2015). The structures of the tetralones were characterised by ^{1}H NMR, ^{13}C NMR and mass spectrometry, and the physical data are given in the supplementary material.

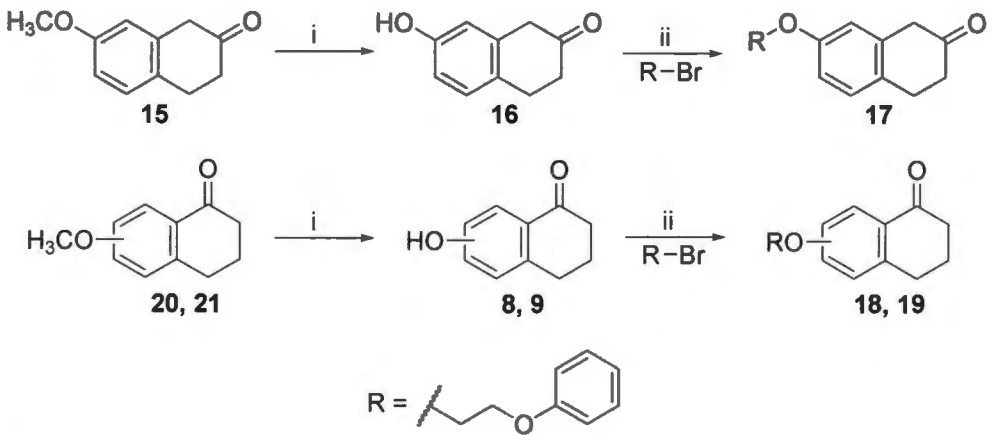


Fig. 2. Synthetic pathway to C6- or C7-substituted ethers of α-tetralone and β-tetralone. Reagents and conditions: (i) $AlCl_3$, toluene; reflux; (ii) acetone, K_2CO_3, reflux.

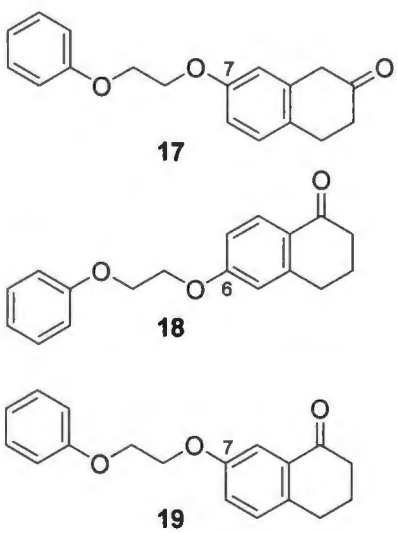
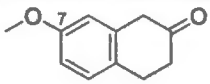
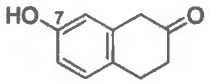
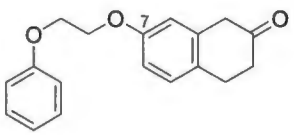
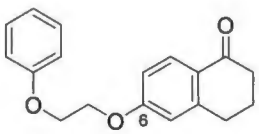
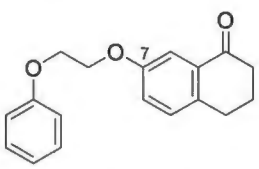


Fig. 3. The structures of tetralone derivatives **17–19**.

The MAO inhibition properties of the 2-phenoxyethoxy-substituted tetralones, compounds **17–19**, were characterised with the aim of comparing their inhibition potencies.

The MAO inhibition potencies of the starting material, 7-methoxy-2-tetralone (**15**), and intermediate, 7-hydroxy-2-tetralone (**16**), in the synthesis of **17** were also measured for comparison. The MAO inhibition properties of 6-hydroxy-1-tetralone (**8**) and 7-hydroxy-1-tetralone (**9**) have been reported (Table 2) (Legoabe *et al.*, 2014; Legoabe *et al.*, 2015). Since the inhibition data given in tables 1 and 2 were recorded under identical conditions as this study, direct comparison of the IC_{50} values are possible. For the inhibition studies, recombinant human MAO-A and MAO-B were used as enzyme sources with kynuramine serving as substrate for both isoforms. The formation of 4-hydroxyquinoline, the ultimate product of kynuramine oxidation by MAO, were measured by fluorescence spectrophotometry (Mostert *et al.*, 2015). After measuring the rate of kynuramine oxidation in the absence and presence of the test inhibitor (at concentrations spanning at least 3 orders of magnitude) sigmoidal dose-response were constructed from which IC_{50} values were estimated (Fig. 4). These values are given in table 3.

Table 3. The measured IC_{50} values for the inhibition of recombinant human MAO-A and MAO-B by tetralone derivatives **17–19** as well as by 7-methoxy-2-tetralone (**15**) and 7-hydroxy-2-tetralone (**16**).

		IC_{50} (μM)[a]		SI[b]
		MAO-A	MAO-B	
15		38.1 ± 1.00	59.8 ± 1.28	0.64
16		70.5 ± 1.83	>100[c]	–
17		56.2 ± 3.44	0.033 ± 0.004	1703
18		1.96 ± 0.72	0.022 ± 0.0013	89
19		1.81 ± 0.326	0.0043 ± 0.0012	421

[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 IC_{50}(MAO-A)/IC_{50}(MAO-B).

[c] No inhibition observed at maximum tested concentration of 100 μM.

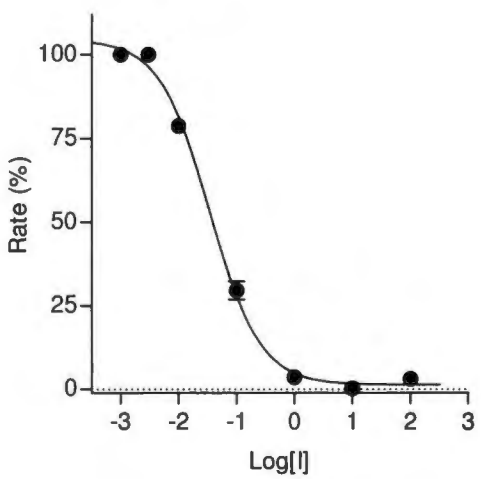


Fig. 4. The sigmoidal dose-response curves for the inhibition of MAO-B by **17**. Data were measured in triplicate and data points are given as mean ± standard deviation (SD).

As anticipated, the C7-substituted β-tetralone ether, compound **17**, proved to be a potent MAO-B inhibitor with an IC_{50} value of 0.033 μM. The inhibition potency of this compound is therefore in the same range as that of the reference MAO-B inhibitors lazabemide (IC_{50} = 0.091 μM) and safinamide (IC_{50} = 0.048 μM) (Petzer *et al.*, 2013). Based on this comparison, **17** may thus be regarded as a potent MAO-B inhibitor. The C6- and C7-substituted α-tetralone ethers, **18** and **19**, also inhibited MAO-B with good potencies and displayed IC_{50} values of 0.022 and 0.0043 μM. The α-tetralone compound **19** is thus a significantly more potent MAO-B inhibitor than **17**.

The results also show that the C7-substituted α-tetralone ethers, **18** (IC_{50} = 1.96 μM) and **19** (IC_{50} = 1.81 μM), are significantly more potent MAO-A inhibitors than β-tetralone homologue **17** (IC_{50} = 56.2 μM). According to the selectivity index values (SI > 89), all of these compounds are, however, selective MAO-B inhibitors with **17** (SI = 1703) being the most selective. The molecular basis for the lower MAO-A and MAO-B inhibition potencies of **17** compared to **18** and **19** is not apparent and will be investigated with molecular docking below. As anticipated, the 2-phenoxyethoxy substituent is required for MAO-A and MAO-B inhibition since 7-hydroxy-2-tetralone (**16**) is a very weak inhibitor of both MAO isoforms.

Employing dialysis, the reversibility of MAO-B inhibition by **17** was investigated. Since **17** proved to be a weak MAO-A inhibitor, the reversibility of MAO-A inhibition was not examined. For comparison the reversibility of MAO-B inhibition by **18** and **19** were also investigated. Interestingly, previous reports suggest that MAO-B inhibition by selected C7-substituted α-tetralone ethers is not completely reversible and these compounds may exhibit tight-binding to the enzyme. To investigate reversibility, the test inhibitors (at concentrations of 4 × IC_{50}) were incubated with MAO-B for 15 min and subsequently dialysed for 24 h.

The mixtures were diluted twofold with the addition of kynuramine and the residual activities were measured. As controls, similar dialysis experiments were carried out in the presence of the irreversible MAO-B inhibitor, (R)-deprenyl (positive control), as well as in the absence of inhibitor (negative control signifying 100% residual activity). The residual MAO-B activity of undialysed mixtures of MAO-B and the test inhibitors were also recorded for comparison. The results show that the test compounds, **17–19**, are reversible MAO-B inhibitors since dialysis restores enzyme activity to levels of 64–149% of the negative control (Fig. 5). Inhibition, however, persists in undialysed mixtures of MAO-B and the test inhibitors with the enzyme activities at 16–48%. As expected, dialysis does not restore MAO-B activity after inactivation with (R)-deprenyl (5%).

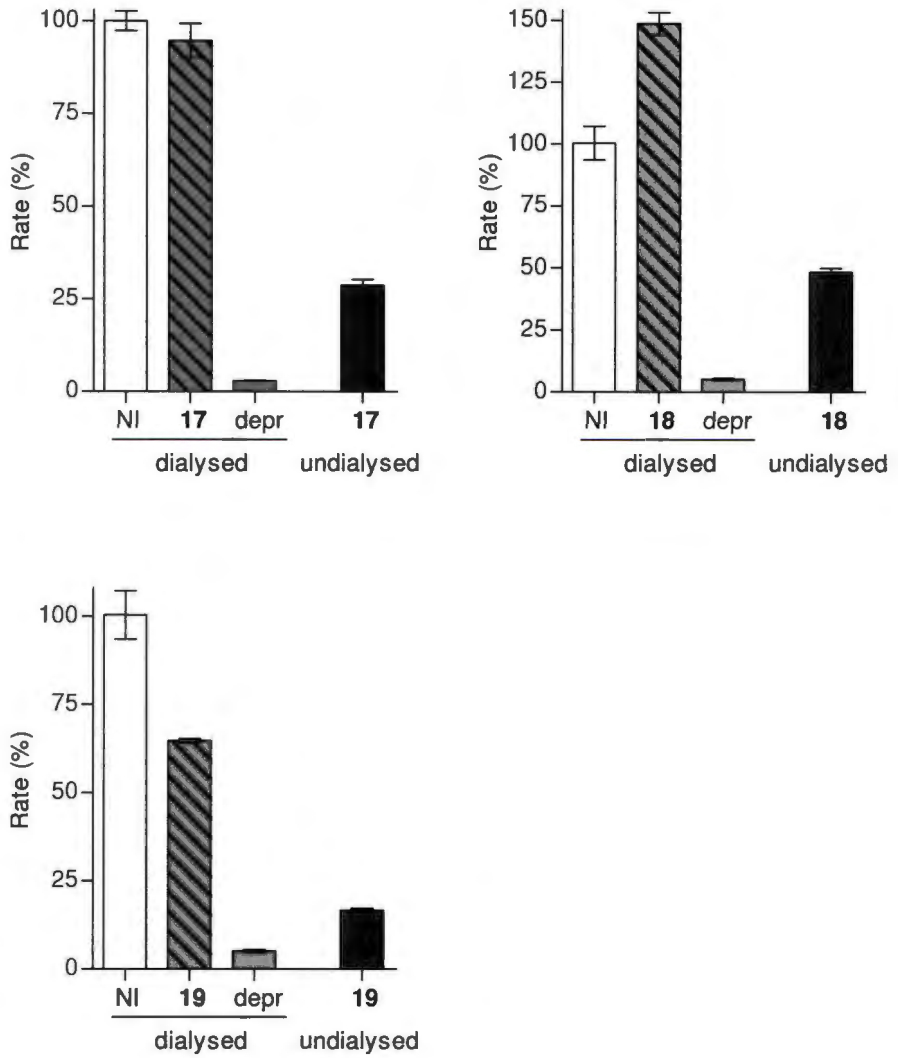


Fig. 5. Compounds **17–19** inhibit MAO-B reversibly. MAO-B and the test inhibitors (at 4 × IC_{50}) were preincubated for 15 min, dialysed for 24 h and the residual enzyme activity was measured (**17–19** dialysed). Similar incubation and dialysis of MAO-B in the absence inhibitor (NI dialysed) and presence of the irreversible inhibitor, (R)-deprenyl (depr dialysed), were also carried out. The residual activities of undialysed mixtures of MAO-B with the test inhibitors were also recorded (**17–19** undialysed).

Since **17** is a reversible MAO-B inhibitor, the enzyme-inhibitor dissociation constant (K_i value) was measured. For this purpose a set of six Lineweaver-Burk plots was constructed using the following inhibitor concentrations: 0 μM, ¼ × IC_{50}, ½ × IC_{50}, ¾ × IC_{50}, 1 × IC_{50} and 1¼ × IC_{50}. For each plot eight concentrations of kynyramine (15–250 μM) were used.

The set of Lineweaver-Burk plots is given in Fig. 6 and is indicative of competitive inhibition since the linear lines intersect on the y-axis. Global (shared) fitting of the inhibition data directly to the Michaelis-Menten equation gave a K_i value of 0.128 ± 0.032 μM (R^2 = 0.98). This value is similar to that (0.056 μM) obtained from a plot of the slopes of the Lineweaver-Burk plots versus inhibitor concentration where the x-axis intercept equals $-K_i$.

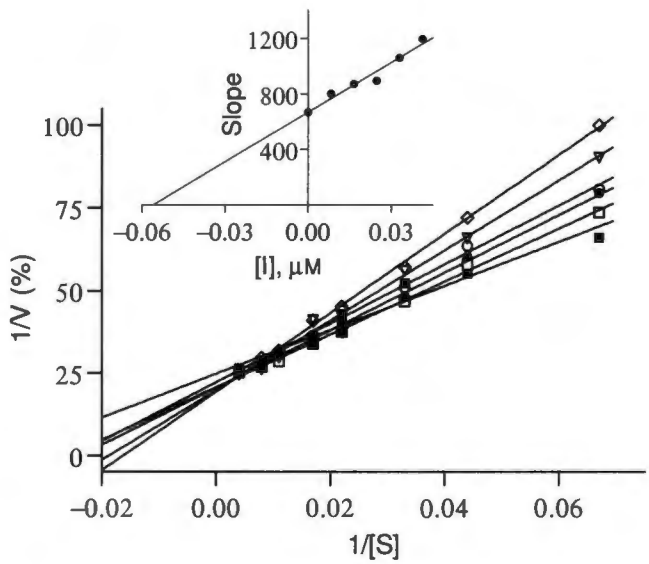


Fig. 6. Lineweaver-Burk plots for the inhibition of MAO-B by **17**. The inset is a graph of the slopes of the Lineweaver-Burk plots versus inhibitor concentration.

To determine possible binding modes and key interactions of **17–19**, the inhibitors were docked into the MAO active sites using the CDOCKER docking algorithm of Discovery Studio 3.1 (Accelrys). For this purpose the literature procedure was followed (Mostert *et al.*, 2015). As enzyme models, the crystal structures of human MAO-A (PDB code: 2Z5X) (Son *et al.*, 2008) and MAO-B (PDB code: 2V5Z) (Binda *et al.*, 2007) were employed. As shown in Fig. 7, compounds **17–19** bind in the MAO-B active site with the tetralone moieties located in the substrate cavity, in proximity to the flavin adenine dinucleotide (FAD) cofactor.

The 2-phenoxyethoxy side chains of these inhibitors project into the entrance cavity of MAO-B (the space beyond Ile-199). These binding orientations are similar to those observed for related compounds, such as 7-(3-chlorobenzyloxy)-4-formylcoumarin (**22**), in crystallographic studies (Fig. 8) (Binda *et al.*, 2007).

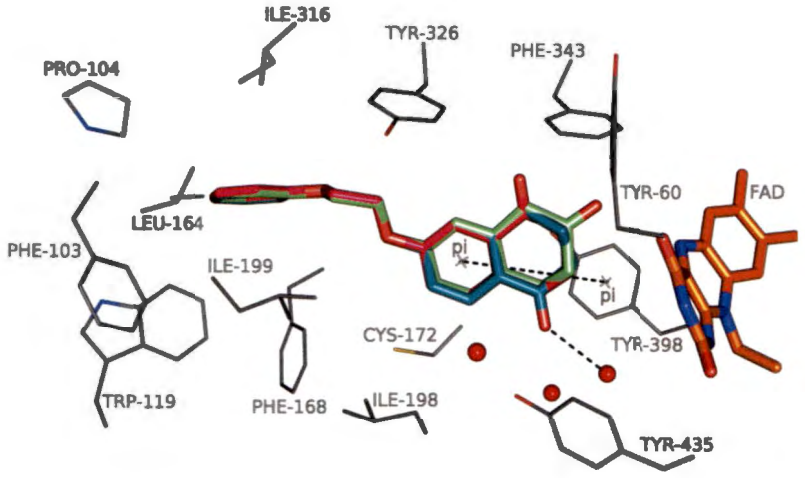
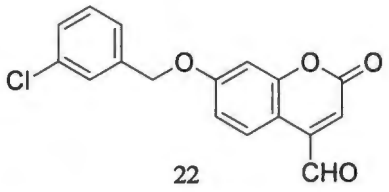


Fig. 7. The docked binding orientations of **17–19** in MAO-B. Key: **17** (light green); **18** (dark green); **19** (magenta).



22

Fig. 8. The structure of 7-(3-chlorobenzyloxy)-4-formylcoumarin (**22**).

Most noteworthy is that the binding orientations of **17–19** are virtually super imposable, which may explain the finding that these compounds are all highly potent MAO-B inhibitors ($IC_{50} < 0.033$ μM).

The principal difference in binding is that, for **18**, the carbonyl oxygen projects towards residues Tyr-398 and Tyr-435 and establishes hydrogen bonding with a water molecule.

For compounds **17** and **19**, the carbonyl oxygen project towards the space lined by Phe-343 and hydrogen bonding is not observed. Since **17–19** are all potent MAO-B inhibitors, hydrogen bonding appears to be of lesser importance and for inhibitor stabilisation, and Van der Waals and π–π-interactions (with Tyr-398) most likely play leading roles. Since the entrance cavity is reported to be a lipophilic space, Van der Waals interactions with the 2-phenoxyethoxy side chains are probably important stabilising interactions (Binda *et al.*, 2002).

Similar to MAO-B, **17–19** binds in MAO-A with the tetralone moieties in proximity to the FAD (Fig. 9). In MAO-A, the positioning of the inhibitors, however, differ notably from each other, particularly with regards to placement of the tetralone moieties. Thus only **18** forms π–π-interactions with Tyr-407, and only **17** and **18** undergoes hydrogen bonding with Tyr-444. Although it would be speculative to correlate a specific interaction with enhanced inhibition potency, the observation that, in MAO-A, the inhibitors are differently placed and thus undergo different interactions may explain their wide range of MAO-A inhibition potencies (IC_{50} = 1.81–56.2 μM). An explanation for the higher MAO-B inhibition potencies (compared to MAO-A), however, is not apparent from the docking studies. Because of limitations imposed by the MAO-A active site, particularly Phe-208, larger inhibitors such as **17–19** studied here, are reported to be better accommodated in MAO-B, and such compounds often are MAO-B selective inhibitors (Hubálek *et al.*, 2005). In MAO-B the residue corresponding to Phe-208 in MAO-A is Ile-199. In contrast to Phe-208, the side chain of Ile-199 may rotate from the MAO-B active site cavity to allow to larger inhibitors to extend into the entrance cavity.

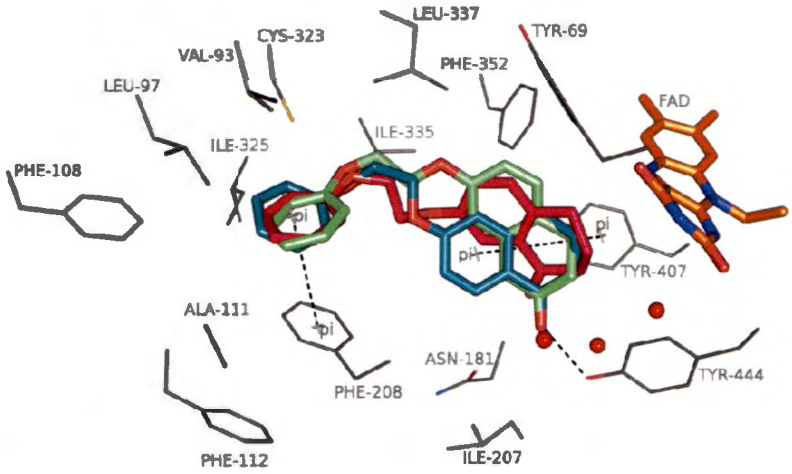


Fig. 9. The docked binding orientations of **17–19** in MAO-A. Key: **17** (light green); **18** (dark green); **19** (magenta).

In conclusion, the present study shows that β-tetralone **17** is a potent and selective MAO-B inhibitor. It is also established that this compound inhibits MAO-B reversibly. Although the α-tetralone homologues **18** and **19** are more potent MAO-B inhibitors, compound **17** displays better isoform selectivity.

For the design of therapies for disorders such as Parkinson's and Alzheimer's diseases, where selective MAO-B inhibition is relevant, **17** may thus represent a suitable lead. The validity of C7-substituted β-tetralone ethers for MAO-B inhibition is thus established.

Acknowledgements

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Conflict of Interest

The authors have no conflicts of interest to declare

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Supplementary Material

The evaluation of 2-phenoxyethoxy-substituted tetralones as inhibitors of monoamine oxidase

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1. Materials and methods

Chemicals, solvents and biological agents: All reagents and solvents for the synthetic chemistry and enzymology were obtained from Sigma-Aldrich and were used without further purification. This included the MAO substrate, kynuramine. 2HBr, and insect cell microsomes containing recombinant human MAO-A and MAO-B (5 mg protein/mL). *NMR spectroscopy*: ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker Avance III 600 spectrometer in $CDCl_3$. The chemical shifts are reported in parts per million (δ) and were referenced to the residual solvent signal at 7.26 ppm (^{1}H NMR) and 77.0 ppm (^{13}C NMR). The following notations were used to indicate spin multiplicities: s (singlet), d (doublet), dd (doublet of doublets), t (triplet) 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 (mp) were measured with a Buchi B-545 melting point apparatus and are uncorrected. *Thin-layer chromatography (TLC)*: Reaction progress was monitored with silica gel 60 aluminium coated TLC sheets (Merck). The sheets were developed in a mobile phase consisting of ethyl acetate:petroleum ether (40:60), and visualised under an UV-lamp at a wavelength of 254 nm and/or by staining with iodine vapour.

Fluorescence spectrophotometry: The fluorescence emission of 4-hydroxyquinoline was measured with a Varian Cary Eclipse fluorescence spectrophotometer.

2. Synthesis of 7-(2-phenoxyethoxy)-3,4-dihydronaphthalen-2(1*H*)-one (17)

While in an atmosphere of nitrogen, 7-methoxy-2-tetralone (**15**) (28.4 mmol) was added to a suspension of anhydrous aluminum trichloride ($AlCl_3$, anhydrous) (71 mmol) in toluene (125 mL) at room temperature. The mixture was heated (110 °C) under reflux, for 2 h. The reaction was cautiously quenched with ice water (125 mL). The reaction was extracted to ethyl acetate (150 mL × 3) and the combined ethyl acetate phases were subsequently washed with water (150 mL × 3). The ethyl acetate phase was dried over $MgSO_4$ (5 g), filtered and removed by rotary evaporation to yield a beige-orange solid residue. The product was recrystallised from toluene (28 mL) at room temperature (for 14 h) to yield 7-hydroxy-2-tetralone (**16**). Crystallised 7-hydroxy-2-tetralone (**16**) appeared as light beige crystals. **16**: yield of 41%; mp 111–114.6 °C (toluene).

7-Hydroxy-2-tetralone (**16**) (1.85 mmol) was suspended in acetone (15 mL) containing K_2CO_3 (3.70 mmol). The reaction was treated with 2-bromoethyl phenyl ether (2.35 mmol) and heated (85 °C) under reflux for 6 h. The reaction progress was monitored using silica gel TLC with ethyl acetate:petroleum ether (40:60) as mobile phase. The workup consisted of filtration of the reaction through a pad of celite, and the crude was purified with silica gel column chromatography using ethyl acetate:petroleum ether (40:60) as mobile phase, to yield 7-(2-phenoxyethoxy)-3,4-dihydronaphthalen-2(1*H*)-one (**17**) (Legoabe *et al.*, 2014; Legoabe *et al.*, 2015).

Compound **17** was prepared in a yield of 0.65%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.53 (t, 2H, J = 6.7 Hz), 2.99 (t, 2H, J = 6.7 Hz), 3.54 (s, 2H), 4.22 – 4.38 (m, 4H), 6.71 (d, 1H, J = 2.6 Hz), 6.80 (dd, 1H, J = 2.6, 8.3 Hz), 6.93 – 7.00 (m, 3H), 7.13 (d, 1H, J = 8.3 Hz), 7.25 – 7.33 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 27.5, 38.5, 45.2, 66.4, 66.7, 113.1, 114.4, 114.6, 121.1, 128.6, 129.2, 129.5, 134.5, 157.6, 158.5, 210.6.

3. Synthesis of compounds 18 and 19

6-Methoxy-1-tetralone (**20**) or 7-methoxy-1-tetralone (**21**) (28.4 mmol) was added to a suspension of anhydrous aluminum trichloride ($AlCl_3$, anhydrous) (71 mmol) in toluene (125 mL) at room temperature. The mixture was heated (85 °C) under reflux for 2 h. The reaction was cautiously quenched with ice water (125 mL). The reaction was extracted to ethyl acetate (150 mL × 3) and the combined ethyl acetate phases were washed with water (150 mL × 3). The ethyl acetate phase was dried over $MgSO_4$ (5 g), filtered and removed by rotary evaporation. The product was recrystallised from ethyl acetate (28 mL) at room temperature to yield 6-hydroxy-1-tetralone (**8**) and 7-hydroxy-1-tetralone (**9**). **8**: yield of 49%; mp 155.5–156.6 °C (ethylacetate). **9**: yield of 34%; mp 154–167 °C (ethylacetate).

6-Hydroxy-1-tetralone (**8**) or 7-hydroxy-1-tetralone (**9**) (1.85 mmol) was suspended in acetone (15 mL) containing K_2CO_3 (3.70 mmol). The reaction was treated with 2-bromoethyl phenyl ether (2.35 mmol) and heated (85 °C) under reflux for 6 h. The reaction progress was monitored using silica gel TLC with ethyl acetate:petroleum ether (40:60) as mobile phase. The acetone was removed by rotary evaporation and the solid residue was suspended in water (35 mL). The suspension was extracted with 35 mL of ethyl acetate and the ethyl acetate phase was washed with water (35 mL). The ethyl acetate phase was dried over $MgSO_4$, filtered and removed by rotary evaporation.

The product was recrystallised from boiling ethanol (10 mL) at room temperature to yield compounds **18** and **19** (Legoabe *et al.*, 2014; Legoabe *et al.*, 2015).

Compound **18** was prepared in a yield of 6.32%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.58 (s, 0.4H), 3.00 (s, 1.7H), 3.02 (t, 2H, J = 6.4 Hz), 3.40 (t, 2H, J = 6.1 Hz), 4.78 – 4.85 (m, 2H), 4.86 – 4.95 (m, 2H), 7.40 – 7.51 (m, 5H), 7.79 (d, 2H, J = 7.7 Hz), 8.32 (d, 1H, J = 9.6 Hz). ^{13}C NMR (151 MHz, DMSO) δ 23.0, 29.3, 38.4, 66.1, 66.7, 113.3, 113.7, 114.5, 120.9, 125.9, 128.8, 129.6, 147.3, 158.2, 162.2, 196.2; APCI-HRMS *m/z*: calcd for $C_{18}H_{19}O_3$ (MH^+), 283.1334, found 283.1346.

Compound **19** was prepared in a yield of 14.9%. mp 116.1 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.57 (s, 0.4H), 2.99 (s, 1.6H), 3.07 (d, 2H, J = 5.7 Hz), 3.36 (t, 2H, J = 6.0 Hz), 4.75 – 4.87 (m, 4H), 7.44 (t, 1H, J = 7.3 Hz), 7.45- 7.50 (m, 2H), 7.68 (dd, 1H, J = 2.8, 8.4 Hz), 7.75 – 7.82 (m, 3H), 7.88 (d, 1H, J = 2.8). ^{13}C NMR (151 MHz, DMSO) δ 23.1, 28.1, 38.5, 66.2, 66.6, 109.7, 114.5, 120.8, 121.5, 129.6, 130.5, 133.0, 137.4, 156.9, 158.3, 197.4; APCI-HRMS *m/z*: calcd for $C_{18}H_{19}O_3$ (MH^+), 283.1334, found 283.1328.

4. Measurement of IC_{50} values for the inhibition of MAO

The protocol for the measurement of IC_{50} values for the inhibition of MAO-A and MAO-B has been reported in literature (Mostert *et al.*, 2015; Mostert *et al.*, 2016). For this purpose the recombinant human MAO-A and MAO-B enzymes were used. *Enzyme reactions*: The enzyme reactions were carried out in white 96-well microtiter plates (Eppendorf) to a final volume of 200 µL. Potassium phosphate buffer at pH 7.4 (100 mM, made isotonic with KCl) served as reaction medium. The reactions contained kynuramine (50 µM) and the test inhibitors at concentrations of 0.003–100 µM.

Stock solutions of kynuramine were prepared in the reaction buffer while stock solutions of the test inhibitors were prepared in DMSO. The inhibitors were added to the reactions to yield a final concentration of 4% DMSO.

For each inhibitor evaluated, control reactions carried out in the absence of inhibitor (but still containing 4% DMSO) were included. 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. *Measurement and data analysis*: At endpoint, the reactions were terminated with the addition of 80 µL sodium hydroxide (2 N) and the fluorescence of 4-hydroxyquinoline, the oxidation product of kynuramine, was measured (λ_{ex} = 310; λ_{em} = 400 nm) (Navoroli *et al.*, 2005). To quantify 4-hydroxyquinoline, a linear calibration curve was constructed with authentic 4-hydroxyquinoline (0.047–1.56 µM). The rate of 4-hydroxyquinoline formation was fitted to the one site competition model of the Prism 5 software package (GraphPad) to obtain sigmoidal plots from which the IC_{50} values were estimated. All enzyme reactions were carried out in triplicate and IC_{50} values are given as the mean ± standard deviation (SD).

5. Investigation of reversibility of inhibition by dialysis

The protocol for investigating the reversibility of MAO inhibition by dialysis has been reported in literature (Mostert *et al.*, 2015; Mostert *et al.*, 2016). For dialysis, 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. *Preincubation*: Recombinant human MAO-B (0.03 mg/mL) and the test compounds (**17–19**) were firstly preincubated for 15 min at 37 °C. The volume of these preincubations was 0.8 mL and potassium phosphate buffer (100 mM, pH 7.4) containing 5% sucrose, served as solvent.

Stock solutions of the test compounds were prepared in DMSO and added to the preincubations to yield 4% DMSO and an inhibitor concentration of 4 × IC_{50}. *Dialysis*: These mixtures were subsequently dialysed at 4 °C in 80 mL of dialysis buffer, which consisted of potassium phosphate buffer (100 mM, pH 7.4) containing 5% sucrose.

The dialysis buffer was replaced with fresh buffer at 3 h and 7 h after the start of dialysis. *Assay*: Following 24 h of dialysis, the reactions were diluted 2-fold (into 1.5 mL microcentrifuge tubes) with the addition of kynuramine (dissolved in potassium phosphate buffer, 100 mM, pH 7.4, made isotonic with KCl). This yielded a final concentration of kynuramine of 50 µM. The final concentration of the test inhibitor in these reactions was 2 × IC_{50}, and the final volume of the reactions was 500 µL. The reactions were incubated for 20 min at 37 °C in a water bath and were terminated with the addition of NaOH (400 µL of 2 N) and 1000 µL water. *Measurement and data analysis*: The fluorescence of 4-hydroxyquinoline in these samples was measured (λ_{ex} = 310; λ_{em} = 400 nm) employing a 3.5 mL quartz cuvette (pathlength 10 × 10 mm) (Navoroli *et al.*, 2005). To quantify 4-hydroxyquinoline, a linear calibration curve was constructed with authentic 4-hydroxyquinoline (0.047–1.56 µM). *Controls*: MAO-B was similarly pre-incubated and dialysed in the absence of inhibitor (negative control) as well as in the presence of the irreversible inhibitor, (R)-deprenyl (positive control). The concentration of (R)-deprenyl in the preincubations was 4 × IC_{50} (IC_{50} = 0.079 µM) (Petzer *et al.*, 2012). *Undialysed mixtures*: For comparison, undialysed mixtures of MAO-B and the test inhibitors were maintained at 4 °C for 24 h and diluted and assayed as above. All reactions were carried out in triplicate and the residual enzyme catalytic rates are expressed as mean ± SD.

6. Construction of Lineweaver-Burk plots

To illustrate that compound **17** acts as a competitive MAO-B inhibitor, a set of six Lineweaver-Burk plots was constructed. The first plot was constructed in the absence of inhibitor, while the remaining five plots were constructed in the presence of the following concentrations of **17**: ¼ × IC_{50}, ½ × IC_{50}, ¾ × IC_{50}, 1 × IC_{50} and 1¼ × IC_{50}. For each plot, kynuramine was used at concentrations of 15–250 μM.

The enzyme reactions were carried out to a volume of 500 μL in 1.5 mL microcentrifuge tubes and the concentration of MAO-B was 0.015 mg protein/mL. All enzyme reactions and activity measurements were carried out as described above for the dialysis experiments. The K_i value was estimated by global (shared) fitting of the inhibition data directly to the Michaelis-Menten equation using the Prism 5 software package. For comparison, the K_i value was also estimated from a plot of the slopes of the Lineweaver-Burke plots versus inhibitor concentration (x-axis intercept equals $-K_i$).

7. Protocol for docking with CDOCKER

Docking of the test inhibitors in the MAO active sites was carried out with the Windows-based Discovery Studio 3.1 software package (Accelrys). Unless otherwise specified, the default settings of Discovery Studio were used for all calculations. As protein models, the reported X-ray crystal structures of human MAO-A (PDB code 2Z5X) (Son *et al.*, 2008) and human MAO-B (PDB code 2V5Z) (Binda *et al.*, 2007) were obtained from the Brookhaven Protein Data Bank. The pKa values and protonation states of the ionisable amino acids were firstly calculated and hydrogen atoms were added at pH 7.4 to the protein models. When necessary, the valences of the FAD cofactors and co-crystallised ligands were corrected, and the FAD cofactors were set to the oxidised state.

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 minimised using the Smart Minimizer algorithm (maximum steps, 50000) and the implicit generalized Born solvation model with molecular volume. The co-crystallised ligands and backbone constraints were removed from the models and the binding sites were identified from an analysis of the protein cavities.

With the exception of 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, all waters of the models were subsequently deleted. These active site waters in the MAOs are considered to be conserved and were thus retained. The structures of the ligands to be docked were drawn in Discovery Studio, the geometries were briefly optimised using a Dreiding-like forcefield (5000 iterations) and the structures were prepared for docking with the Prepare Ligands protocol. Atom potential types and partial charges were subsequently assigned to the ligands with the Momany and Rone CHARMm forcefield. Docking was carried out with the CDOCKER algorithm. Ten random conformations were generated for each ligand, the heating target temperature was set to 700 K and full potential mode was used. The docking solutions were finally refined using in situ ligand minimisation with the Smart Minimizer algorithm and the illustrations were prepared with PyMol (DeLano, 2002).

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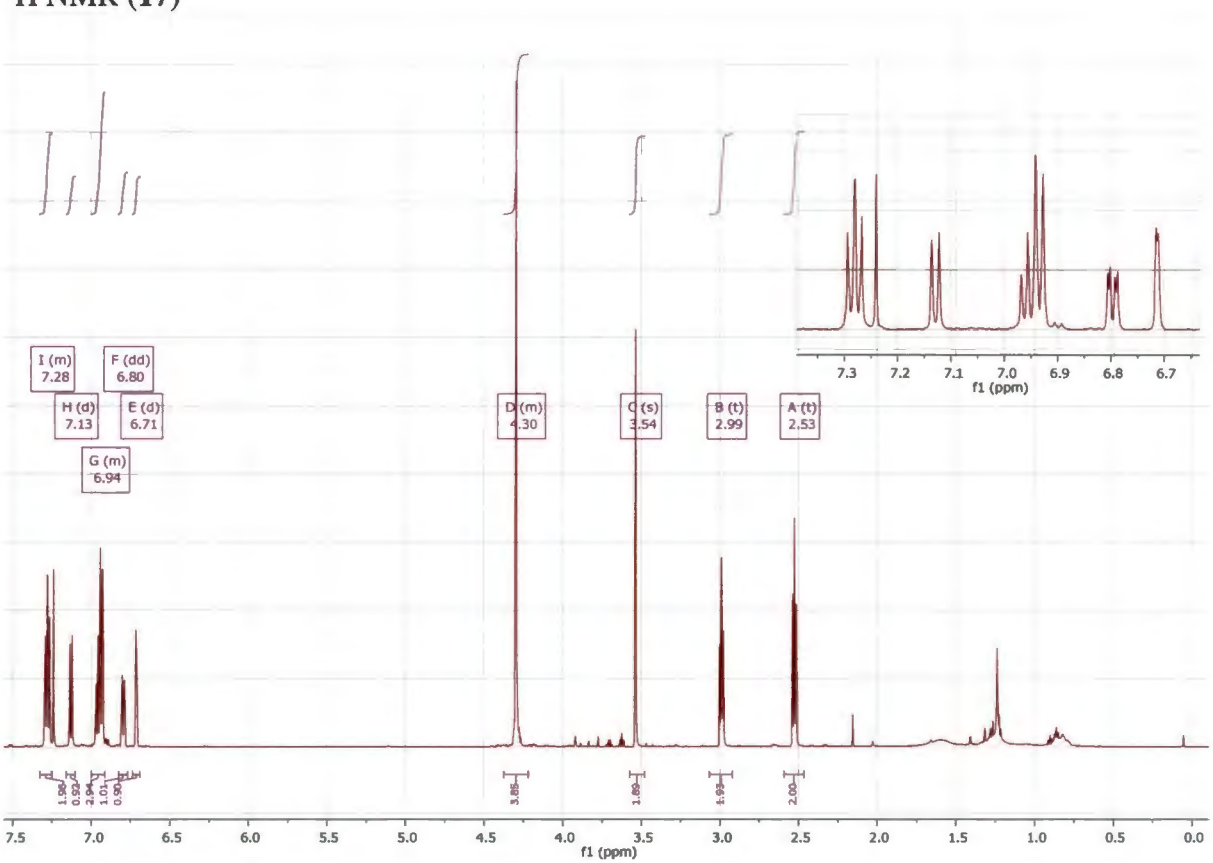
^{1}H NMR (17)



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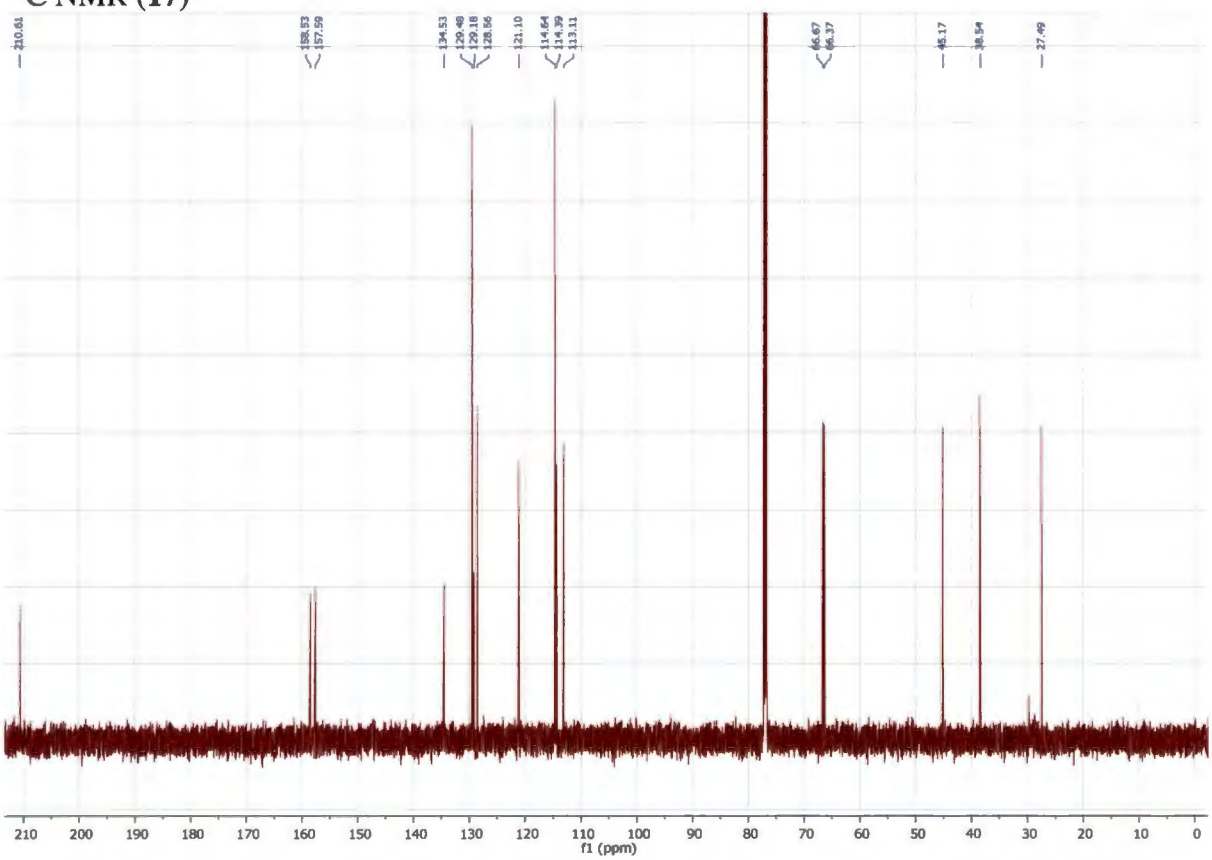
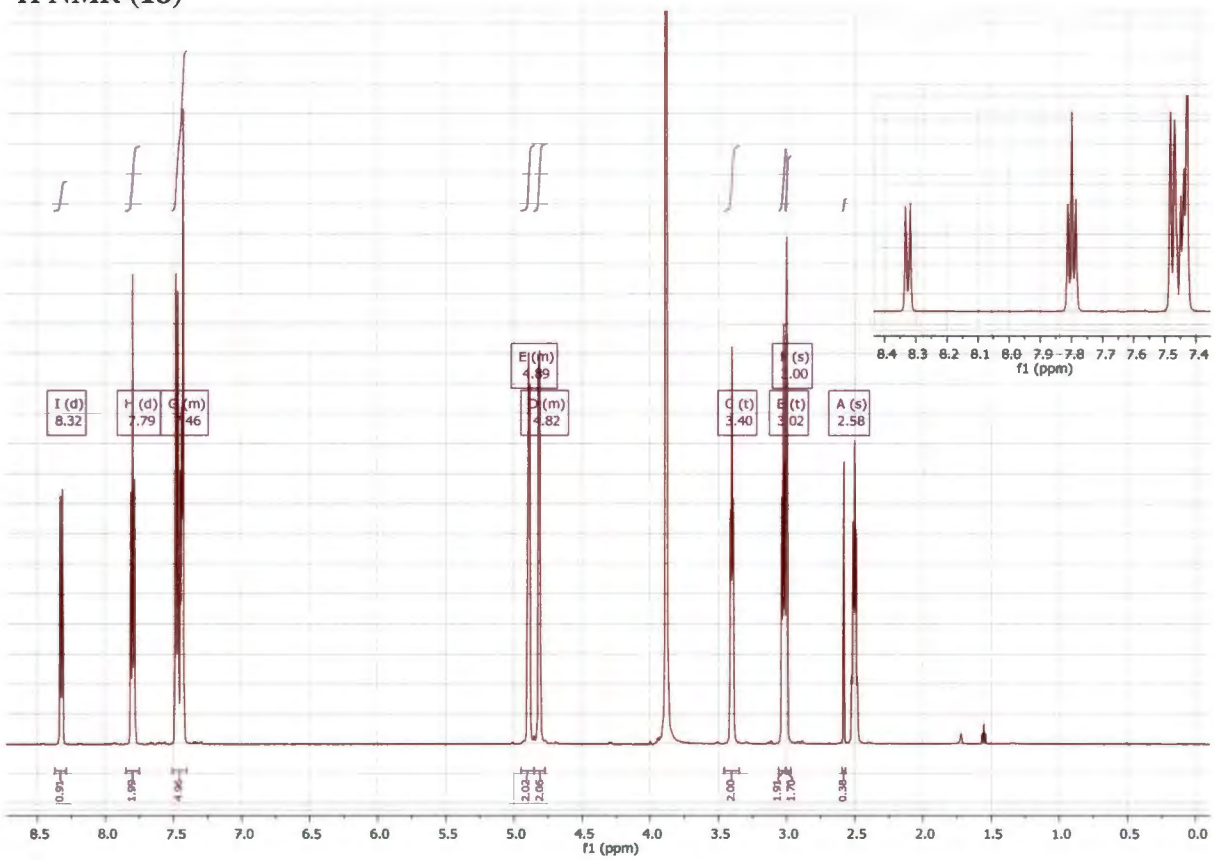
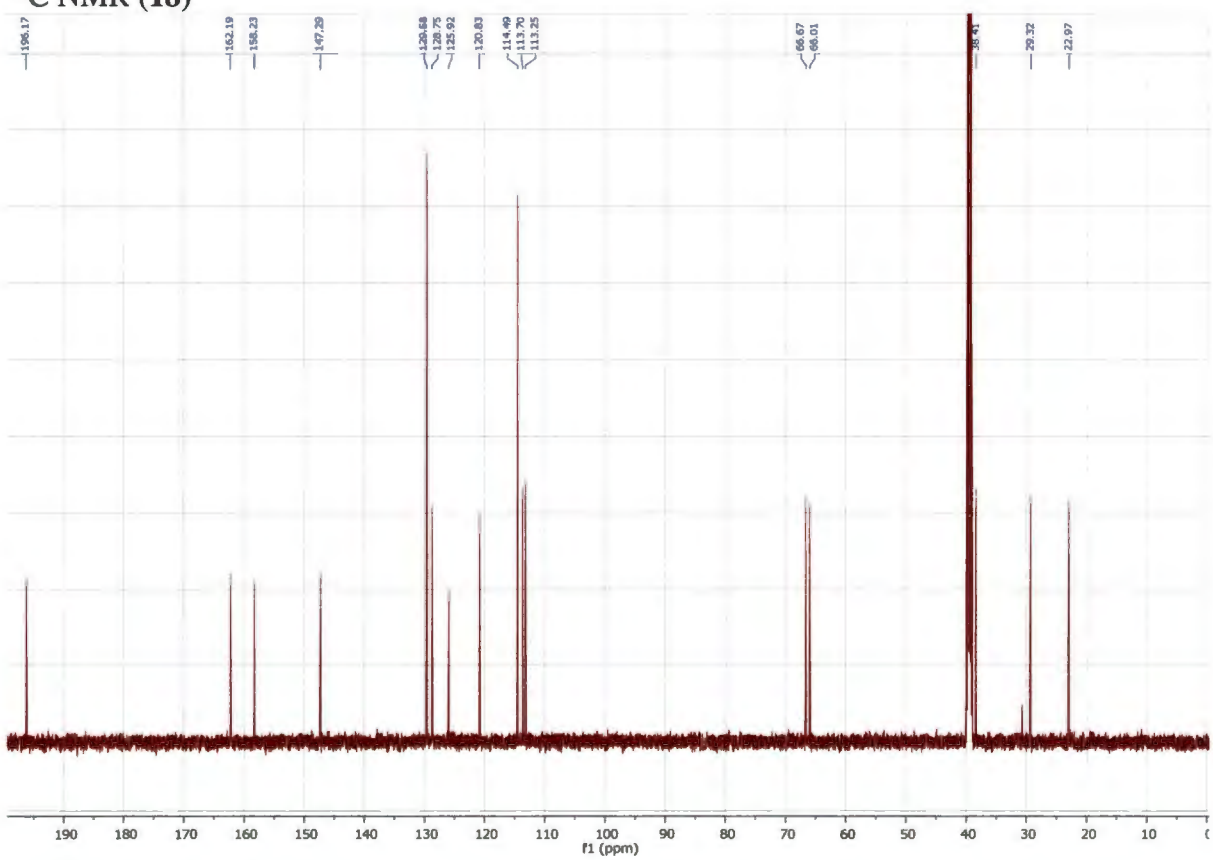


^{1}H NMR (**18**)



^{13}C NMR (**18**)



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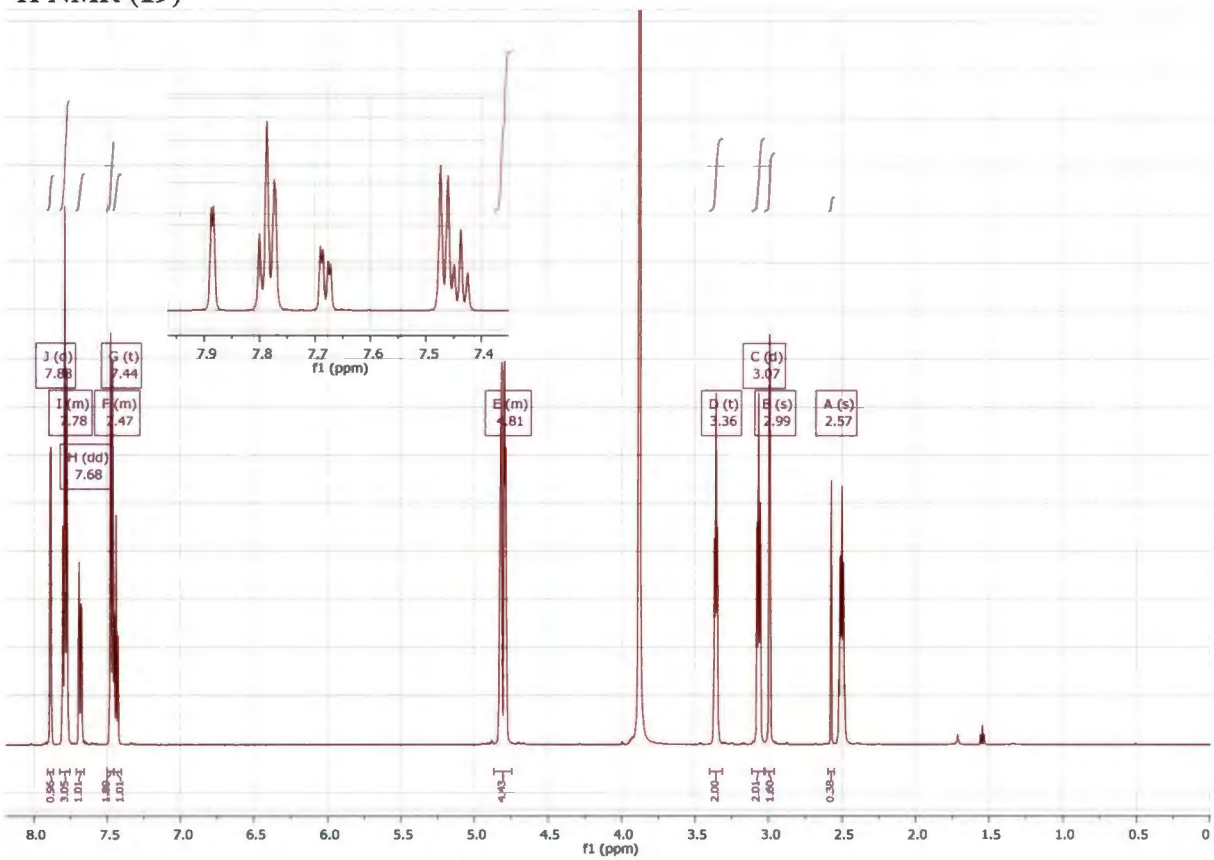
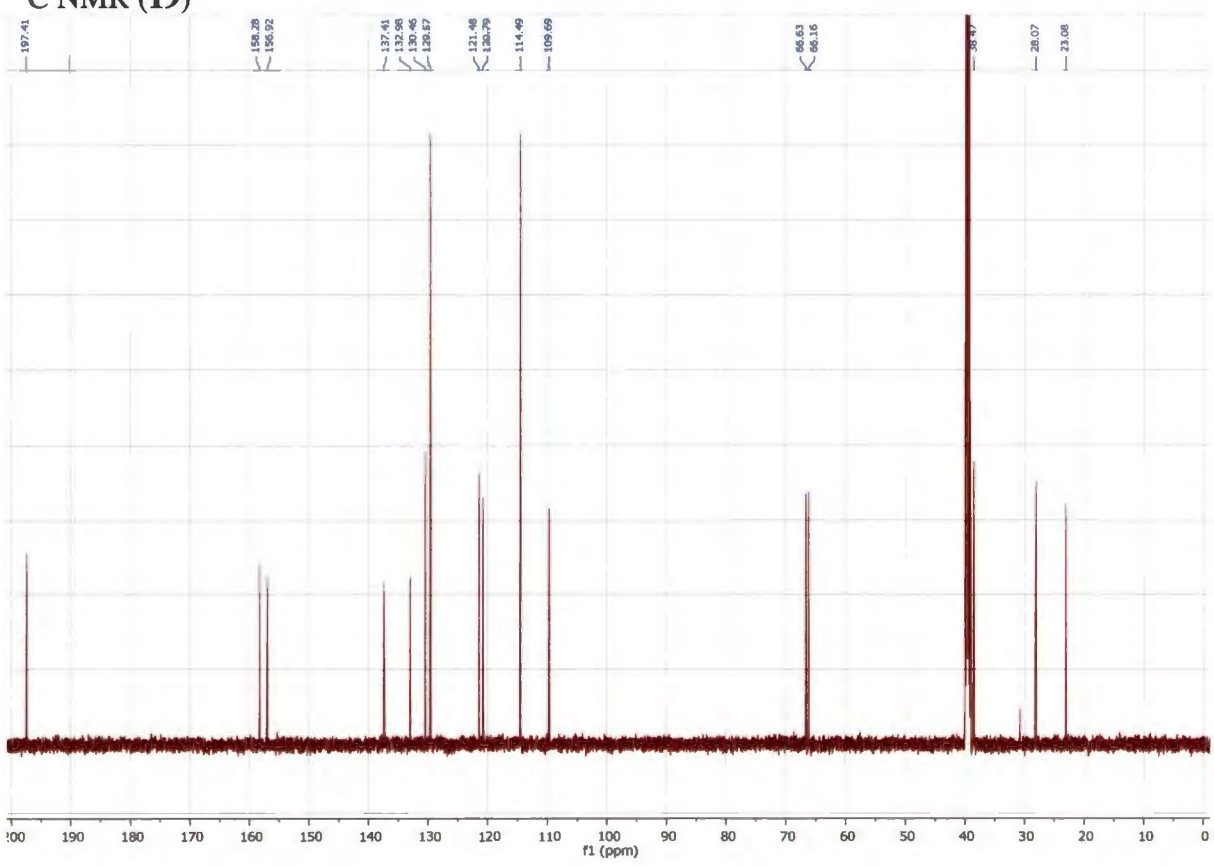


^{13}C NMR (**19**)



CHAPTER 6

MANUSCRIPT D

The evaluation of N-propargylamine-2-aminotetralin as an irreversible inhibitor of monoamine oxidase

Authors' contributions:

- The experimental work, interpretation of results and documentation of this study was carried out by L. Meiring.
- 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 L. Meiring's Ph.D thesis.

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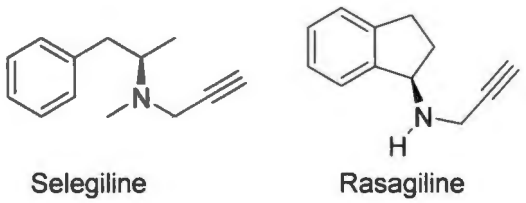
Running title: N-propargylamine-2-aminotetralin, an irreversible MAO inhibitor
Keywords: monoamine oxidase, MAO, inhibition, irreversible, propargylamine, selegiline, (R)-deprenyl, rasagiline, 2-PAT.

Abstract: Monoamine oxidase B (MAO-B) inhibitors are established therapy for Parkinson's disease and act, in part, by blocking the MAO-catalysed metabolism of dopamine in the brain. Two propargylamine-containing MAO-B inhibitors, selegiline [(R)-deprenyl] and rasagiline, are currently used in the clinic for this purpose. These compounds are mechanism-based inactivators and, after oxidative activation, form covalent N(5) adducts with the FAD co-factor. An important consideration is that selegiline and rasagiline display specificity for MAO-B over the MAO-A isoform thus reducing the risk of tyramine-induced changes in blood-pressure. In the interest of discovering new propargylamine MAO inhibitors, the present study synthesises racemic N-propargylamine-2-aminotetralin (2-PAT), a compound that may be considered as both a six-membered ring analogue of rasagiline and a semi-rigid N-desmethyl ring-closed analogue of selegiline. The in vitro human MAO inhibition properties of this compound were measured and compared to those of rasagiline, selegiline and N-methyl-N-propargylamine-2-aminotetralin (2-MPAT), the latter a known MAO inhibitor and close structural analogue of 2-PAT. The results show that 2-PAT is a 20-fold more potent inhibitor of MAO-A (IC_{50} = 0.721 μM) compared to MAO-B (IC_{50} = 14.6 μM). Interestingly, dialysis studies found that 2-PAT is a reversible MAO-A inhibitor, while acting as an inactivator of MAO-B. Since reversible MAO-A inhibitors are much less liable to potentiate tyramine-induced side effects than MAO-A inactivators, it is reasonable to suggest that 2-PAT could be an useful and safe therapeutic agent for disorders such as Parkinson's disease and depression.

Parkinson's disease is a progressive disorder which involves the degeneration of specific neuronal pathways in the central nervous system. In Parkinson's disease, the dopamine-containing neurons of the nigrostriatal pathway are thus destroyed, resulting in a deficiency of dopamine at the nerve terminals in the corpus striatum (Olanow *et al.*, 2009). Replacing the lost dopamine with L-3,4-dihydroxyphenylalanine (L-dopa), the direct metabolic precursor of dopamine, remains the most valuable approach for the treatment of the symptomatic Parkinson's disease (Carlsson, 2002). L-Dopa is, however, extensively metabolised in the periphery which reduces its therapeutic efficiency. For this reason, L-dopa is often co-administered with inhibitors of key L-dopa metabolising enzymes such as dopa decarboxylase and catechol-O-methyl transferase (Perdosa & Timmermann, 2013; Talati *et al.*, 2009). The inhibition of these enzymes in the peripheral tissues increases the availability of L-dopa for uptake into the brain and allows for the effective L-dopa dose to be reduced (Fahn *et al.*, 2004). This approach thus decreases the occurrence of adverse effects as a result of large L-dopa dosages. The efficacy of L-dopa may also be enhanced by inhibiting the metabolism of dopamine with monoamine oxidase (MAO) type B inhibitors (Youdim *et al.*, 2006). The inhibition of MAO-B in the brain conserves central dopamine and, when co-administered with L-dopa, further enhances the levels of central dopamine derived from L-dopa (Shoulson *et al.*, 2002; Fernandez & Chen, 2007; Finberg *et al.*, 1998). MAO-B inhibitors are thus established therapy in Parkinson's disease and as monotherapy in early Parkinson's disease may delay the need for L-dopa therapy (Pålhagen *et al.*, 1998). Two MAO-B inhibitors, (R)-deprenyl (also known as selegiline) and rasagiline, are registered for the treatment of Parkinson's disease (Fig. 1) (Youdim *et al.*, 2006). These are irreversible MAO-B inhibitors containing the propargylamine functional group.

Reversible MAO-B inhibitors such as safinamide, which has completed phase III development for the management of Parkinson´s disease, are also under consideration as future medications (Borgohain *et al.*, 2014; Dézsi and Vécsei, 2014).



Selegiline Rasagiline

Fig. 1. The structures of the irreversible MAO-B inhibitors, selegiline and rasagiline.

Two isoforms of MAO are expressed in mammals, MAO-A and MAO-B (Youdim & Bakhle, 2006). Although both isoforms metabolise dopamine in the brain, only MAO-B inhibitors are used for the treatment of Parkinson's disease (Youdim *et al.*, 2006). This is mainly due to potentially fatal adverse effects that may occur when MAO-A inhibitors are combined with tyramine-containing food such as cheese and wine. MAO-A inhibitors prevent the metabolism of tyramine in the gastrointestinal mucosa and hepatic tissues leading to high systemic concentrations of tyramine and an elevation in blood-pressure (Da Prada *et al.*, 1988; Flockhart, 2012). This sympathomimetic response may be further exacerbated when MAO-A inhibitors and L-dopa are combined, hence the avoidance of MAO-A inhibitors in Parkinson's diseasetherapy (Teychenne *et al.*, 1975). With appropriate dietary restrictions MAO-Ainhibitors are, however, used for the treatment of major depressive disorder (Schwartz, 2013; Lum & Stahl, 2012). In depression MAO-A inhibitors act by inhibiting the central metabolism of serotonin and norepinephrine, thereby enhancing neurotransmission mediated by these neurotransmitters (Youdim *et al.*, 2006).

MAO-A inhibitors that have been used for the treatment of depression include phenelzine, isocarboxazid, tranylcypromine and iproniazid (Fig. 2) (Youdim *et al.*, 2006; Finberg, 2014). These are irreversible mechanism-based MAO inhibitors and, after oxidation by the enzyme, form covalent adducts with the flavin adenine dinucleotide (FAD) co-factor of MAO (Edmondson *et al.*, 2004; Binda *et al.*, 2004; Binda *et al.*, 2005; Milczek *et al.*, 2008). Reversible inhibitors such as moclobemide and toloxatone have also been used in the therapy of depression.

In contrast to irreversible MAO-A inhibitors, reversible inhibitors are safer and better-tolerated and are not associated with tyramine-induced changes in blood-pressure (Bonnet, 2003; Provost *et al.*, 1992). This is presumably because with reversible inhibition, the increasing amounts of tyramine compete with and displace the reversible inhibitor from MAO-A thus allowing for normal metabolism to occur. This limits the amount of tyramine that reaches the systemic circulation. MAO-B inhibitors have also recently found application in depression therapy. A transdermal delivery system of selegiline has been developed and reported to be effective in the treatment of major depressive disorder in clinical trials (Pae *et al.*, 2014). With this formulation selegiline does not inhibit MAO-A in the gastrointestinal and hepatic systems, and thus the risk of tyramine-induced adverse effects is low.

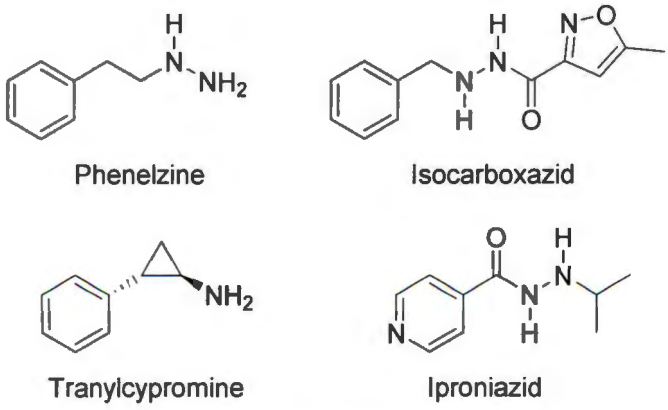


Fig. 2.The structures of the irreversible MAO inhibitors, phenelzine, isocarboxazid, tranylcypromine and iproniazid.

In spite of the risks associated with their use, irreversible inhibitors of the MAOs are an important class of therapeutic agents. Irreversible MAO inhibitors used in the clinic are mechanism-based inhibitors and contain the following functional groups: propargylamine [e.g. (R)-deprenyl, rasagiline], cyclopropylamine (e.g. tranylcypromine), hydrazine (e.g. phenelzine, isocarboxazid), haloallylamine (mofegiline) and N-(2-aminoethyl)carboxamide (e.g. lazabemide) (Fig. 3).

Mechanism-based inhibitors, also known as suicide inhibitors, are metabolised by the MAOs to reactive intermediates which form covalent adducts with the FAD co-factor at the N(5) and C(4a) positions of the isoalloxazine ring (Edmondson *et al.*, 2004; Binda *et al.*, 2004; Binda *et al.*, 2005; Milczek *et al.*, 2008). Motivated by the academic challenge of designing and discovering high potency and isoform-specific MAO inhibitors that may be useful therapeutic agents, the present study synthesises N-propargylamine-2-aminotetralin (2-PAT), and evaluates the racemic mixture as an in vitro inhibitor of the human MAOs (Fig. 4). This compound may be considered as both a six-membered ring analogue of rasagiline and a semi-rigid N-desmethyl ring-closed analogue of selegiline. Even though structurally very similar to rasagiline, the MAO inhibition properties of 2-PAT may be very different. For example, literature reports that N-methylation of rasagiline significantly affects the inactivation kinetics, inhibition potency and isoform selectivity (Hubálek *et al.*, 2004). Furthermore, the MAOs are much less sensitive to inhibition by the *S*-enantiomer of rasagiline. The N-methyl analogue of 2-PAT, N-methyl-N-propargylamine-2-aminotetralin (2-MPAT), has been studied. In vitro inhibition of rat brain MAO-A and MAO-B found that the enantiomeric pure forms as well as the racemate are potent inhibitors of both MAO isoforms (Table 1) (Hazelhoff *et al.*, 1985). The (+)-enantiomer, however displays highest MAO-B inhibition and best isoform selectivity (over MAO-A).

An earlier study also found that racemic 2-MPAT is a selective inhibitor of MAO-B when using rat liver MAO (Tipton *et al.*, 1982). Via washing of mitochondria it was shown that 2-MPAT inhibits rat liver MAO irreversibly.

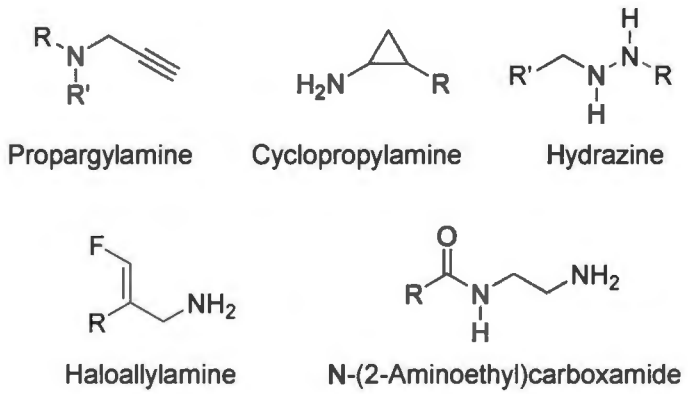


Fig 3. Functional groups responsible for mechanism-based inhibition of the MAOs: propargylamine, cyclopropylamine, hydrazine, haloallylamineand N-(2-aminoethyl)carboxamide.

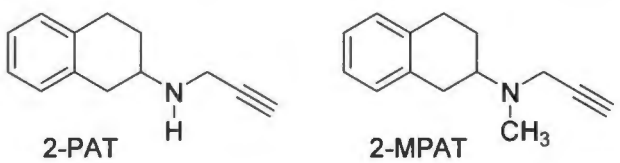


Fig. 4. The structures of N-propargylamine-2-aminotetralin (2-PAT) and N-methyl-N-propargylamine-2-aminotetralin (2-MPAT).

Table 1. The reported IC_{50} values for the inhibition of rat brain MAO-A and MAO-B by 2-MPAT.

	IC_{50} (μM)[a]		**SI[b]**
	MAO-A	**MAO-B**	
(±)2-MPAT	0.051	0.041	1.2
(+)2-MPAT	0.140	0.016	8.8
(–)2-MPAT	0.046	0.088	0.5

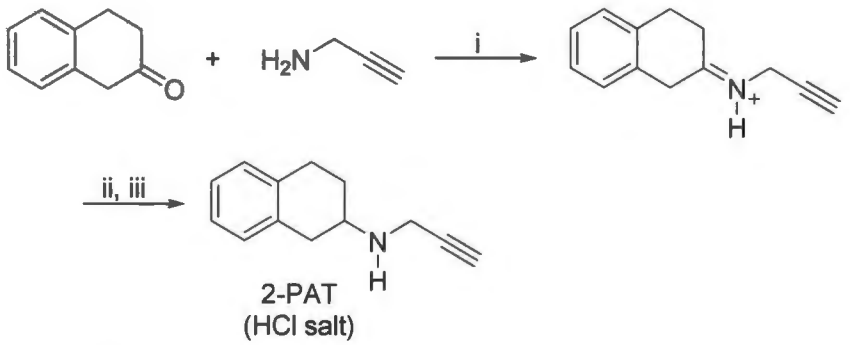
[a] Values obtained from reference (Hazelhoff *et al.*, 1985).

[b]The selectivity index (SI) equals the ratio of IC_{50}(MAO-A)/IC_{50}(MAO-B).

A number of synthetic approaches are possible for the synthesis of propargylamine derivatives. Rasagiline and derivatives thereof are synthesised by alkylation of 1-aminoindan with propargyl chloride or propargyl bromide in the presence of base, usually potassium carbonate (Guozhen *et al.*, 2014).

In the present study we have explored an alternative route for the synthesis of 2-PAT (Scheme 1). Commercially available β–tetralone (1 equiv.) and propargylamine (5 equiv.) were firstly dehydrated in a Dean-Stark trap in the presence of p-toluenesulfonic acid (0.056 equiv.) (Zhuang *et al*, 1993). After reduction with sodium cyanoborohydride ($NaCNBH_4$) and a workup that involves acidification with HCl, washing with diethyl ether and extraction to dichloromethane the hydrochloric acid salt of 2-PAT was obtained in low yield as a powder (7.9%). Curiously this salt is soluble in water, dichloromethane, chloroform, methanol and weakly in acetone while being insoluble in most other organic solvents (e.g. diethyl ether, ethyl acetate). Both the ^{1}H and ^{13}C NMR spectra, recorded in $CDCl_3$ and CD_3OD, corresponded with the proposed structure. In CD_3OD the most characteristic ^{13}C signals included the CH_2 (79 ppm) and the acetylenic carbons [55(CH) and 75(C) ppm] of the propargyl group. On the ^{1}H NMR spectrum the propargyl CH_2 (3.30–3.23 ppm) was observed to correlate with the carbon signal at 79 ppm while the acetylenic CH (3.72–3.62 ppm) correlates with the carbon signal at 55 ppm. In $CDCl_3$ the exchangeable NH (10 ppm) was visible as a broad singlet, integrating for 2 protons as expected for the HCl salt. Interestingly on the FT-IR spectrum a characteristic absorption band at 2100 $cm^{-1}$for the terminal alkyne (due to stretching vibrations) was absent. This absorption band also was not observed for propargyl amine or the free base of 2-PAT (prepared with Na_2CO_3), while reported to be present at low intensity at 2100 cm^{-1} in the FT-IR spectrum of rasagiline free base (Başköse *et al.*, 2012). The carbonyl stretch vibration of β-tetralone at 1711 cm^{-1}, however, was absent in the IR spectra of both the hydrochloric acid salt and free base of 2-PAT.

The mass spectrum for hydrochloric acid salt of 2-PAT yielded a mother ion [MH^+] of 186.1274, in close agreement with the calculated value.



Scheme 1.Synthetic pathway to 2-PAT. Reagents and conditions: (i) p-toluenesulfonic acid, benzene, Dean-Stark trap; (ii) $NaCNBH_4$, methanol, N_2, rt; (iii) 10% HCl.

The MAO inhibition properties of 2-PAT was investigated in vitro using recombinant human MAO-A and MAO-B (Novaroli *et al.*, 2005). To measure MAO catalytic rates, kynuramine served as substrate for both isoforms and is oxidised to 4-hydroxyquinoline as the ultimate product. After alkalinisation of the enzyme reactions at endpoint (after 20 min incubation), 4-hydroxyquinoline was quantified by fluorescence spectrophotometry (Mostert *et al.*, 2015). By thus measuring the rate of kynuramine oxidation in the absence and presence of 2-PAT (at concentrations of 0.003–100 μM) sigmoidal dose-response curves were constructed in triplicate from which IC_{50} values were estimated (Fig. 5). The results show that 2-PAT inhibits MAO-A with an IC_{50} value of 0.721 ± 0.030μM while MAO-B is inhibited an IC_{50} value of 14.6 ± 0.282μM (Table 2). Based on IC_{50} values, 2-PAT is therefore 20-fold more potent as an inhibitor of MAO-A compared to MAO-B. For comparison, the propargylamine MAO inhibitors, clorgyline, pargyline and selegiline inhibits the human MAOs with IC_{50} values of 0.0026 (MAO-A), 13 (MAO-A) and 0.079 (MAO-B) μM, respectively, under identical conditions (Strydom *et al.*, 2012; Petzer *et al.*, 2012). Compared to clorgyline and selegiline, the MAO inhibition potencies of 2-PAT is therefore modest.

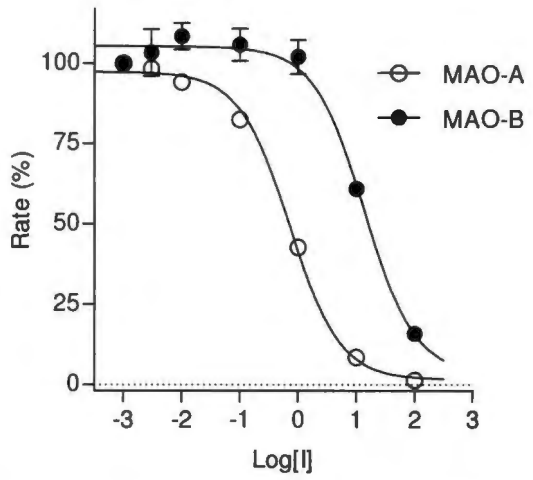


Fig. 5. The sigmoidal dose-response curves of the inhibition of MAO-A and MAO-B by 2-PAT. Rates were measured in triplicate and are shown as mean ± standard deviation (SD).

Table 2. The IC_{50} values for the inhibition of recombinant human MAO-A and MAO-B by 2-PAT and reference inhibitors.

	IC_{50} (μM)[a]		**SI[b]**
	MAO-A	**MAO-B**	
(±)2-PAT	0.721 ± 0.030	14.6 ± 0.282	0.05
Pargyline	15.6 ± 0.424	0.782 ± 0.035	20
Selegiline	29.7 ± 1.44	0.095 ± 0.013	313

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

[b] Ratio of IC_{50}(MAO-A)/IC_{50}(MAO-B).

To verify that 2-PAT acts indeed irreversibly as expected for propargylamine derived MAO inhibitors, the reversibility of inhibition was investigated using dialysis (Mostert *et al.*, 2015). For this purpose 2-PAT (at a concentration of 4 × IC_{50}) was incubated with MAO-A and MAO-B for 15 min and the mixture was subsequently dialysed for 24 h.

As negative controls similar incubations of the enzymes were carried out in absence of inhibitor, while as positive controls MAO-A and MAO-B were incubated in the presence of the irreversible inhibitors, pargyline and selegiline, respectively, with the inhibitor concentrations also at 4 × IC_{50}. After dialysis, the mixtures were diluted twofold with the addition of kynuramine and the formation 4-hydroxyquinoline was measured at an endpoint of 20 min. The MAO activities of the negative control samples signified 100% residual activity. For comparison, the residual activities of undialysed mixtures of MAO-A and MAO-B and 2-PAT were also measured. The results of these studies show that for MAO-A, inhibition by 2-PAT is reversed by dialysis with the enzyme activity recovering to 102% (Fig. 6). In contrast, enzyme activity is not recovered by dialysis when MAO-A is inhibited by pargyline, with the residual activity at 0.9%. Inhibition persisted in undialysed mixtures of MAO-A and 2-PAT with the residual activity at 46%. Since dialysis clearly restores enzyme activity it may be concluded that 2-PAT is a reversible inhibitor of human MAO-A. For MAO-B, dialysis however does not reverse inhibition by 2-PAT with the residual activity at only 7.5%. This is similar to the residual MAO-B activity after inhibition with selegiline (3.9%) and subsequent dialysis. The almost complete inhibition observed with 2-PAT and selegiline, which cannot be reversed by dialysis, is evidence that these compounds are irreversible human MAO-B inhibitors. Complete inhibition (0.3%) is also observed in undialysed mixtures of MAO-B and 2-PAT.

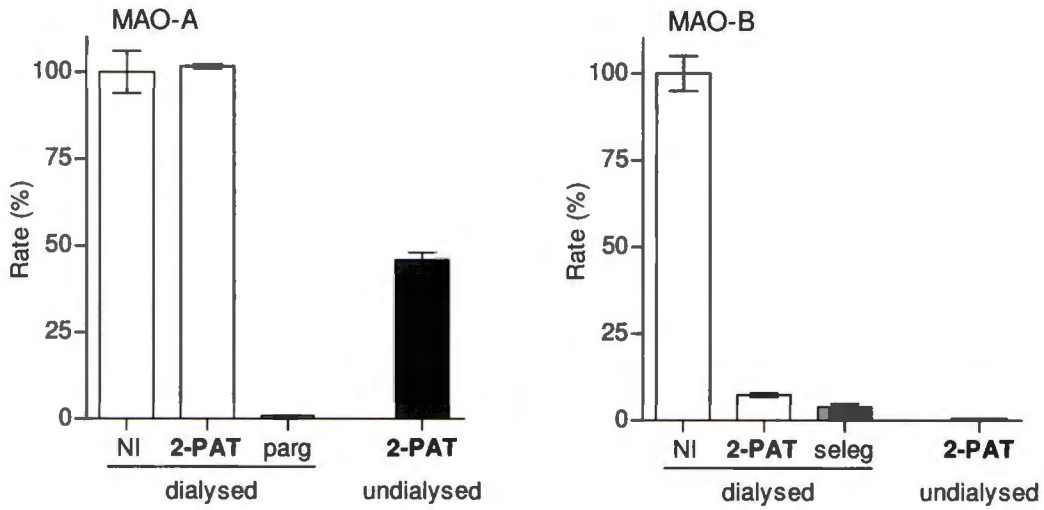


Fig. 6.2-PAT is a reversible MAO-A inhibitor and an irreversible MAO-B inhibitor. The MAO enzymes and 2-PAT (at a concentration of 4 × IC_{50}) were incubated for 15 min, dialysed for 24 h and the residual enzyme activity was measured (2-PAT dialysed). Similar incubation and dialysis of the MAOs in the absence (NI dialysed) and presence of the irreversible inhibitors, pargyline (parg dialysed) and selegiline (seleg dialysed) were also carried out. The residual MAO activity of undialysed mixtures of the MAOs with 2-PAT was also recorded (2-PAT undialysed).

To further investigate the reversible interaction between 2-PAT and MAO-A, the enzyme-inhibitor dissociation constant (K_i value) was measured. For this purpose six Lineweaver-Burk (double-reciprocal) plots was constructed employing the following six inhibitor concentrations: 0 μM, ¼ × IC_{50}, ½ × IC_{50}, ¾ × IC_{50}, 1 × IC_{50} and 1¼ × IC_{50}. For each plot eight concentrations of kynyramine (15–250 μM) were used. The set of Lineweaver-Burk plots is shown in Fig. 7. Since the lines are linear and intersect on the y-axis, it may be concluded that the inhibition of MAO-A by 2-PAT is competitive. From global (shared) fitting of the inhibition data directly to the Michaelis-Menten equation, a K_i value of 0.941 ± 0.079μM (R^2 = 0.99) is estimated. A similar value of (1.32μM) is obtained by graphing the slopes of the Lineweaver-Burk plots versus inhibitor concentration (x-axis intercept equals – K_i).

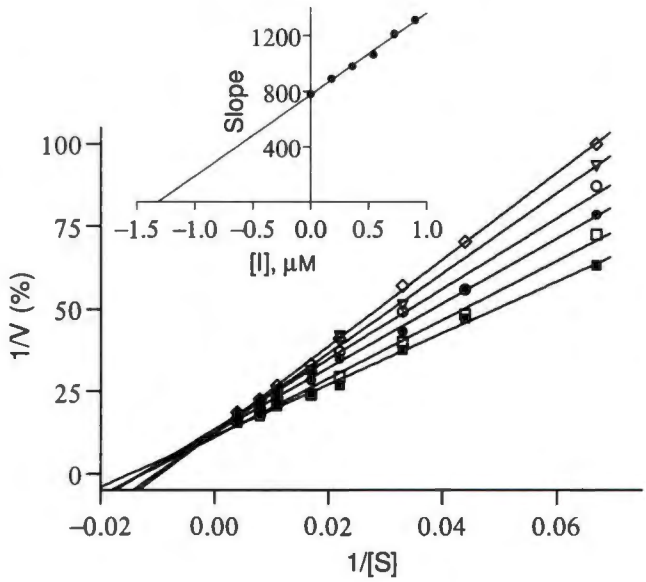


Fig.7. Lineweaver-Burk plots for the inhibition of human MAO-A by 2-PAT. The inset is a graph of the slopes of the Lineweaver-Burk plots versus inhibitor concentration.

An attempt was made to gain insight into the interactions between the MAOs and 2-PAT on the molecular level. For the reversible interaction between 2-PAT and the MAOs, both enantiomers of the inhibitor were docked into the active sites of the enzymes using the CDOCKER docking algorithm of Discovery Studio 3.1 (Accelrys). For this purpose the literature protocol was followed (Mostert *et al.*, 2015) and the reported crystal structure of human MAO-A (PDB code: 2Z5X) and human MAO-B (PDB code: 1S2Q) served as enzyme models (Son *et al.*, 2008, Binda *et al.*, 2004). As shown in Fig. 8, 2-PAT fits in both MAO-A and MAO-B active site cavities. In this respect 2-PAT binds in proximity to the FAD cofactor for both isoforms, and in MAO-B, is thus situated in the substrate cavity. The enantiomers of 2-PAT exhibit similar binding orientations to the respective MAO isoforms.

Interestingly, the propargylamine group of 2-PAT is directed towards the FAD in MAO-A, while being directed towards the entrance of the active site in MAO-B. Based on the reversibility of MAO-A inhibition and irreversibility of MAO-B inhibition by 2-PAT, this is contrary to what is expected. For an irreversible inhibitor, the propargylamine is expected to bind close to the FAD where it may act as a suicide substrate. This inconsistency between the experimental results and docking study suggests that 2-PAT may bind with several orientations to the MAOs, and for MAO-B not all orientations lead to mechanism-based inactivation. This point of view is supported by the observation that 2-PAT is a relatively small molecule compared to the active site cavities of the MAOs.

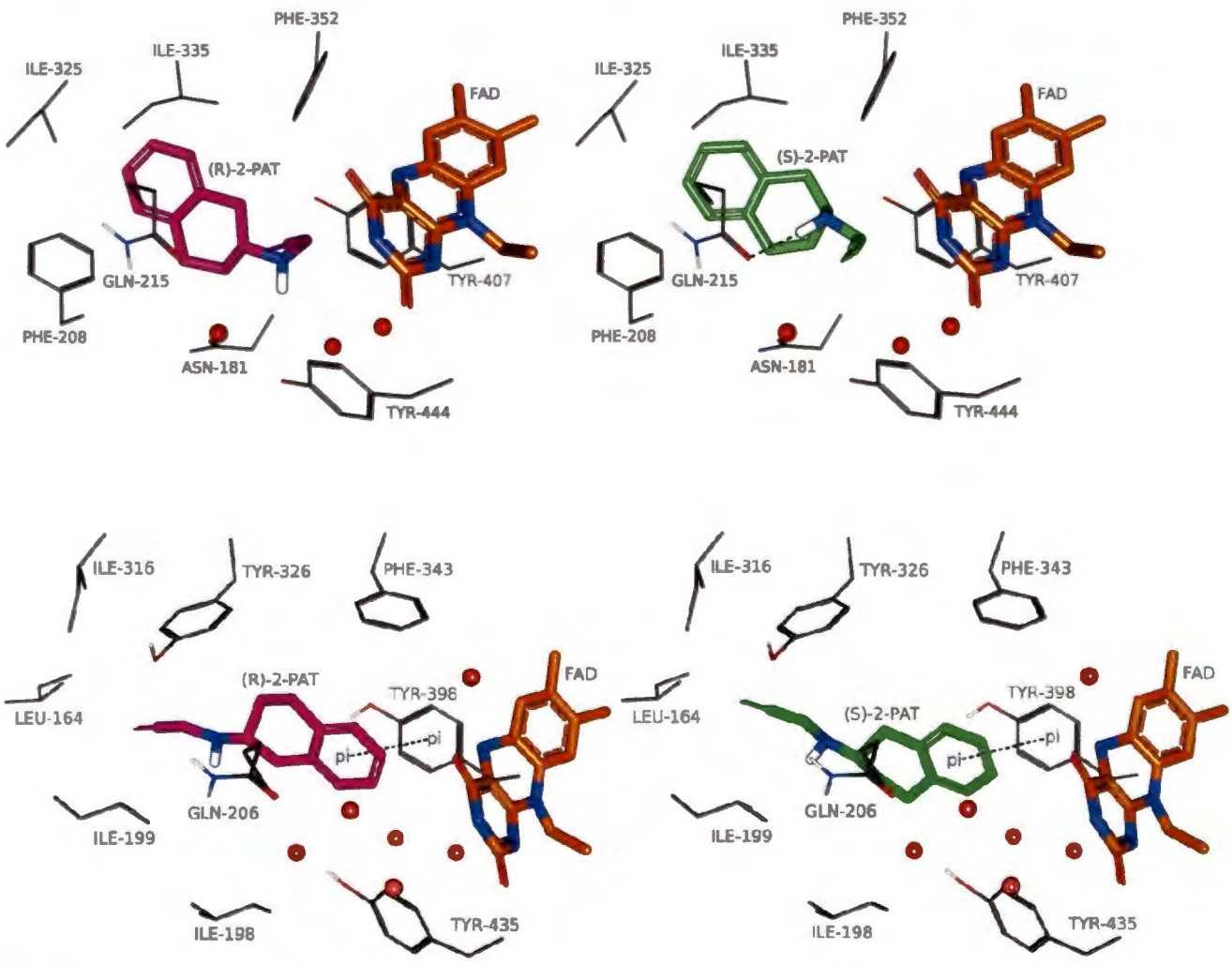


Fig. 8.The proposed reversible interactions between 2-PAT and MAO-A (top) and MAO-B (bottom).

The results in Fig. 9 shows that, in MAO-A, both enantiomers of rasagiline bind with the propargylamine group directed towards the FAD. In MAO-B, orientations with the propargylamine group directed towards the FAD as well as in the opposite direction, were recorded. This further underscores the proposal that small molecule inhibitors may adopt different binding orientations in the relatively large MAO active sites, and for propargylamines, not all orientations lead to inactivation. Key interactions of 2-PAT and rasagiline in the MAOs include hydrogen bonding to Gln-215 (MAO-A), Gln-206, Tyr-398 and water molecules (MAO-B). π-π interactions with Tyr-398 in MAO-B is another important interaction, particularly where 2-PAT and rasagiline binds with the propargylamine group directed towards the FAD.

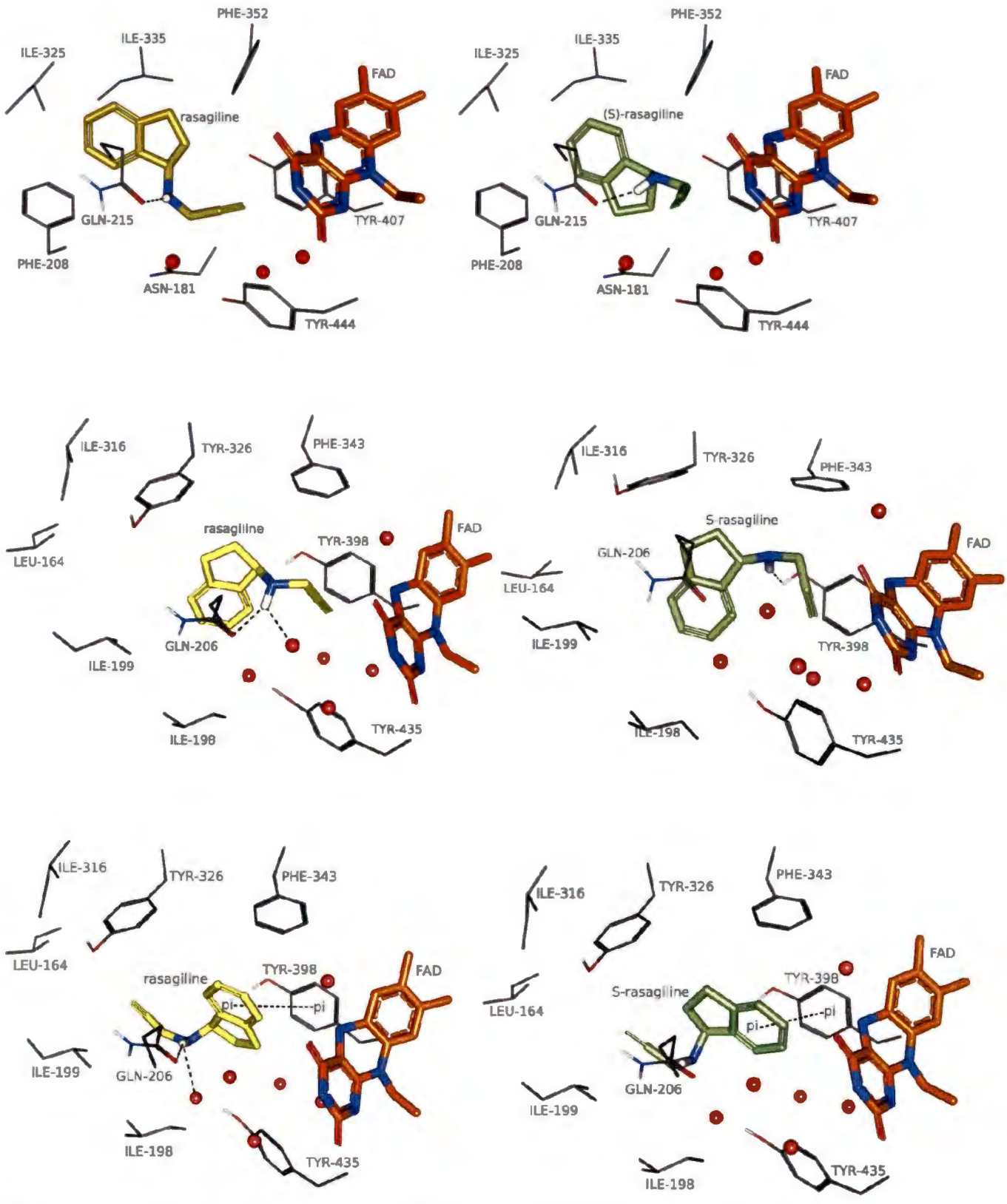


Fig. 9.The proposed reversible interactions of rasagiline and its S-enantiomer with MAO-A (top) and MAO-B (middle and bottom).

In conclusion, this study shows that racemic 2-PAT is mechanism-based inactivator of human MAO-B while acting as a reversible inhibitor of human MAO-A. In this regard 2-PAT may be considered as a relatively potent MAO-A inhibitor with an IC_{50} value of 0.721 μM. Since 2-PAT is approximately fivefold more potent thantoloxatone (IC_{50} = 3.92 μM), a clinically used antidepressant and reversible MAO-A inhibitor, the MAO-A inhibition of 2-PAT may thus be clinically relevant (Petzer *et al.*, 2013). Based on its reversibility of MAO-A inhibition, 2-PAT is unlikely to potentiate tyramine-induced side effects as expected for MAO-A inactivators. Although not nearly as potent as selegiline as a MAO-B inhibitor (based on IC_{50}), 2-PAT would still be expected to produce effective MAO-B inhibition in vivo since inhibition is time-dependent and thus cumulative upon chronic dosage regimen. This is the case for pargyline, an effective MAO inhibitor in vivo while displaying a modest IC_{50} in vitro. Based on these considerations 2-PAT could be useful and safe therapeutic agent for disorders such as Parkinson's disease, particularly where depression is a comorbidity of Parkinson's disease. In a follow-up study the MAO inhibition properties of the individual enantiomers of 2-PAT needs to be investigated in order to determine if either of the pure enantiomers provides enhanced benefits compared to the racemate. A protocol for the synthesis of 2-PAT with improved yields is, however, required for such studies.

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Conflict of Interest

The authors have no conflicts of interest to declare

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Supplementary Material

The evaluation of N-propargylamine-2-aminotetralin as anirreversible inhibitor of monoamine oxidase

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1. Materials and methods

Chemistry: All starting materials and reagents were obtained from Sigma-Aldrich (unless otherwise noted) and were used without further purification. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a 600 MHz spectrometer employing chloroform-*d*or methanol-$d_4$as NMR solvents. Chemical shifts are reported in parts per million (δ) downfield from the signal of tetramethylsilane and were referenced to the residual solvent signal at 7.26 ppm (^{1}H NMR) and 77.0 ppm (^{13}C NMR) for chloroform-*d* and 3.31 ppm (^{1}H NMR) and 49.00 ppm (^{13}C NMR) for methanol-d_4. Spin multiplicities are given as s (singlet), d (doublet), t (triplet), qd (quartet of doublets) or m (multiplet). High resolution mass spectra (HRMS) were recorded on a BrukermicrOTOF-Q II mass spectrometer in atmospheric-pressure chemical ionization (APCI) mode (positive mode). Melting points (mp) were determined with a BuchiB-545 melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was carried out using silica gel 60 (Merck) with UV_{254} fluorescent indicator, employing a mobile phase of ethyl acetate:petroleum ether (40:60). The developed sheets were visualised by staining with iodine vapour.

Biology: All solvents and chemicals used for the biology were obtained from Sigma-Aldrich (unless otherwise noted). These include microsomes from insect cells containing recombinant human MAO-A and MAO-B (5 mg/mL), kynuraminedihydrobromide, selegiline hydrochloride and pargyline hydrochloride. Fluorescence spectrophotometry was carried out with a Varian Cary Eclipse fluorescence spectrophotometer.

2. Synthesis of the hydrochloric acid salt of N-propargylamine-2-aminotetralin (2-PAT)

β–Tetralone (5 mmol) and propargylamine (25 mmol) were dissolved in 40 mL benzene and p-toluenesulfonic acid (0.282 mmol) was added. The mixture was heated under refluxed for 2 h while water was separated with a Dean-Stark distilling trap. The reaction solvent was evaporated in vacuo and the residue, a dark orange oil, was dissolved in 11 mL methanol. Under an atmosphere of nitrogen, $NaCNBH_4$ (3.06 mmol) was added and the reaction was stirred for 30 min at room temperature. Water (11 mL) was added and the reaction was subsequently acidified with HCl (10%) to a pH of 1 and extracted to diethyl ether (3 × 30 mL). The pH of the aqueous phase was adjusted to 8–9 with the addition of solid $NaHCO_3$, and extracted to dichloromethane (3 × 30 mL). The dichloromethane phases were combined and evaporated in vacuo to yield a light yellow solid, which was suspended and washed in boiling ethyl acetate (40 mL). The resulting cream coloured solid was collected by filtration and appeared pure by TLC (Zhuang *et al*, 1993).

Yield of 7.9%; mp 127.5 °C; APCI-HRMS m/z: calcd for $C_{13}H_{16}N$ (MH^+), 186.1277, found 186.1274.

^{1}H NMR (600 MHz, Methanol-d_4) δ 7.19 – 7.08 (m, 4H), 4.10 (d, J = 2.6 Hz, 2H), 3.72 – 3.62 (m, 1H), 3.30 – 3.23 (m, 2H), 3.04 – 2.87 (m, 3H), 2.39 – 2.29 (m, 1H), 1.93 – 1.82 (m, 1H); ^{13}C NMR (151 MHz, Methanol-d_4) δ 135.86, 132.89, 130.23, 129.74, 127.86, 127.43, 79.14, 74.76, 55.12, 34.95, 32.84, 28.21, 26.91.

^{1}H NMR (600 MHz, Chloroform-*d*) δ 10.07 (s, 2H), 7.17 – 7.00 (m, 4H), 3.96 (qd, J = 16.9, 2.6 Hz, 2H), 3.80 – 3.68 (m, 1H), 3.35 – 3.17 (m, 2H), 3.04 – 2.82 (m, 2H), 2.54 (t, J = 2.6 Hz, 1H), 2.52 – 2.44 (m, 1H), 2.12 (qd, J = 12.0, 5.8 Hz, 1H); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 134.48, 131.87, 129.24, 128.69, 126.70, 126.27, 78.08, 72.90, 52.86, 33.40, 31.86, 27.67, 25.84.

3. Procedure for the measurement of IC_{50} values

The procedure for the measurement of IC_{50} values for the inhibition of MAO-A and MAO-B has been detailed in the literature (Mostert *et al.*, 2015; Mostert *et al.*, 2016). For these studies the recombinant human enzymes and kynuramine served as enzyme sources and substrate, respectively. MAO oxidises kynuramine to ultimately yield 4-hydroxyquinoline, which may be measured by fluorescence spectrophotometry (λ_{ex} = 310 nm; λ_{em} = 400 nm). After recording the catalytic rates of the MAOs in the presence of different concentrations of the test inhibitors (0.003–100 μM), sigmoidal dose-response plots were constructed. From these the IC_{50} values were determined. All measurements were conducted in triplicate and the IC_{50} values are given asthe mean ± standard deviation (SD).

4. Recovery of enzyme activity after dialysis

The procedure for the dialysis studies has been detailed in the literature (Mostert *et al.*, 2015; Mostert *et al.*, 2016). The MAO enzymes and the test inhibitors were combined and preincubated for 15 min at 37 °C. The concentrations of the inhibitors were fourfold the IC_{50} value for the inhibition of the relevant MAO. The mixtures were dialysed at 4 °C for 24 h, during which the dialysis buffer was replaced twice, and diluted twofold with the addition of kynuramine. The residual enzyme catalytic rates were subsequently determined by measuring the formation of 4-hydroxyquinoline.

As positive and negative controls, respectively, MAO-A and MAO-B were similarly preincubated and dialysed in the presence of the irreversible inhibitors, pargyline and selegiline, and in the absence of inhibitor. The enzyme activities of undialysed mixtures of the MAOs and the test inhibitors, which were maintained at 4 °C for 24 h, were also recorded. Dialysis and rate measurements were carried out in triplicate and the residual enzyme catalytic rates are given as mean ± SD.

5. The construction of Lineweaver-Burk plots and calculation of K_i value

The reversible interaction of 2-PAT with MAO-A was investigated by constructing a set of six Lineweaver-Burk plots. The procedure for the construction of Lineweaver-Burk plots has been reported in the literature (Mostert *et al.*, 2015; Mostert *et al.*, 2016). The first plot was constructed in the absence of inhibitor while the remaining five plots were constructed in the presence of the following concentrations of 2-PAT: ¼ × IC_{50}, ½ × IC_{50}, ¾ × IC_{50}, 1 × IC_{50} and 1¼ × IC_{50}. For each plot the substrate, kynuramine, was used at eight different concentrations (15–250 μM).

After incubation of the reactions containing the substrate, inhibitor and enzyme (0.015 mg/mL), the MAO-generated 4-hydroxyquinoline was quantified by fluorescence spectrophotometry. The K_i value for the inhibition of MAO-A was 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 to the Michaelis-Menten equation.

6. Procedure for molecular docking and dynamics simulations

The procedure for docking inhibitors into the MAO active sites has been reported in literature (Mostert *et al.*, 2015). The Windows-based Discovery Studio 3.1 software package (Accelrys) was used for these studies and the reported X-ray crystal structures of human MAO-A (PDB code 2Z5X) (Son *et al.*, 2008) and human MAO-B (PDB code 1S2Q) (Binda *et al.*, 2004) served as protein models. Preparation of the protein models included the calculation of pKa values and protonation states of the ionisable amino acids to which hydrogen atoms were subsequently added at pH 7.4. After verifying that the FAD is in the oxidised state, a fixed atom constraint was applied to the protein backbones. The protein models were energy minimised using the Smart Minimizer algorithm and the co-crystallised ligands and waters were removed. Only three waters in each model were retained: 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 test ligands were drawn in Discovery Studio and docking was performed with the CDOCKER algorithm. For this purpose full potential mode was used, ten random conformations were generated for each ligand and the heating target temperature was set to 700 K. After docking, the ligand orientations and interactions were further refined using in situ ligand minimisation with the Smart Minimizer algorithm. The illustrations were prepared with PyMol (DeLano, 2002).

7. References

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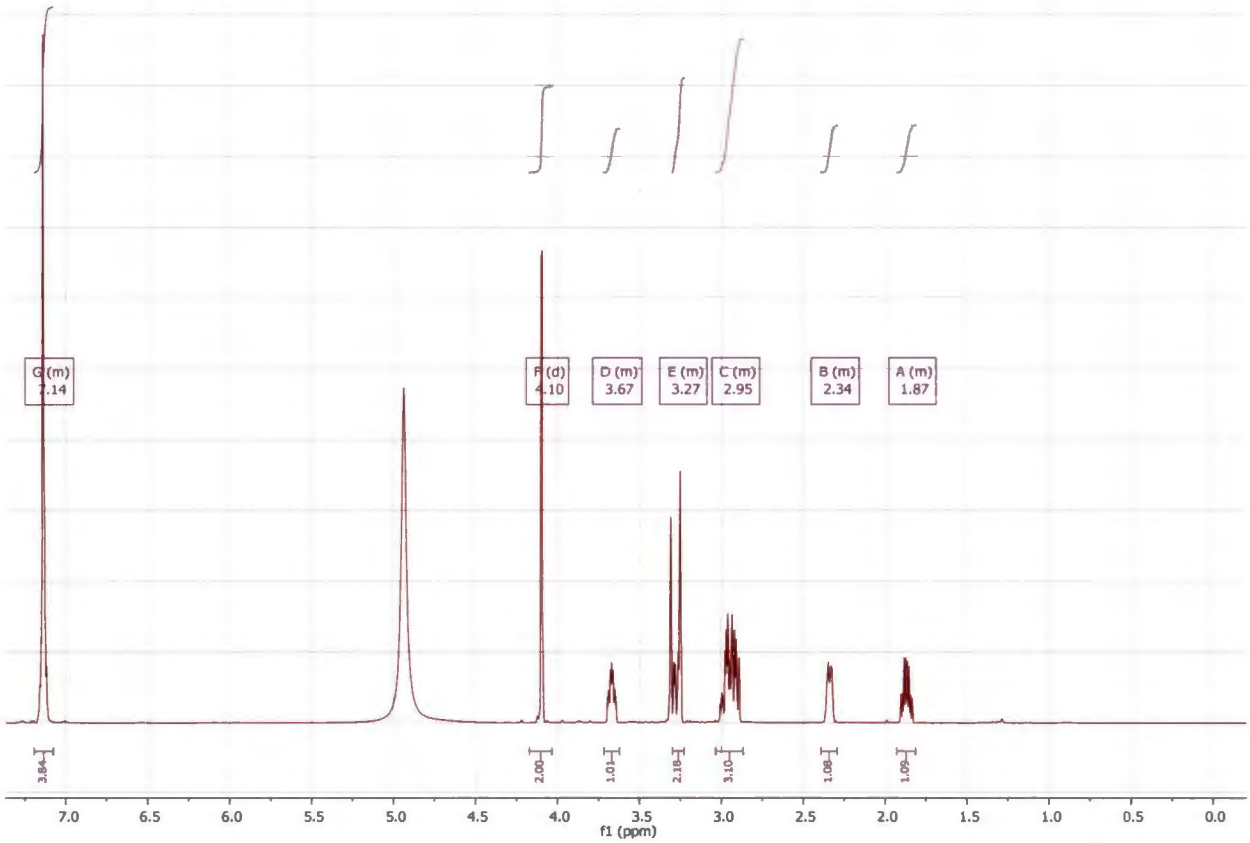
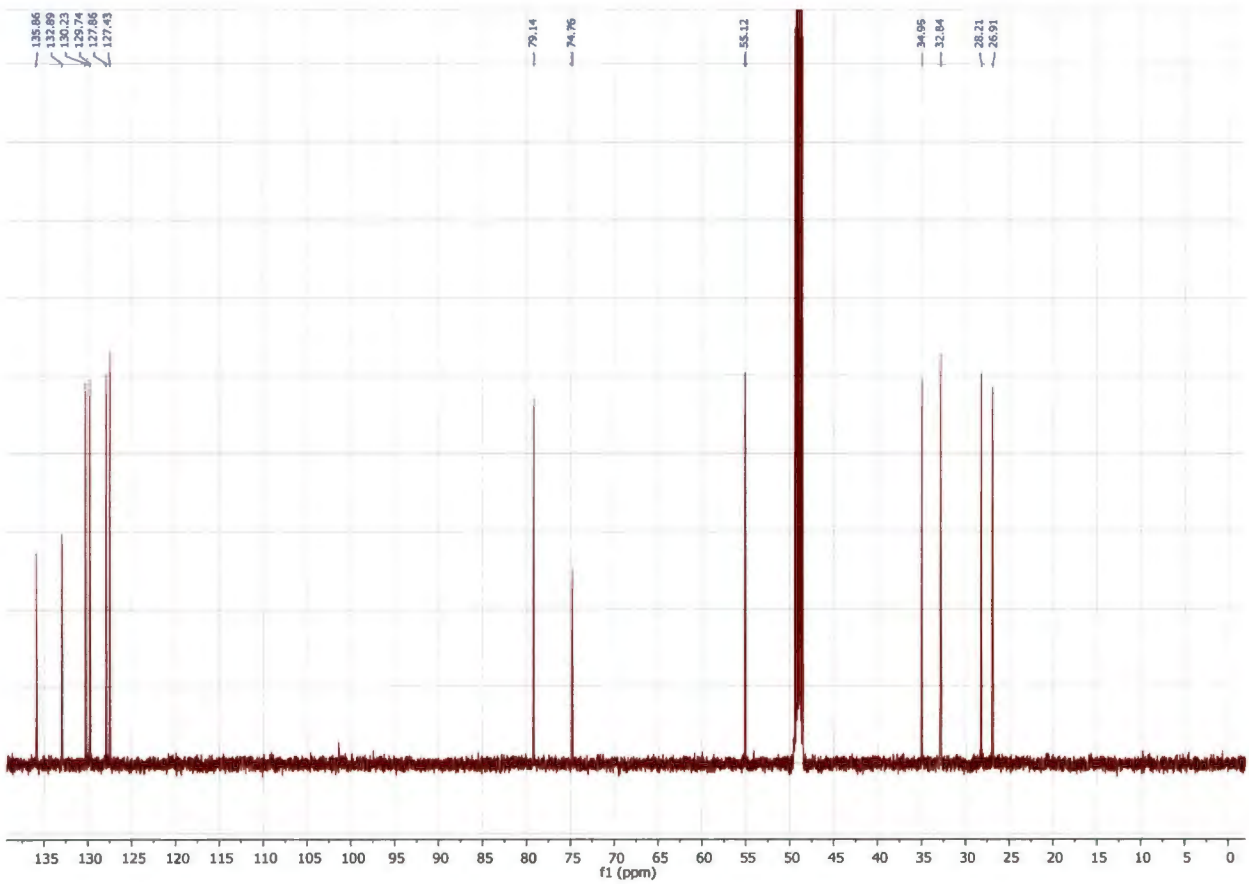
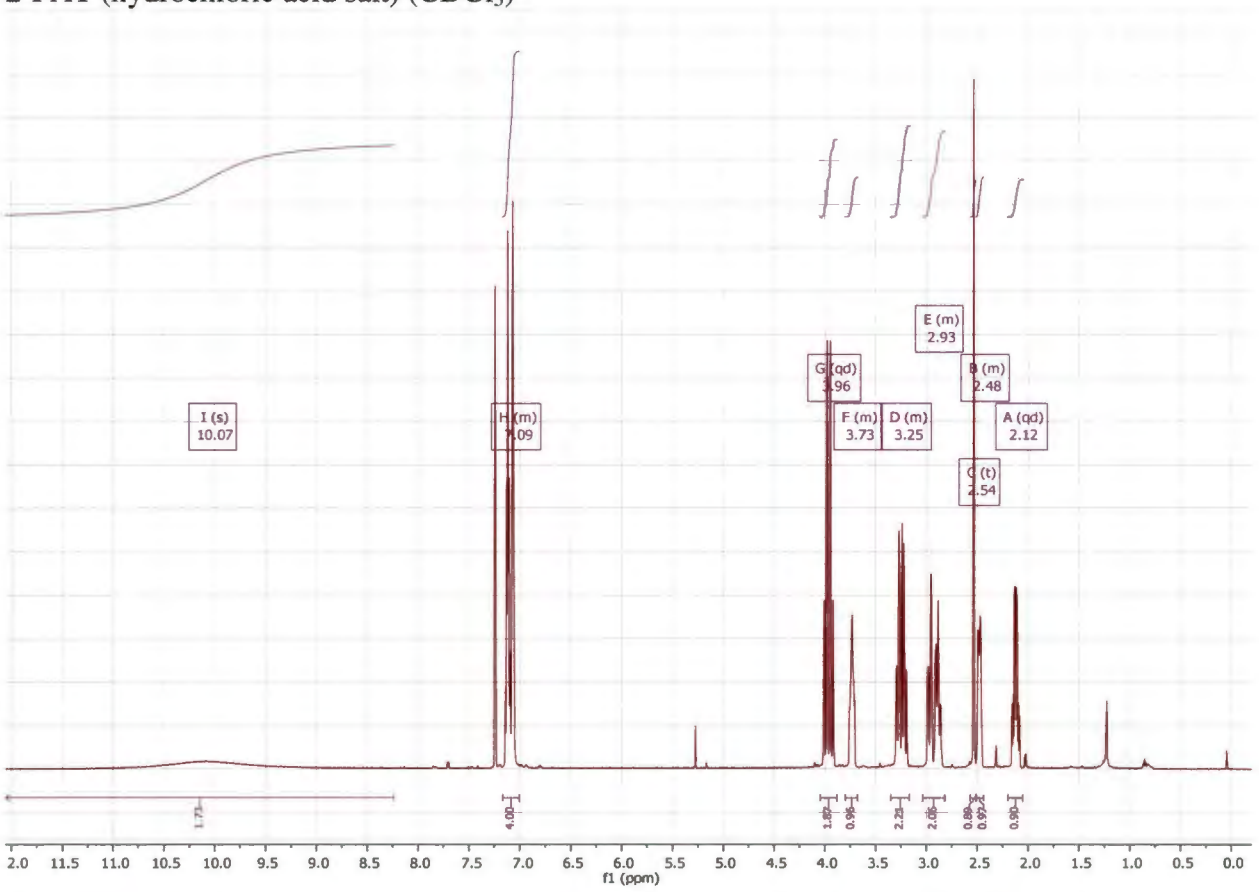
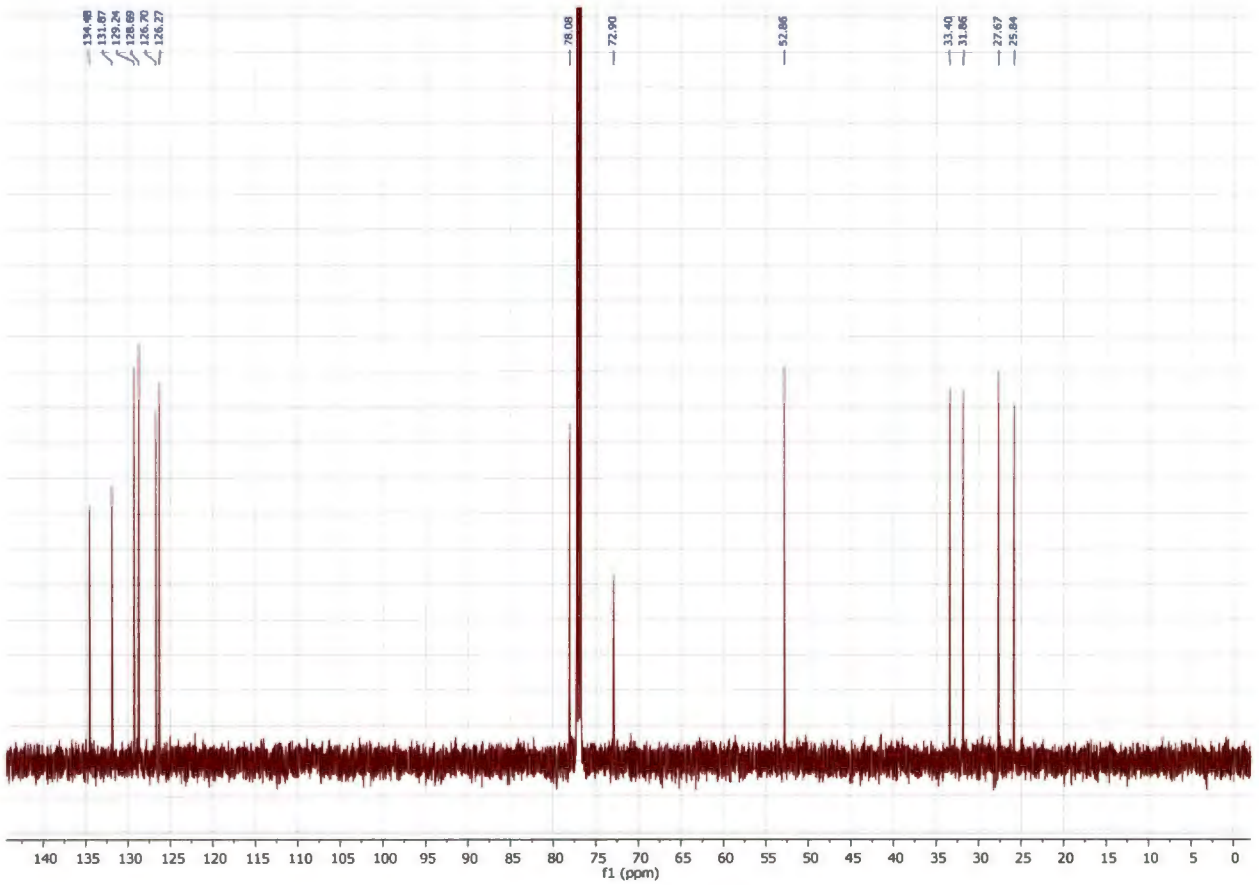
2-PAT (hydrochloric acid salt) (CD_3OD)





2-PAT (hydrochloric acid salt) ($CDCl_3$)





CHAPTER 7

CONCLUSION

Introduction: In the current study three chemical classes were synthesised and evaluated as recombinant human MAO inhibitors. These include (1) C6- and C7-substituted 3,4-dihydro-2(1*H*)-quinolinones, (2) 2-phenoxyethoxy-substituted tetralones and (3) N-propargylamine-2-aminotetralin (2-PAT).

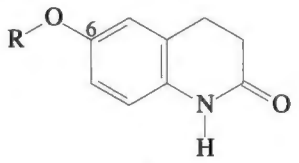
MAO consists of two isoenzymes, MAO-A and MAO-B. The MAOs are mitochondrial bound FAD containing enzymes. MAO-A is mainly present in the placenta and gastrointestinal tract, whilst MAO-B is the main form in the basal ganglia, blood platelets and liver. MAO-A is responsible for the deactivation of catecholamines in circulation as well as vasopressors such as tyramine ingested in the diet. MAO-A inhibitors inhibit the oxidation of serotonin and noradrenaline in the central nervous system, making these drugs useful in the treatment of depression. Side effects of MAO-A inhibitors include the cheese reaction, which occur with the irreversible inhibition of MAO-A (Youdim *et al.*, 2006).

Since DA is mainly oxidised by MAO-B in the basal ganglia of the midbrain, inhibitors of MAO-B are useful in the treatment of PD. The oxidation of DA is associated with the formation of by-products such as hydrogen peroxide and aldehydes, which may lead to oxidative stress and neurotoxicity if not inactivated or cleared (Fernandez & Chen, 2007). Hydrogen peroxide and aldehydes produced by the MAO catalytic cycle may contribute to neurodegeneration in PD. By preventing the formation of these potentially neurotoxic metabolic by-products, MAO-B inhibitors may act as neuroprotective agents. MAO-B inhibitors may also slow the depletion of DA stores and elevate the levels of endogenous DA (Youdim *et al.*, 2006). This analysis shows that MAO-B inhibitors are valuable as symptomatic and potentially neuroprotective agents for the treatment of PD.

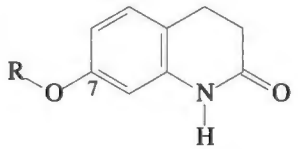
Selection of compounds: Quinolinone and β-tetralone derivatives are structurally related to chemical classes which have been reported to inhibit the MAOs. These chemical classes include α-tetralones, 1-indanones and 3-coumaranones (Meiring *et al.*, 2013; Legoabe *et al.*, 2014; Legoabe *et al.*, 2015).

In this study a series of fourteen 3,4-dihydro-2(1*H*)-quinolinone derivatives were synthesised (**1a–g** and **2a–g**) by substitution of 3,4-dihydro-2(1*H*)-quinolinone on C6 and C7 with a variety of substituents (Table 7.1). A second series of three β-tetralone derivatives were also synthesised: 6-(2-phenoxyethoxy)-3,4-dihydronaphthalen-1(2*H*)-one (**10**), 7-(2-phenoxyethoxy)-3,4-dihydronaphthalen-1(2*H*)-one (**11**) and 7-(2-phenoxyethoxy)-3,4-dihydronaphthalen-2(1*H*)-one (**12**) (Figure 7.1).

Table 7.1. The structures of the C6- and C7-substituted quinolinones (**1a–g** and **2a–g**) that were synthesised in this study.



1a-g



2a-g

	R		R
1a	4-$ClC_6H_4CH_2$–	**2a**	4-$ClC_6H_4CH_2$–
1b	4-$BrC_6H_4CH_2$–	**2b**	4-$BrC_6H_4CH_2$–
1c	3-$CH_3C_6H_4CH_2$–	**2c**	3-$CH_3C_6H_4CH_2$–
1d	4-$CH_3C_6H_4CH_2$–	**2d**	4-$CH_3C_6H_4CH_2$–
1e	3-$CH_3C_6H_4(CH_2)_2$–	**2e**	4-$CH_3C_6H_4(CH_2)_2$–
1f	4-$CH_3C_6H_4(CH_2)_2$–	**2f**	$C_6H_5O(CH_2)_2$–
1g	$C_6H_5O(CH_2)_2$–	**2g**	4-$ClC_6H_4O(CH_2)_2$–

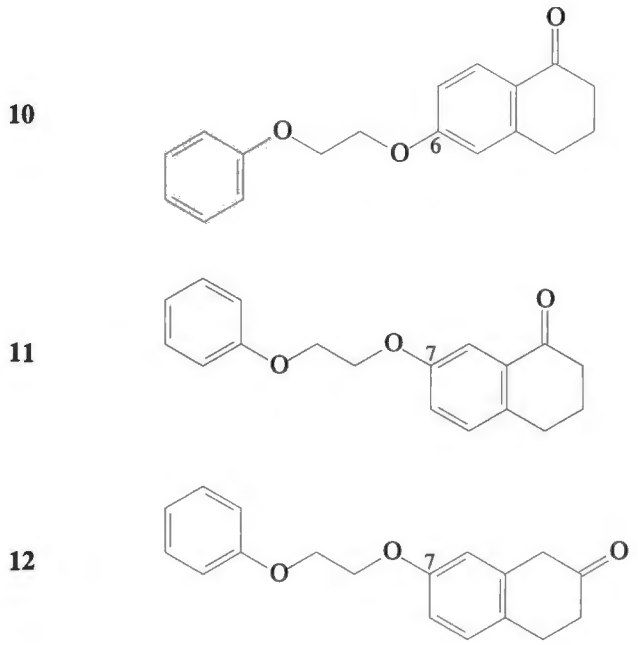
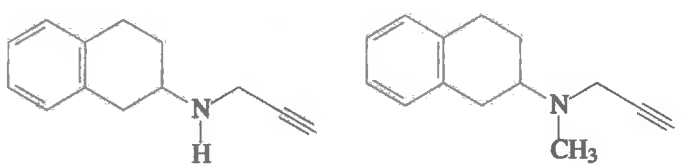


Figure 7.1: The structures of 6-(2-phenoxyethoxy)-3,4-dihydronaphthalen-1(2*H*)-one (**10**), 7-(2-phenoxyethoxy)-3,4-dihydronaphthalen-1(2*H*)-one (**11**) and 7-(2-phenoxyethoxy)-3,4-dihydronaphthalen-2(1*H*)-one (**12**).

Finally, 2-PAT, structurally very similar to rasagiline and the previously studied 2-MPAT, was also synthesised and evaluated as a MAO inhibitor. This study was motivated by the academic challenge of designing and discovering high potency and isoform-specific MAO inhibitors that may be useful therapeutic agents in PD.



2-PAT 2-MPAT

Figure 7.2: The structures of N-propargylamine-2-aminotetralin (2-PAT) and N-methyl-N-propargylamine-2-aminotetralin (2-MPAT).

Synthesis: The C6- and C7-substituted 3,4-dihydro-2(1*H*)-quinolinone and 2-phenoxyethoxy tetralone derivatives were synthesised by reacting 6- or 7-hydroxy-3,4-dihydro-2(1*H*)-quinolinone and 6- or 7-hydroxytetralone, respectively, with an appropriate alkyl bromide in the presence of base (Shigematzu, 1961; Meiring *et al.*, 2013; Legoabe *et al.*, 2014; Legoabe *et al.*, 2015).

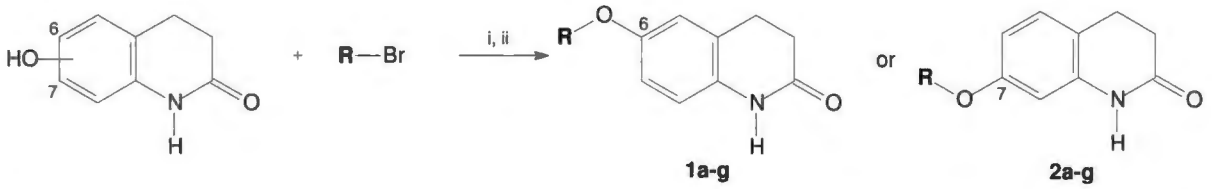


Figure 7.3: The synthesis of 3,4-dihydro-2(1*H*)-quinolinone derivatives **1a–g** and **2a–g**. Reagents and conditions: (i) KOH, ethanol, reflux; (ii) recrystallisation from ethanol.

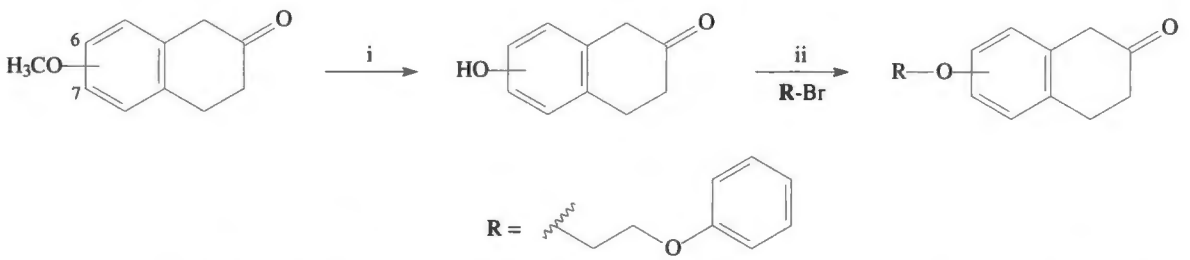


Figure 7.4: The synthesis of C6- or C7-substituted ethers of β-tetralone. Reagents and conditions: (i) $AlCl_3$, toluene; reflux; (ii) acetone, K_2CO_3, reflux.

2-PAT was synthesised by dehydrating commercially available β–tetralone and propargylamine in the presence of p-toluenesulfonic acid. After reduction with sodium cyanoborohydride ($NaCNBH_4$) and a workup that involves acidification with HCl, washing with diethyl ether and extraction to dichloromethane the hydrochloric acid salt of 2-PAT was obtained in low yield (Zhuang *et al*, 1993).

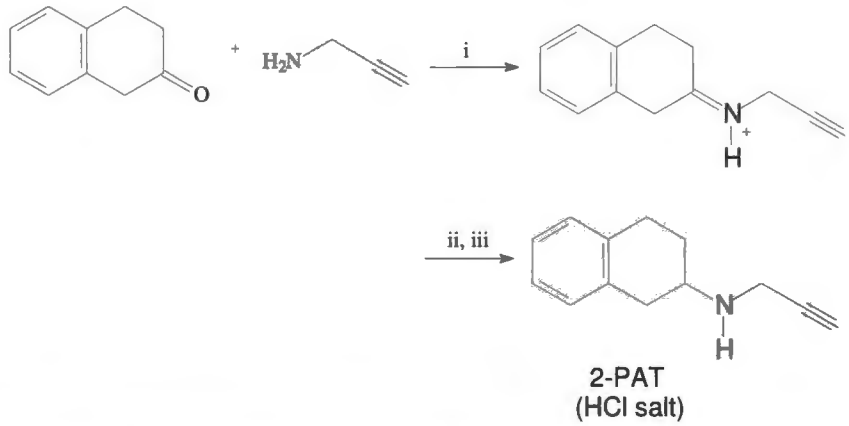


Figure 7.5: The synthesis of 2-PAT. Reagents and conditions: (i) p-toluenesulfonic acid, benzene, Dean-Stark trap; (ii) $NaCNBH_4$, methanol, N_2, rt; (iii) 10% HCl.

Enzymology: The synthesised compounds were evaluated as inhibitors of the recombinant human MAO-A and MAO-B enzymes, which are commercially available (Novaroli *et al.*, 2005). To measure MAO activities, the MAO-A/B mixed substrate, kynuramine, was employed. Kynuramine is oxidised by the MAO enzymes to yield 4-hydroxyquinoline. Since 4-hydroxyquinoline is fluorescent, the formation of 4-hydroxyquinoline was measured by fluorescence spectrophotometry. Sigmoidal dose-response curves were constructed and the inhibition potencies, the corresponding IC_{50} values, were calculated (Mostert *et al.*, 2015; Mostert *et al.*, 2016).

To determine the reversibility of inhibition of MAO by selected derivatives, dialysis was employed. For reversible inhibition, sets of Lineweaver-Burk plots were constructed to determine the modes of inhibition. Finally, the inhibitors were docked into the MAO active sites using the CDOCKER docking algorithm of Discovery Studio 3.1 (Accelrys). This was done to gain insight into the interactions between the enzyme and selected inhibitors (Mostert *et al.*, 2015; Mostert *et al.*, 2016; Silverman, 1995; Son *et al.*, 2008; Binda *et al.*, 2004).

The results of the first study document that the 3,4-dihydro-2(1*H*)-quinolinone derivatives are MAO-B specific with high inhibition potencies. Most notably among these are 6-(4-bromobenzyloxy)-3,4-dihydro-2(1*H*)-quinolinone (**1b**), 7-(4-chlorobenzyloxy)-3,4-dihydro-2(1*H*)-quinolinone (**2a**) and 7-(4-bromobenzyloxy)-3,4-dihydro-2(1*H*)-quinolinone (**2b**) which display IC_{50} values <0.006 μM. The most potent inhibitor, **2a**, displayed an IC_{50} value of 0.0014 μM, and is significantly more potent than the reference MAO-B inhibitors lazabemide (IC_{50} = 0.091 μM) and safinamide (IC_{50} = 0.048 μM) (evaluated under the same experimental conditions). The 3,4-dihydro-2(1*H*)-quinolinone derivatives also display high specificities for the MAO-B enzyme with selectivities for MAO-B ranging from 99 to 40,000-fold.

Based on dialysis experiments, it was concluded that **1b** is a reversible MAO-B inhibitor. The Lineweaver-Burk plots constructed for the inhibition of MAO-B by **1b** were linear and intersected on the y-axis, indicating that this compound is a competitive MAO-B inhibitor. An analysis of the Lineweaver-Burk plots indicated that **1b** inhibits MAO-B with a K_i value of 2.8 nM. An analysis of the structure-activity relationships for MAO-B inhibition by 3,4-dihydro-2(1*H*)-quinolinones shows that C7 substitution yields compounds with higher MAO-B inhibition potencies than C6 substitution. For each pair of homologues, the corresponding C7-substituted compounds display more potent inhibition although differences in inhibition potencies are in many instances very small.

The results for the second study document that the β-tetralone derivative, 7-(2-phenoxyethoxy)-3,4-dihydronaphthalen-2(1*H*)-one (**12**) , is a weak inhibitor of human MAO-A (IC_{50} = 56.2 μM) compared to the corresponding α-tetralone derivatives, 6-(2-phenoxyethoxy)-3,4-dihydronaphthalen-1(2*H*)-one (**10**) (IC_{50} = 1.96 μM) and 7-(2-phenoxyethoxy)-3,4-dihydronaphthalen-1(2*H*)-one (**11**) (IC_{50} = 1.81 μM). Compound **12**, however, was a potent human MAO-B inhibitor (IC_{50} = 0.033 μM). It was also established that **12** is a reversible and competitive MAO-B inhibitor (K_i = 0.128 μM). This is the first report of the MAO inhibition properties of a β-tetralone derivative.

The results for the final study shows that 2-PAT is a 20-fold more potent inhibitor of MAO-A (IC_{50} = 0.721 μM) compared to MAO-B (IC_{50} = 14.6 μM) and 2-PAT is approximately fivefold more potent than toloxatone (IC_{50} = 3.92 μM), a clinically used antidepressant and reversible MAO-A inhibitor.

Interestingly, dialysis studies found that 2-PAT is a reversible MAO-A inhibitor, while acting as an inactivator of MAO-B. This is the first report that a propargylamine compound acts as reversible inhibitor of one MAO isoform, while inhibiting the other irreversibly.

Conclusion: In conclusion, the first study shows that 3,4-dihydro-2(1*H*)-quinolinones are MAO-B specific inhibitors with compounds, **1b**, **2a** and **2b** showing high potency MAO-B inhibition (IC_{50}< 0.006 μM). The second study shows for the first time that a β-tetralone derivative, compound **12**, is a potent and selective MAO-B inhibitor. It was also established that this compound inhibits MAO-B reversibly. Although the α-tetralone homologues **10** and **11** are more potent MAO-B inhibitors, compound **12** displays better isoform selectivity. It may thus be concluded that, in contrast to the α-tetralones reported to date, **12** is a more selective MAO-B inhibitor. Specific MAO-B inhibitors such as these represent leads for the design of therapeutic agents for PD with lower risk for MAO-A related side effects.

The final study found that 2-PAT is a potent reversible MAO-A inhibitor, which may be clinically relevant since this compound is approximately fivefold more potent than toloxatone (IC_{50} = 3.92 μM). 2-PAT is unlikely to potentiate tyramine-induced side effects as seen with MAO-A inactivators, since 2-PAT is a reversible inhibitor of MAO-A. Although not as potent as current MAO-B inhibitors such as selegiline, 2-PAT would still be expected to inhibit MAO-B *in vivo* since inhibition is time-dependent and thus cumulative with a chronic dosage regimen. Based on the above considerations, 2-PAT could be a safe and useful agent for the treatment of PD, specifically where depression is a comorbidity of PD.

This study therefore concludes that the objectives given in Chapter 1 have been achieved and the hypotheses proven. 3,4-Dihydro-2(1*H*)-quinolinones and β-tetralones are suitable scaffolds for the design of MAO-B inhibitors and appropriate substitution yields highly potent MAO-B inhibitors. Such compounds are thus suitable leads for the design of antiparkinsonian therapies. 2-PAT possesses an interesting MAO inhibition profile that may be a useful in the treatment of PD.

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ADDENDUM A

Author guidelines: Mini-Reviews in Medicinal Chemistry

INSTRUCTIONS FOR AUTHORS

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It is advisable that the document files related to a manuscript submission should always have the name of the corresponding author as part of the file name, i.e., "Cilli MS text.doc", "Cilli MS Figure 1" *etc.*

It is imperative that before submission, authors should carefully proofread the files for special characters, mathematical symbols, Greek letters, equations, tables, references and images, to ensure that they appear in proper format.

References, figures, tables, chemical structures etc. should be referred to in the text at the appropriate place where they have been first discussed. Figure legends/captions should also be provided.

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Single Topic Issues:

These peer-reviewed issues will be restricted to invited or uninvited review/mini-review articles. A Single Topic Issue Editor will offer a short perspective and co-ordinate the solicitation of manuscripts between 3-5 (for a mini-thematic issue) to 6-10 (for full-length thematic issue) from leading scientists. Authors interested in editing a single topic issue in an emerging topic of medicinal chemistry may submit their proposal to the Editor-in-Chief at mrmc@benthamscience.org for consideration.

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Full-Length Reviews:

Full-length reviews should be 8000-40000 words excluding figures, structures, photographs, schemes, tables *etc.*

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Mini-reviews should be 3000- 6000 words excluding figures, structures, photographs, schemes, tables *etc.*

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- Copyright Letter
- Title
- Title Page
- Structured Abstract
- Graphical Abstract
- Keywords

- Text Organization
- Conclusion
- List of Abbreviations (if any)
- Conflict of Interest
- Acknowledgements
- References
- Appendices
- Figures/Illustrations (if any)
- Chemical Structures (if any)
- Tables (if any)
- Supportive/Supplementary Material (if any)

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- [1] Bard, M.; Woods, R.A.; Bartón, D.H.; Corrie, J.E.; Widdowson, D.A. Sterol mutants of *Saccharomyces cerevisiae*: chromatographic analyses. *Lipids*, 1977,*12*(8), 645-654.
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Book Reference:

- [3] Crabtree, R.H. *The Organometallic Chemistry of the Transition Metals*, 3rd ed.; Wiley & Sons: New York, 2001.

Book Chapter Reference:

- [4] Wheeler, D.M.S.; Wheeler, M.M. Stereoselective Syntheses of Doxorubicin and Related Compounds In: *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed. Elsevier Science B. V: Amsterdam, 1994; Vol. *14*, pp. 3-46.

Conference Proceedings:

- [5] Jakeman, D.L.; Withers, S.G.E. In: *Carbohydrate Bioengineering: Interdisciplinary Approaches* In: Proceedings of the 4th Carbohydrate Bioengineering Meeting, Stockholm, Sweden, June 10-13, 2001; Teeri, T.T.; Svensson, B.; Gilbert, H.J.; Feizi, T., Eds. Royal Society of Chemistry: Cambridge, UK, 2002; pp. 3-8.

URL (WebPage):

- [6] National Library of Medicine. Specialized Information Services: Toxicology and Environmental Health. sis.nlm.nih.gov/Tox/ToxMain.html (Accessed May 23, 2004).

Patent:

- [7] Hoch, J.A.; Huang, S. Screening methods for the identification of novel antibiotics. U.S. Patent 6,043,045, March 28, 2000.

Thesis:

- [8] Mackel, H. *Capturing the Spectra of Silicon Solar Cells*. PhD Thesis, The Australian National University: Canberra, December 2004.

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