Biochemical characterisation of six novel monoamine oxidase inhibitors identified in tobacco smoke

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Abstract

Background: Tobacco smoking is supposed to be the most difficult addiction to give up, and nicotine has been observed as the main addictive agent found in tobacco smoke. However, research is showing that nicotine alone does not account for tobacco dependence. One hypothesis is that monoamine oxidase (MAO) inhibition from non-nicotine compounds in tobacco smoke contributes to nicotine addiction. Six new MAO inhibitors in tobacco smoke have been identified before this PhD project.

Overall research aim: The overall research aim for this project was to characterize these six novel monoamine oxidase (MAO) inhibitors identified in tobacco smoke and study their interaction with MAO A and B enzymes.

Methods: First, non-nicotinic components of tobacco smoke were tested for MAO inhibitory activity, using the kynuramine assay and recombinant human MAO enzymes. Next, a centrifugation-ultrafiltration method and a time-dependent assay were used as the primary tests of reversibility of the phenolic compounds. Then, Lineweaver-Burke (LB) plots were prepared to understand the kinetics and mechanism of inhibition of recombinant human MAO enzymes by the polyunsaturated fatty acids (PUFAs). Finally, molecular docking and in silico studies using SwissADME and PreADMET web tools were performed.

Results: Catechols and hydroquinone showed potent irreversible MAO A inhibition. Among these, 4-methylcatechol displayed the highest activity for MAO A with an IC_{50} value of 0.267 μM after 1h preincubation. Two PUFAs, α-linolenic acid and linoleic acid displayed potent inhibitory effect for MAO A with IC_{50} values of 15.74 and 23.8 μM, respectively. Kinetic analysis revealed that α-linolenic acid and linoleic acid are competitive inhibitors of MAO A and MAO B. Molecular docking studies suggest that ternary complexes [MAO B-linoleic $acid_2$ species (EII)] may be formed.

Conclusions: This PhD project showed that six novel MAOIs in tobacco smoke inhibited human MAO A and MAO B isoenzymes. The catechols and hydroquinone are irreversible MAO inhibitors, suggesting they may play a role in contribution to the addictive effects of nicotine and the low incidence of Parkinson's disease in smokers. In addition, α-linolenic acid and linoleic acid are found to be reversible MAO inhibitors, suggesting these PUFAs may play a role in the lower MAO levels or activity in smokers. Overall, these findings suggest that MAO inhibitors from tobacco smoke may have pharmaceutical possibilities, perhaps in smoking cessation, or in relief of anxiety or depression or in Parkinson's and Alzheimer disease.

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Declaration

The presented thesis consists of six chapters including two chapters with experimental work in this study. Partial contents of all chapters are structured as manuscripts that have either been published, or planned to be submitted to peer-reviewed journals. The published manuscripts include other authors who provided expert advice and contributed to the final shape of the manuscript, including PhD Supervisors in Massey University and Victoria University of Wellington.

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List of Abbreviations

3-MT	3-methoxytyramine
5-HIAA	5-hydroxindole acetic acid
5-HT	5-hydroxytryptamine
AADC	Aromatic L-amino acid decarboxylase
ACh	Acetylcholine
AD	Alzheimer's disease
ADME	Absorption, distribution, metabolism, and excretion
ADT	AutoDockTools
ALDH	Aldehyde dehydrogenase
ALR	Aldehyde reductase
BBB	Blood-brain barrier
CNS	Central nervous system
COMT	Catechol-*O*-methyltransferase
COPD	Chronic obstructive pulmonary disease
COSY	Correlation spectroscopy
COX	Cytochrome c oxidase
CYP2A6	Cytochrome P450 2A6
CYP2D6	Cytochrome P450 2D6
Cyt*c*	Cytochrome *c*
DA	Dopamine
Da	Dalton
DAD	Diode array detection
DBH	Dopamine β-hydroxylase
DOPAC	3,4-dihydroxyphenylacetic acid

DOPAL	3,4-dihydroxyphenylacetaldehyde
ELSD	Evaporative light scattering detector
EPR	Electron paramagnetic resonance
ETC	Electron transport chain
EtOH	Ethanol
FAD	Flavin adenine dinucleotide
GABA	Gamma-aminobutyric acid
GC-MS	Gas chromatography-mass spectroscopy
GSH	Glutathione peroxidase
HIA	Human gastrointestinal absorption
HPLC	High performance liquid chromatography
H_2O_2	Hydrogen peroxide
HVA	Homovanillic acid
LB	Lineweaver-Burke
L-dopa	L-dihydroxyphenylalanine
MAO	Monoamine oxidase
MAO A	Monoamine oxidase A
MAO B	Monoamine oxidase B
Mfsd2a	Major facilitator superfamily domain-containing protein 2a
MHPG	3-methoxy-4-hydroxyphenylglycol
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MTCA	2-methylthiazolidine-4-carboxylic acid
NAc	Nucleus accumbens
nAChRs	Nicotinic acetylcholine receptors
NMR	Nuclear magnetic resonance

PBS	Phosphate buffered saline
PCET	Proton-coupled electron transfer
PC12	Pheochromocytoma
PD	Parkinson’s disease
PDB	Protein data bank
PET	Positron emission tomography
Pgp	P-glycoprotein
PH	Phenylalanine hydroxylase
PPB	Plasma protein binding
PSDVB	Poly(styrene-divinylbenzene)
PUFAs	Polyunsaturated fatty acids
RCT	Randomized controlled trial
SET	Single electron transfer
SI	Selectivity index
SOD	Superoxide dismutase
TH	Tyrosine hydroxylase
THβCs	1,2,3,4-tetrahydro-β-carbolines
TIQs	Tetrahydroisoquinolines
TPH	Tryptophan hydroxlase
TPM	Total particulate matter
TPSA	Topological polar surface area
VTA	Ventral tegmental area

Chapter 1

Introduction

This chapter provides background for this thesis and outlines the rationale and overall research aim of the PhD study. The chapter concludes with an outline of the thesis structure.

1.1. Background

Tobacco use is a leading cause of premature disability and death in the world (Prochaska & Benowitz, 2019). Nicotine, the major psychoactive chemical found in tobacco (Wonnacott et al., 2005), binds to nicotinic cholinergic receptors in the brain to stimulate dopamine and other neurotransmitters, sustaining tobacco addiction (Benowitz, 2010). However, nicotine by itself has modest potential for addiction (Dome et al., 2010; Rose, 2006). One hypothesis is that non-nicotinic components in smoke that inhibit monoamine oxidase (MAO) reduce the breakdown of neurotransmitters in brain reward pathways and synergistically increase the addictive effects of nicotine in smokers (Hogg, 2016).

MAO enzymes (EC 1.4.3.4) are oxidoreductases that catalyze the oxidative deamination of monoamine substrates, including neurotransmitters such as serotonin, histamine, dopamine, norepinephrine and epinephrine (Youdim et al., 2006). There are two isoforms of MAO, MAO A and MAO B, with different inhibitor sensitivity and with different substrate specificity (Shih, 2018). Serotonin (5-hydroxytryptamine, 5-HT) is oxidized by MAO A, while beta phenethylamine and benzylamine are oxidized by MAO B. Both isoforms metabolize dopamine, norepinephrine, epinephrine, tyramine and tryptamine (Youdim et al., 2006). The MAO enzymes are also known targets for neurological disorders. MAO inhibitors have been used for treatment of depression, panic disorder, social phobia, bipolar, and anxiety (Menkes et al., 2016; Sub Laban & Saadabadi, 2021). MAO B inhibitors are also used for the symptomatic treatment of Parkinson's disease (PD) (Schapira, 2011).

Positron emission tomography (PET) studies have shown that smokers have lower MAO A (28%) and MAO B (40%) activity in the brain relative to non-smokers (Fowler et al., 1996a; Fowler et al., 1996b). These findings raised questions about specific components in tobacco leaf and/or smoke responsible for the lowering of MAO activity in the brains of human smokers (Castagnoli & Murugesan, 2004). It is reported that several components exhibiting MAO inhibition *in vitro* have been purified from tobacco and tobacco smoke and found to be absorbed in blood or plasma and/or platelets in people who smoke (Hogg, 2016; Rommelspacher et al., 2002; Talhout et al., 2007). Hogg (2016) has suggested that MAO inhibition in smokers may arise from additive or synergistic properties of components in, or derived from, tobacco smoke. An alternative explanation is the possibility that low platelet MAO linked to personality and vulnerability to psychiatric disorders may also predispose to smoking (Fowler et al., 2003).

However, it has been suggested that platelet MAO may not represent MAO levels or activity in the brains (Hogg, 2016).

Many studies have been performed to identify and characterize components in tobacco and tobacco smoke that inhibit MAO A and MAO B (Herraiz & Chaparro, 2005; Khalil et al., 2006; Khalil et al., 2000). Two beta-carbolines, harman and norharman, have been suggested as potent competitive MAO inhibitors from tobacco smoke (Herraiz & Chaparro, 2005). Harman inhibited the human MAO A (IC_{50} 0.34 μM), while it has no effect on MAO B up to 25 μM concentrations. Norharman showed both human MAO A inhibition (IC_{50} 6.47 μM) and human MAO B inhibition (IC_{50} 4.68 μM). However, these two inhibitors have been found to contribute to under 10% of the total MAO A inhibitory activity of tobacco smoke suggesting other inhibitors may contribute significantly to total inhibitory activity (Truman et al., 2017).

Characterising MAO inhibitors in tobacco smoke will be very important in helping to understand tobacco dependence. This PhD study will enhance our knowledge of the way tobacco smoke affects MAO activity in smokers and will set the foundation for a better understanding of the place of MAO inhibition in tobacco dependence. This knowledge can be used to provide better advice to help smokers quit smoking. Further, if specific MAO inhibitors are proven to promote addiction to smoking, they could be used with nicotine to produce a more effective form of nicotine replacement therapy (NRT), with the potential to help many more smokers to stop smoking. MAO inhibitors have also been used clinically to control depression and anxiety, and the symptoms of Parkinson's disease. The MAO inhibitors from tobacco smoke have the potential to provide alternative methods of symptom control in these smokers, but without the health risks associated with smoking.

1.2. Rationale for this PhD Study

It has been proposed that monoamine oxidase (MAO) inhibitors in, or derived from, tobacco smoke reduces the breakdown of neurotransmitters involved in the brain reward pathways and synergistically increase the addictive effects of nicotine in smokers (Hogg, 2016). Several studies have shown that MAO inhibition increases self-administration of nicotine in rats (Guillem et al., 2005, 2006; Villegier et al., 2007). Therefore, to fully understand the underlying mechanism of nicotine addiction, it is important to characterize unidentified non-nicotinic components of tobacco smoke that display inhibitory activity against MAO enzymes. It was reported that cigarette smoke extracts inhibited MAO reversibly and irreversibly (Castagnoli

and Murugesan 2004). In addition, characterizing irreversible MAO inhibitors from tobacco smoke has been proposed (Villegier et al., 2007). On the other hand, although the mechanism is unclear, serotonergic systems may be linked to the enhancing effect of MAO inhibition on nicotine self-administration (Sved et al., 2022).

In a research breakthrough, identification of seven new MAO inhibitors in tobacco smoke was completed prior to the start of this PhD, by Penny Truman, Sa Weon Hong, Rob Keyzers, Peter Northcote, and Ali Heydari, but not yet published for intellectual property protection reasons. Six of the seven inhibitors are commercially available. One inhibitor (4-vinylcatechol) has been successfully synthesized, but was not followed up because of its known instability leading to polymerization. These six new MAO inhibitors (catechol, 4-methylcatechol, 4-ethylcatechol, hydroquinone, α-linolenic acid, and linoleic acid) will be characterized further in this PhD. The MAO inhibitory activity, selectivity and reversibility, and the potential binding mode and interactions to MAO enzymes will be investigated.

1.3. Overall research aim

The overall research aim for this thesis, is in depth characterisation of six novel monoamine oxidase (MAO) inhibitors identified from tobacco smoke and examination of their interaction with the monoamine oxidase isoforms, MAO A and MAO B.

1.4. Research Questions

The four research questions to address the overall research aim are noted in Sections 1.4.1 – 1.4.4 below.

1.4.1. Research Question 1

What already known non-nicotinic components in tobacco and tobacco smoke could contribute the total MAO inhibitory activity in cigarette smoke?

Many studies were carried out to identify and characterize MAO inhibitors in tobacco and tobacco smoke. However, knowledge of the MAO inhibitors responsible for MAO inhibition in the brains of smoker appears to be incomplete. The aim of the literature review is to discuss components in tobacco and tobacco smoke with MAO inhibitory activity especially for their MAO selectivity and reversibility.

1.4.2. Research Question 2

Do the six novel MAO inhibitors that have been identified from tobacco smoke show potent inhibition of human MAO A and MAO B isoenzymes?

In previous work, Truman et al. (2017) found that harman and norharman, two potent reversible MAO inhibitors from tobacco smoke comprised under 10% of the total MAO A inhibitory activity in cigarette smoke and suggested that other either known or unknown MAO inhibitors may make significant contributions to total MAO inhibitory activity. The kynuramine assay will be used to determine MAO inhibitory activity of the novel MAO inhibitors identified. In addition, norharman will be used as a positive control for MAO A and MAO B inhibition.

1.4.3. Research Question 3

Do the six novel MAO inhibitors identified show reversible inhibition or irreversible inhibition against human MAO A and MAO B?

It has long been suggested that tobacco smoke results in irreversible MAO inhibition. We suggested that 1,4-benzoquinone may play a role in the irreversible inhibition of MAO A (Hong et al., 2022). Many studies have been conducted to isolate MAO inhibitors from tobacco smoke. However, the identification of irreversible inhibitors in tobacco smoke has remained unanswered. To determine the reversibility of the novel MAO inhibitors identified, a time-dependent assay and a centrifugation-ultrafiltration method (commonly named repeated washing) is used.

1.4.4. Research Question 4

What are the modes of inhibition and potential binding sites of the novel reversible MAO inhibitors to human MAO enzymes?

It has been reported that non-nicotinic compounds in tobacco are commonly reversible inhibitors of MAO and show competitive inhibition, non-competitive, or mixed-type inhibition (Hong et al., 2022). Kinetic analysis of the MAO inhibition by the two novel PUFA inhibitors will be explored to determine the mode of inhibition, and molecular docking studies will be conducted to characterize the potential binding sites of the novel inhibitors to MAO.

1.5. Thesis Structure

This PhD study follows the format of thesis by publication. The thesis chapters are presented below with identification of those chapters that have been published, or in preparation for publication.

Chapter 1 discusses the background and rationale for this PhD study. It also introduces and describes the overall research aim and four research questions to be addressed in this PhD project.

Chapter 2 is a literature review and summarizes behavioural effects reported for nicotine, cotinine, and the non-nicotinic components in tobacco and tobacco smoke. This study was published in Frontiers in Neuroscience in 2022 and is entitled, "Biologically active compounds present in tobacco smoke: Potential interactions between smoking and mental health".

Chapter 3 also reviews the current knowledge on the components in tobacco and tobacco smoke with MAO inhibitory activity particularly for their MAO selectivity and reversibility and the research gaps. This study was published in Neurotoxicology in 2022 and is entitled, "A review of monoamine oxidase (MAO) inhibitors in tobacco or tobacco smoke". This literature review addresses research question 1 (Section 1.4.1).

Chapter 4 presents the first experimental chapter of this PhD study, focusing on the novel irreversible monoamine oxidase (MAO) inhibitors in tobacco smoke and their biological activities, and addresses Research question 2 and 3 (Section 1.4.2 and 1.4.3). This study has been prepared for submission to Neurotoxicology.

Chapter 5 presents the second experimental chapter of this PhD study, focusing on the novel reversible monoamine oxidase (MAO) inhibitors in tobacco smoke and their biological activities, and addresses Research question 2 and 4 (Section 1.4.2 and 1.4.4). This study has been prepared for submission to Neurotoxicology.

Chapter 6 presents the overall discussion and conclusion of the PhD study focusing on the key findings of the study. This Chapter also presents the limitations of the study and suggestions for future research work.

The Appendix section contains supporting documents for this thesis and includes research outputs related to this PhD study, including a list of publications and awards (Appendix 1). It also contains copies of the two published journal articles (Appendices 2), and other relevant information for the three in silico studies (Molecular docking, SwissADME, and PreADMET tools; Appendices 3-5).

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Chapter 2

Literature Review 1

This chapter begins with an overview on the pharmacologic effects of nicotine in the human body, followed by behavioural effects reported for nicotine, cotinine, and the non-nicotinic components in tobacco and tobacco smoke.

This chapter contains the article. “Biologically active compounds present in tobacco smoke: Potential interactions between smoking and mental health.”, co-authored by Sa Weon Hong (first author) (SH), Paul Teesdale-Spittle (PT-S), Rachel Page (RP), Bart Ellenbroek (BE), and Penelope Truman (PT). This article was published in Frontiers in Neuroscience in 2022. Author contributions: PT, RP, and PT-S guided the initial literature search, performed by SH. SH and PT wrote the manuscript. BE added expert advice. All authors contributed to the final shape of the manuscript.

Section 2.2 becomes the Frontiers in Neuroscience paper, of which the sections on tobacco alkaloids and MAO inhibitors were most strongly contributed by PT. SH was the primary author responsible for both Tables, for information of endogenous formation of MAO inhibitors, and for information on the behavioural and biochemical effects of a variety of tobacco components.

Biologically active compounds present in tobacco smoke

2.1. Mechanisms of nicotine addiction

Smoking is still a major cause of premature death and preventable disease in the world (Gowing et al., 2015; West, 2017). Smoking behaviour is not only affected by the pharmacologic effects, but also by environmental factors like smoking cues, smoking friends, stress, and product marketing. Other factors that affect susceptibility involve age, sex, genetic preposition, psychiatric disorder and substance abuse (Fig. 2.1) (Benowitz, 2010).

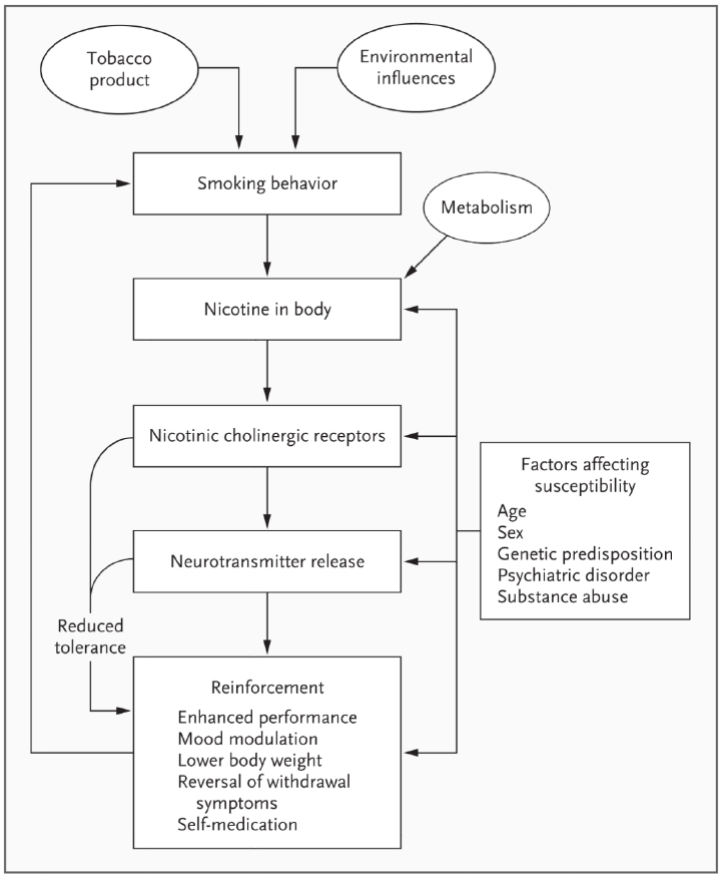


Figure 2.1. The Biology of nicotine addiction.
Reproduced with permission, from Benowitz (2010). Copyright Massachusetts Medical Society.

Nicotine from tobacco causes pleasure and relieves stress and anxiety. However, nicotine withdrawal induces stress and anxiety, which encourages people to start smoking again. Nicotine addiction occurs when smokers become dependent on smoking for modulation of

mood and arousal, relief of withdrawal symptoms, or both (Benowitz, 2010). Nicotine (3-(1-methylpyrrolidin-2-yl) pyridine) is the principal alkaloid, found in tobacco leaves, and is important as the addictive substance in tobacco (Hukkanen et al., 2005). In general, nicotine is not classified with 'harder' addictive drugs like heroin or cocaine, but with continued use it is often difficult to quit smoking (Laviolette & Van Der Kooy, 2004). Nicotine is mainly converted to its major metabolite cotinine in the liver, by the liver enzyme cytochrome P450 2A6 (CYP2A6). Consequently, cotinine is converted to 3′-hydroxycotinine which is the primary nicotine metabolite present in urine (Hukkanen et al., 2005). By breathing in smoke from a cigarette, volatilised nicotine is transported to the lungs with smoke particles and is quickly absorbed into the pulmonary circulation. Next, it travels rapidly to the brain, readily diffuses into the brain tissue, and binds to the ligand-gate ion channels, nicotinic acetylcholine (ACh) receptors (nAChRs) (Benowitz, 2009).

Neuronal nAChRs consist of pentameric subunits which are placed around a central pore forming a cation channel (Greenbaum & Lerer, 2009). Each subunit includes a large N-terminal extracellular domain, a transmembrane domain (M1-M4) and a variable cytoplasmic domain (Changeux, 2010). Nine α-subunits (α2-10) and three β-subunits (β2-4) are expressed and the primary types of nAChRs are α4β2 heteromers, α7 homomers and α6-containing heteromers in the brain. These receptors are targeted by numerous approved and experimental drugs for various conditions like Alzheimer's disease, Parkinson's disease, and neuropathic pain (Dhara et al., 2020). The α4β2 heteromer is characterized by a high affinity and slow desensitization, while α7 homomer is distinguished by a low affinity, a fast activation, and a high Ca^{2+} permeability (Changeux, 2010). Most neuronal nAChRs in the CNS regulate the release of dopamine, serotonin, ACh, glutamate, gamma-aminobutyric acid (GABA), and norepinephrine, and are found presynaptically. Postsynaptic nAChRs can also be found on the dopaminergic neurons in the ventral tegmental area (VTA), an important brain region for drug reinforcement (Herman et al., 2014; Valentine & Sofuoglu, 2018). Nicotine stimulates dopamine release in the mesolimbic area, the corpus striatum, and the frontal cortex (Benowitz, 2010) (Figure 2.2). Burst firing in dopaminergic neurons in VTA is caused by stimulation of the α4β2 nAChR, which increases dopamine release in the nucleus accumbens (NAc). Like other drugs of abuse, this dopamine release in the NAc mediates nicotine-induced pleasure and reward, which is presumed to be important mechanism for the onset and maintenance of nicotine dependence (Herman et al., 2014). However, nicotine leads to a lesser increase in dopamine levels than other drugs of abuse (Marenco et al., 2004; Tsukada et al., 2002). It has

been proposed that non-nicotinic components in tobacco smoke also play a part in the NAc by reducing the rate of MAO degradation and maintaining a high concentration of catecholamines and dopamine at their action sites due to synergy with nicotine (Lewis et al., 2007). Nicotine has reinforcement enhancement action which strongly increases behaviours leading to the delivery of nonpharmacological stimuli (Palmatier et al., 2006).

nAChR can exist in three main functional states which consist of resting state, open/activated state, and the desensitized state. In the desensitized state, the binding sites are occupied, but the channel is closed (non-conductive) (Wittenberg et al., 2020). Repeated exposure to nicotine results in tolerance (neuroadaptation) to some effects of nicotine. As neuroadaptation progresses, the number of sites that bind to nAChRs in the brain increases, possibly in response to nicotine-mediated desensitization of receptors (Benowitz, 2009). It has been assumed that cravings and withdrawal symptoms begin with smokers when desensitized α4β2* nAChRs become sensitive during abstinence periods, like night sleep. As a result, during smoking, nicotine binding to these receptors alleviates cravings and withdrawal (Benowitz, 2010).

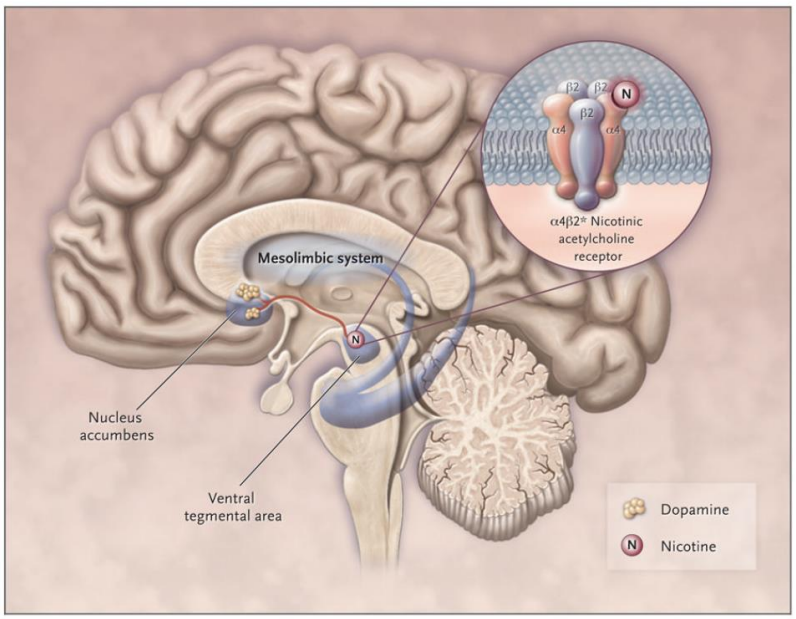


Figure 2.2. Role of the mesolimbic dopamine system in nicotine activity.
Nicotine activates α4β2* (* represents other subunits of the pentamer) receptors in the ventral tegmental area, resulting in dopamine release in the shell of the nucleus accumbens. Reproduced with permission, from Benowitz (2010). Copyright Massachusetts Medical Society.

2.2. Behavioural studies of MAO inhibitors in tobacco and tobacco smoke

2.2.1. Introduction

Smoking is a major cause of preventable premature death and disability, believed to cause six million deaths worldwide each year with smokers, on average, losing 10 years of their lives (West, 2017). The negative health effects of prolonged smoking are well established. Smoking primarily damages lung and cardiovascular health, as well as impacting negatively on every organ of the body (Office of the Surgeon et al., 2004). The reason people keep smoking, despite knowing that this habit is likely to eventually kill them, is that smoking is addictive (Henningfield & Faro, 1999). The major addictive component of tobacco smoke is nicotine, but it is increasingly evident that tobacco dependence is multi-faceted (West & Cox, 2022). Nicotine in laboratory tests is much less addictive than the lived experience of smokers would suggest (Balfour, 2009; Jain, 2003). The explanations are varied, ranging from societal and behavioural influences (Moolchan et al., 2003), to strong cue association and cognitive effects (Chiamulera, 2005; Sacco et al., 2004) and to the influence of tobacco companies (Hoek et al., 2012).

Smokers themselves report smoking to relieve stress or anxiety, or to aid concentration (Benowitz, 2010) and smokers with a variety of mental health conditions report using smoking as a form of self-medication (Aubin et al., 2012; Leonard et al., 2001). Unsurprisingly, the interpretation of these reports varies widely. The only mental health condition where smoking is proven to have protective effects is Parkinson's Disease (Castagnoli & Murugesan, 2004; Gigante et al., 2017), with smoking having a well-established neuroprotective effect against this disease (Veljkovic et al., 2018).

A strong theme coming through the literature is that components in tobacco smoke other than nicotine, possibly monoamine oxidase (MAO) inhibitors, may enhance tobacco dependence, or may have positive effects on mood (Fowler et al., 2003; Harris et al., 2020; Hogg, 2016; Rose, 2006). Thus, apart from the effects of nicotine, the observed difficulty that people have in stopping smoking could be influenced by other chemical components of the tobacco smoke. These types of interpretation are disputed. For instance, the high rate of relapse in smokers attempting to stop smoking is attributed to relief from nicotine withdrawal, rather than thinking of a positive effect on mood or concentration from smoking as reinforcing nicotine dependence (Steven Moylan et al., 2013).

It is completely accepted that tobacco smoking is a harmful habit, due to the many toxic and carcinogenic components of the smoke. However, this review aims to focus attention on areas of the literature suggesting pharmacological drivers behind tobacco dependence other than the immediate effects of nicotine in inducing dependence, and our current understanding of the short-term effects of tobacco smoke components on smokers. Are some of these effects positive, as in neuroprotection against Parkinson's Disease? Are there known tobacco components which could have a positive effect?

2.2.2. Nicotine

Nicotine acts at the nicotinic acetylcholine receptors (nAChRs) to cause flow-on effects in specific areas of the brain. These nAChRs are believed to be important in coordinating brain responses, by action of the natural transmitter, acetylcholine (ACh). Nicotine binds to nAChRs mimicking the action of ACh and altering responses within the brain (Balfour, 2009).

The key addictive response is stimulation of nAChRs in the ventral tegmental area of the brain, which causes the release of dopamine in the nucleus accumbens, believed to be central to the development of all addictive responses(Di Chiara & Imperato, 1988). Nicotine's pharmacology has been well reviewed by others (Benowitz, 1996; Laviolette & Van Der Kooy, 2004) as have the complexities of the nicotinic receptors in the brain (Albuquerque & Pereira, 2009; Wu & Lukas, 2011). The nAChRs belong to the ionotropic family of receptors, with five proteins forming a channel. Seventeen different proteins have been identified, leading to a wide diversity of nicotinic receptor subtypes. Thus the diversity of nicotinic receptor types and their varied localisation within the brain allows for much more nuanced and complex responses to nicotine than simple dopamine release.

Several milligrams of nicotine are present in each gram of tobacco, and nicotine reaches around 0.2 micromolar concentrations in the blood of smokers, sufficient to cause nAChR responses (Alkondon et al., 2000). Nicotine concentrations rise rapidly when tobacco smoke is inhaled, reaching the brain in under two minutes. It then dissipates slowly, having a half-life of around 2 hours (Benowitz, 2009). As nicotine brain concentrations fall, in the addicted smoker, cravings for nicotine begin, leading to smokers repeating the experience. Many of the effects of smoking tobacco (dopamine release and dependence, and withdrawal effects and relapse back to smoking) can be related back to these key effects of nicotine on the brain.

As the major pharmacologically active component of tobacco smoke, nicotine has also been investigated to see whether it can cause other effects, reported from smoking, such as relief

from anxiety and depression, improved concentration and symptom control in Schizophrenia, Parkinson's Disease, Attention Deficit Hyperactivity Disorder, and Alzheimer's Disease (Mihailescu & Drucker-Colín, 2000; Newhouse et al., 2004a; Veljkovic et al., 2018).

2.2.2.1. Nicotine and cognition/concentration

In utero exposure and exposure of children to tobacco smoke are both believed to interfere with cognitive development, causing deleterious effects on attention span and ability to concentrate, in children (Alhowail, 2021; Hajdusianek et al., 2021). With some caveats as to the strength of the evidence (Chan et al., 2020) it is generally accepted that smoking in pregnancy is bad for the unborn child, although there is some doubt as to whether nicotine causes all of the problems noted (Baler et al., 2008; Chan et al., 2020).

In adults, however, nicotine is believed to have a positive effect on mental acuity (Conley et al., 2021; Nop et al., 2021). Again, this has been suggested by studies in animals and in humans (Kumari et al., 2003; Newhouse et al., 2004b), and trials of the effect of nicotine in older adults have produced some evidence of benefit. A recent meta-analysis by Majdi and coworkers suggested that nicotine has a moderate but positive effect on attentional ability in healthy non-smoking adults (Majdi et al., 2021) and it has been suggested that nicotine could be used to treat late-life depression, with part of this action being mediated by effects on cognition (Gandelman et al., 2018).

Smoking as a long-term enhancer of cognition is not recommended however, since smoking is a risk factor for vascular dementia (López-Arrieta et al., 2001).

2.2.2.2. Nicotine and anxiety/depression

The suggestion that nicotine can relieve stress and help with anxiety and depression is controversial. The key argument is whether the positive effects noted by smokers are real e.g. (Choi et al., 2015; Pomerleau et al., 1984) and act as a reinforcer of tobacco dependence by improving mood, or whether nicotine dependence causes depression and /or anxiety over time, with relief of cravings being misread by smokers as relief from the related mood disorders (Molas et al., 2017; S. Moylan et al., 2013). A systematic review by Fluharty and co-workers (Fluharty et al., 2017) found evidence for causation in both directions and suggested the need for more studies. More recent work tends to look at effects of transdermal nicotine, and has found potential for nicotine to be used in cases of major depressive disorder (Janes et al., 2018), and for relief of later life depression (Conley et al., 2021).

2.2.2.3. Nicotine and schizophrenia

Schizophrenia is also strongly associated with tobacco smoking, and here also the direction of causation is controversial. Scott and coworkers examined the evidence that smoking might cause schizophrenia and suggested nicotine as the causative agent (Scott et al., 2018). Others have suggested that nicotine's effects within the brain might give relief from the symptoms of schizophrenia, providing motivation to continue smoking (Postma et al., 2006). It is possible that nicotine's positive effect on cognition is the mediating mechanism for this (Waterhouse et al., 2018).

2.2.2.4. Nicotine and Alzheimer's Disease

The possibility that nicotine might be useful in treating early stages of Alzheimer's Disease has been studied for some years. As with Parkinson's Disease, smokers are under-represented among those with Alzheimer's Disease. Impaired cholinergic function is an early feature of Alzheimer's Disease and nAChR agonists have been suggested as potentially helpful in ameliorating symptoms (Albuquerque et al., 2001). Transdermal nicotine has been used in trials with positive effects on attention and other cognitive measures (Newhouse et al., 2004b). Nicotine has also been shown to be effective in reducing behavioural and synaptic plasticity deficits in a rat model of Alzheimer's disease (Esteves et al., 2017). In a separate line of enquiry nicotine is also thought to delay formation of amyloid plaque (Zhang et al., 2006), giving another mechanism by which smoking (nicotine) could be helpful in delaying Alzheimer's Disease onset.

2.2.2.5. Nicotine and Parkinson's Disease

While smoking is well known to be protective against Parkinson's Disease (Gigante et al., 2017), the causative agents in this case are likely to include both nicotine, through its action on dopaminergic pathways (Thiriez et al., 2011), and monoamine oxidase inhibitors (Castagnoli & Murugesan, 2004). Nicotine's effect on inhibiting formation of α-synuclein fibrils has also been investigated. Nicotine slowed down the formation of these fibrils (Kardani et al., 2017). However, although nicotine appeared to be neuroprotective in Parkinsonian animals it had no effect in restoring damage in the same experimental system (Huang et al., 2009) and was not significantly effective in Phase II clinical trials (Villafane et al., 2018).

2.2.3. Minor tobacco alkaloids

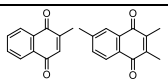
Like nicotine, the other nicotine analogues found in tobacco smoke have also been found to act on the nicotinic receptors. The "minor alkaloids" in tobacco smoke other than nicotine include nornicotine, myosmine, cotinine, anabasine and anatabine. Where nicotine is found at around 10 milligrams per cigarette in tobacco, these minor alkaloids are found in microgram per cigarette amounts (Smith et al., 2015).

Several groups have looked at the effect of these alkaloids *in vivo* (Harris et al., 2015; Marusich et al., 2017; Tan et al., 2022). Such studies have found that the minor tobacco alkaloids can partially substitute for nicotine in behavioural tests but have lower potency than nicotine itself. The different alkaloids are not equivalent in their behavioural effects. Differences in binding to different nAChR variants between the various nicotinic alkaloids would allow for differences in mode of action and potency even if all act through binding nAChRs. The combination of lower potency and lower concentration in tobacco make it unlikely that these alkaloids have significant effects on smoker behaviour.

Cotinine, however, is also a breakdown product of nicotine. It has relatively long half-life in the blood (c.a. 16 hours compared to c.a. 2 hours for nicotine (Hukkanen et al., 2005) and reaches low micromolar concentrations in a smoker's blood, several-fold higher than the concentration of nicotine. It may, therefore, be present in sufficient amounts to have a modulating effect on smoker behaviour, particularly for heavy smokers. Cotinine is a weak agonist of nAChRs, binding less strongly than nicotine (Tan et al., 2021) but has recently been shown to support self-administrative behaviour (Tan et al., 2022) although less robustly than nicotine. Cotinine upregulates the α7nAChRs and this activity is neuroprotective to glial cells (Iarkov et al., 2021). Cotinine also slows down the formation of α-synuclein fibrils, with a potency similar to that of nicotine, and may also have positive cognitive benefits.

Table 2.1. summarises the suggested beneficial effects of nicotine and related alkaloids.

Table 2.1. Suggested behavioural effects of nicotine, cotinine, and selected monoamine oxidase inhibitors in tobacco smoke.

Smoke component	Chemical structure	Mechanism	Disease state affected	References
Nicotine		nAChR activation	Cognitive improvement	(Majdi et al., 2021; Newhouse et al., 2004b)
Nicotine		↓ α-synuclein fibril formation: (cognition ↑)	Parkinson's disease	(Kardani et al., 2017; Villafane et al., 2018)
Nicotine		↓Amyloid ß-peptide aggregation: (cognition↑)	Alzheimer's disease	((Esteves et al., 2017; Zhang et al., 2006)
Nicotine		Cognition↑	Schizophrenia	(Waterhouse et al., 2018)
Nicotine		Cognition↑	Depression	(Conley et al., 2021; Gandelman et al., 2018)
Cotinine		↓ α-synuclein fibril formation, neuroprotection	Parkinson's disease Alzheimer's disease	(Iarkov et al., 2021; Riveles et al., 2008; Terry Jr et al., 2005)
Naphthoquinones		MAO inhibition, neuroprotection (nitric oxide control)	Parkinson's disease	(Venkatakrishnan et al., 2009)
Harman/Norharman		MAO inhibition	Antidepressant	(Farzin & Mansouri, 2006)
Harman/Norharman		MAO inhibition	Anxiolytic	(Smith et al., 2013)
2,3,6-Trimethyl-1,4-naphthoquinone		MAO inhibition	Neuroprotection, Parkinson's disease	(Sari & Khalil, 2015)

Reproduced with permission, from Hong et al. (2022).

2.2.4. Monoamine oxidase inhibitors

Monoamine oxidase inhibitors (MAOIs) in tobacco smoke have long been regarded as having potential significance in modulating the effects of smoking on the brain (Dome et al., 2010; Fowler et al., 2003; Hogg, 2016). Monoamine oxidase (MAO) enzymes in the brain are responsible for clearance of brain transmitters, notably dopamine, serotonin, adrenaline and noradrenaline (Lewis et al., 2007). Since inhibition of MAO activity will lead to reduced clearance of neurotransmitters such as dopamine it is suggested that MAOIs in the tobacco smoke might enhance the dopamine reward from nicotine and the addictiveness of smoking.

A variety of experimental evidence has been produced showing that MAO inhibition enhances behavioural responses to nicotine in rats (Guillem et al., 2005; Harris et al., 2020; Smith et al., 2016b; Villegier et al., 2011; Villegier et al., 2006), but the extension to human smoking behaviour is less clear. MAO activity is well known to be reduced in smokers (Fowler et al., 1996a; Fowler et al., 1996b) with the inhibition being believed to be irreversible (Yu & Boulton, 1987). The time course of recovery of activity after smoking cessation extends over several weeks after smoking cessation, consistent with the equivalent recovery time from inhibition by known irreversible MAO inhibitory drugs (Fowler et al., 2003). Although epigenetic (Launay et al., 2009) and microRNA (Higuchi et al., 2018) mechanisms for MAO

activity reduction have been suggested, direct inhibition by components of tobacco smoke as the major cause of the observed reduction remains a likely mechanism by which MAO inhibition is accomplished in smokers.

The known MAOIs in tobacco smoke include the ß-carbolines harman and norharman, α-naphthylamine, farnesyl acetone and tetrahydroisoquinolines (TIQ's) (Hogg, 2016; Lewis et al., 2007). Harman and norharman have been proposed as the major contributors to the observed MAOI activity in tobacco smoke, causing the observed decrease in MAO activity (Herraiz & Chaparro, 2005; Rommelspacher et al., 2002), with the discrepancy between the amounts of ß-carboline measured in smokers, and the inhibition observed being ascribed to their accumulation in platelets (Rommelspacher et al., 2002) and in brain (Fekkes & Bode, 1993). However, harman and norharman make up less than 1/10th of the total direct MAO inhibitory activity in tobacco smoke (Truman et al., 2017) so the opportunity for other MAOIs in tobacco smoke to contribute substantially to the MAO activity reduction seen in smokers must exist. To our knowledge, no irreversible MAO inhibitors, or inhibitors expected to have long half-lives in the blood have yet been reported from tobacco or tobacco smoke. Together, these data suggest that additional MAO inhibitors are yet to be found.

The question of whether MAO inhibitors in tobacco smoke can affect behaviour remains unresolved. As well as the potential for effects on addiction, MAO enzymes are drug targets for a variety of neurological disorders including depression, mood, anxiety, attention deficit hyperactivity, Tourette's syndrome, Parkinson's disease and Alzheimer's disease (Borroni et al., 2017; Sharma, 2016). Of the known MAO inhibitors in tobacco smoke, high concentrations of harman and norharman can affect responses to nicotine (Harris et al., 2020) and may act as antidepressants (Farzin & Mansouri, 2006; Smith et al., 2013) in animals. However, when used in amounts relevant to smokers, they were not seen to affect rat self-administration of nicotine (Smith et al., 2015) and, to our knowledge, no pharmacological effects have been reported from the known tobacco MAO inhibitors at physiologically relevant concentrations. In contrast, use of tobacco smoke extracts in self-administration or intracranial self-stimulation experiments has been found to affect responses to nicotine (Brennan et al., 2015; Costello et al., 2014; Harris et al., 2010), although there are some inconsistencies in the literature (Gellner et al., 2016). These differences may be in part because we have not yet identified the full range of tobacco smoke MAO inhibitors.

It has also been suggested that further MAO inhibitory activity is formed from smoke components in the body. Acetaldehyde is formed during tobacco combustion, from the sugars in the plant material, and from sugars added as humectants and flavour additives during tobacco and cigarette manufacture. Acetaldehyde is typically present in cigarette smoke amounts ranging from 0.6 to over 2 milligrams per cigarette (Seeman et al., 2002). Acetaldehyde yields locomotor stimulation and reinforcing effects (Quertemont & Tambour, 2004) at concentrations higher than those seen in tobacco smoke and enhances self-administration of nicotine at concentrations similar to those in tobacco smoke (Belluzzi et al., 2005), although this finding was not confirmed by Smith and co-workers in similar trials (Smith et al., 2015).

It is suggested that acetaldehyde acts by reacting with chemicals naturally occurring in the brain to form MAO inhibitors (Talhout et al., 2007). A wide variety of TIQs are formed from acetaldehyde and catecholamines (dopamine, noradrenalin, adrenalin) (Naoi et al., 2004; Patsenka & Antkiewicz-Michaluk, 2004). Of particular note are the cyano derivatives, which inhibit MAO A and B with K_i values between 18 and 38 μM (Méndez-Álvarez et al., 1997). A group of tetrahydro-β-carbolines (THBCs) are also formed from condensation of acetaldehyde and indoleamines (serotonin, tryptamine, tryptophan) (Talhout et al., 2007) and are more potent, inhibiting with K_i values under 10 μM. It seems likely that concentrations of these MAOIs, formed in the body after smoking, are sufficient to have some effect on smoking addiction in humans, as well as in rats, since a randomized double-blind trial of a method to reduce the amount of acetaldehyde entering a smoker's body had some success in encouraging cessation (Syrjänen et al., 2017). Thus far, together with the β-carbolines, acetaldehyde is a leading candidate as a tobacco smoke component causing monoamine oxidase inhibition in smokers sufficient to modulate tobacco dependence, even though it does not directly cause MAO inhibition. The inhibitors formed from acetaldehyde appear to be reversible (Naoi et al., 2004).

The immediate effect on MAO activity of smoking a single cigarette could not be detected using PET methods (Fowler et al., 2003), whereas longer term effects of continued smoking results in an overall, apparently irreversible reduction of 30-40% in both MAO A and B activity, suggesting that long term exposure to tobacco smoke is required for this effect. Until we know which components of tobacco smoke cause the observed inhibition of MAO activity, and their mechanism of action, it will remain difficult to determine the extent, timing and overall effects of MAO inhibition in smokers.

Monoamine oxidase inhibitors in tobacco smoke may well have effects other than enhancing the addictive potential of nicotine. Smith and coworkers (Smith et al., 2016a) have examined the effects of MAO inhibitors and nicotine on brain function, measured using EEG techniques in non-smoking humans. They suggest that MAO inhibition may result changes in brain function which are experienced as lapses in cognition. Nicotine's effect in enhancing cognition is suggested to alleviate this.

MAOI's are a major drug target, of interest for treatment of depression and anxiety, Parkinson's disease and Alzheimer's disease (Sharma, 2016). Thus, it has been suggested that the MAOI activity in tobacco smoke may enhance a smoker's mood (Choi et al., 2015; Farzin & Mansouri, 2006; Pomerleau et al., 1984; Smith et al., 2013) independently of their effects on dopamine reward from nicotine, and may contribute to smoker's dependence on tobacco by this indirect route (Arnold et al., 2014). However, rather than a positive effect on mood, it is possible that MAO inhibition serves to intensify withdrawal symptoms (Malin et al., 2013).

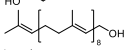
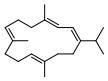
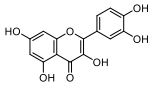
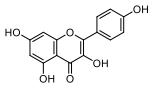
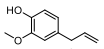
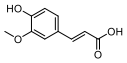
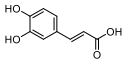
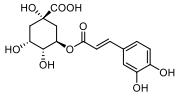
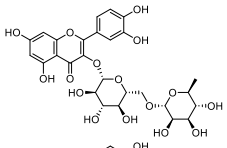
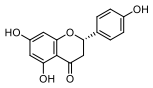
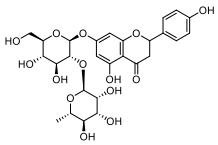
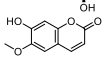
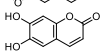
This potential role for MAO inhibition in tobacco dependence has led to suggestions that MAO inhibitors could be used for smoking cessation (Biberman et al., 2003; George & Weinberger, 2008). Current consensus is that, after some promising preliminary results, the known MAO inhibitory drugs are not particularly useful for smoking cessation (Howes et al., 2020).

The proposed effects of MAO inhibitors on smokers are listed in Table 2.1.

2.2.5. Other components of tobacco smoke

Additional components of tobacco and tobacco smoke which have been associated with beneficial effects are listed in Table 2.2, below. They have been identified as a result of investigations of the active components supporting herbal use, but from plants other than tobacco. These compounds are all known components of tobacco (Rodgman & Perfetti, 2013), however, while their biological activity is of interest, their contribution to the overall effects of tobacco smoking is unknown.

Table 2.2. Suggested behavioural effects of additional compounds from tobacco and tobacco smoke.

Component	Chemical structure	Observed effect	Application	Reference
Catechol		↓ amyloid-β fibril formation	Alzheimer's disease	(Huong et al., 2010)
Hydroquinone		↓ a-synuclein fibrillation	Parkinson's disease	(Hong et al., 2009)
Solanesol		Neuroprotection	Stroke	(Rajdev et al., 2020)
Limonene		Neuroprotection	Alzheimer's disease	(Shin et al., 2020)
Cembranoids		Nicotinic activation, neuroprotection	Alzheimer's disease Parkinson's disease	(Ferchmin et al., 2009)
Quercetin		↓cognitive function ↓ amyloid-β fibril formation	Alzheimer's disease Parkinson's disease	(Khan et al., 2019; Paula et al., 2019)
Kaempferol		↑ striatal dopamine, SOD & GSH ↓ malondialdehyde	Parkinson's disease	(Li & Pu, 2011)
Eugenol		↓immobility in forced swim test	Depression	(Irie et al., 2004)
β-asarone		↑efficacy of memantine ↑cognitive deficit	Alzheimer's disease	(Chang & Teng, 2018; Liu et al., 2016) (Han et al., 2020)
Vanillin		↓ oxidative stress response ↓behavioural impairment	Parkinson's disease	(Dhanalakshmi et al., 2016)
Ferulic acid		↑ serotonin and norepinephrine ↓immobility in forced swim test ↓brain capillary constriction	Depression Alzheimer's disease	(Chen et al., 2015; Wang et al., 2021)
Caffeic acid		↓ immobility in forced swim test	Depression	(Takeda et al., 2002)
Chlorogenic acid		↓mitochondrial disfunction ↓ oxidative stress	Parkinson's disease	(Singh et al., 2020)
Rutin		↓ immobility in tail suspension test ↓ effect of chronic induced stress	Depression, Anxiety	(Parashar et al., 2017; Yusha'u et al., 2017)
Naringenin		↓ amyloid-β toxicity ↓ immobility in tail suspension test ↑memory	Alzheimer's disease Depression	(Md et al., 2018; Yang et al., 2014; Yi et al., 2012)
Naringin		↓mitochondrial disfunction ↑memory	Alzheimer's disease	(Sachdeva et al., 2014)
Scopoletin		↓ anxiety-like behaviour	Anxiety	(Luo et al., 2020)
Esculetin		↓ immobility in forced swim test	Depression, Anxiety	(Sulakhiya et al., 2016)
Quinic acid		↓MAO-B, neuroprotection	Dementia	(Liu et al., 2020)

SOD: superoxide dismutase, GSH: glutathione peroxidase, Reproduced with permission, from Hong et al. (2022).

2.2.6. Conclusion

It is accepted that nicotine has positive effects on cognition and attention in adults, even though having negative effects in infant and child development. For this reason, nicotine could be helpful in the management of a variety of mental health conditions and provide an explanation for high levels of smoking in disorders such as schizophrenia. Nicotine and its metabolite cotinine both stimulate cholinergic systems, which can be neuroprotective, and may also prevent ß-amyloid fibre aggregation in Alzheimer's disease, and α-synuclein fibre formation in Parkinson's disease. While the long-term overall effect of smoking is deleterious, the same may not be true for nicotine and cotinine. However, nicotine is thought to have negative effects, including on blood pressure. Long-term exposure to nicotine induces oxidative stress and endothelial dysfunction, which results in hypoperfusion of the cerebral arteries (Whitehead et al., 2021). The therapeutic use of cotinine also deserves further work, since it is expected to have a lower abuse potential than nicotine but may provide similar neuroprotective and cognitive effects.

The other most significant and potentially beneficial biological activity in tobacco smoke is monoamine oxidase inhibitory activity. This is likely to have an impact on the response to nicotine, and to increase the addictiveness of smoking, both by increasing the dopamine rewards from the nicotine, and by its effects (if any) on mood. The extent to which these effects on mood from smoking are due to relief of nicotine cravings, or directly caused by the modulation of monoamine oxidase enzyme activity in the brain remains uncertain. In this respect it is important to recall that the first generation of antidepressant drugs were selective MAO inhibitors, with some still currently used for treatment of major depressive disorders. This question will only be resolved when the causative agents of the MAO inhibition seen in smokers have been identified, and their effects can be studied independently of the effects of nicotine. It may prove possible to separate immediate MAO inhibitory activity from the long-term irreversible inhibitory factors and assess their effects separately once any irreversible inhibitors have been identified.

It is likely that MAO B inhibitory activity in tobacco smoke, perhaps together with the combined neuroprotective effect of a variety of smoke components may be useful for symptom control in Parkinson's disease, while MAO A inhibitory activity is more likely to contribute to the alleviation of mood disorders.

While the contribution of biologically active molecules other than nicotine remains to be established it is clear that tobacco smoke contains many components which might have beneficial effects. While this in no way compensates for the overall deleterious effects of smoking, these effects may help explain the strength of tobacco dependence many smokers experience and are worthy of further study, so that we can disentangle the deleterious and beneficial effects, to better help smokers to stop smoking.

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Chapter 3
Literature Review 2

This chapter begins with a review of monoamine oxidase (MAO) and MAO inhibitors in tobacco or tobacco smoke.

This chapter contains the article. “A review of monoamine oxidase (MAO) inhibitors in tobacco or tobacco smoke”, co-authored by Sa Weon Hong (first author) (SH), Paul Teesdale-Spittle (PT-S), Rachel Page (RP), and Penelope Truman (PT). This article was published in Neurotoxicology in 2022. Author contributions: PT, RP, and PT-S guided the initial literature search, performed by SH. SH wrote the manuscript. All authors contributed to the final shape of the manuscript. The Neurotoxicology paper can be found in Sections 3.1-3.5. We have added Sections 3.2.1-3.2.3 as additional background.

This article addresses research question 1 (Section 1.4.1) for components in tobacco and tobacco smoke with MAO inhibitory activity.

A review of monoamine oxidase (MAO) inhibitors in tobacco or tobacco smoke

3.1. Introduction

Smoking is reputed to be one of the hardest addictions to give up, yet nicotine by itself has only moderate potential for addiction (Rose, 2006). One hypothesis is that monoamine oxidase (MAO) inhibitors in, or derived from, tobacco smoke decrease the breakdown of neurotransmitters associated with reward pathways, such as dopamine, and synergistically increase the addictive effects of nicotine in smokers (Hogg, 2016) by increasing the effective reward signal.

MAO enzymes are responsible for the oxidative deamination of monoamine substrates in the body and brain, including neurotransmitters such as serotonin, histamine, dopamine, norepinephrine and epinephrine. In humans, there are two isoforms, MAO A and MAO B, with different distributions in the body and the brain and with different substrate specificity (Youdim et al., 2006). Together they are involved in complex behaviour, including drug use as well as personality traits (Harro & Oreland, 2016). The MAO enzymes are also known drug targets: their inhibitors affect mood and have been used to treat depression, panic disorder, social phobia, bipolar, and anxiety (Menkes et al., 2016; Sub Laban & Saadabadi, 2021). MAO B inhibitors are also important in treatment of Parkinson's disease (PD) (Duarte et al., 2020).

Interestingly, positron emission tomography (PET) scans have shown that smokers have lowered levels of MAO A (28%; $P < 0.0003$) and MAO B (40%; $P < 0.0002$) activity in the brain relative to non-smokers (Fowler et al., 1996a; Fowler et al., 1996b). Fowler et al. (2003) proposed that smoke-induced MAO inhibition requires prolonged exposure and is not reversed quickly. An earlier *in vitro* study had reported that unidentified components of cigarette smoke showed irreversible inhibition (Yu & Boulton, 1987). Hogg (2016) has suggested that several tobacco-derived substances may cause the MAO inhibition seen in smokers through additive or synergistic effects. Previous studies were conducted to identify and characterize MAO inhibitors in tobacco and tobacco smoke and several compounds that show reversible MAO A and/or MAO B inhibition have been described (Herraiz & Chaparro, 2005; Khalil et al., 2006; Khalil et al., 2000) and reviewed in Lewis et al. (2007). Two review papers have focused on the potential role of MAO inhibitors from tobacco smoke in the treatment of Parkinson's disease (Sari & Khalil, 2015) and describing the available evidence that links tobacco dependence and MAO inhibition by tobacco-derived substances (Hogg, 2016). However, to our knowledge, irreversible MAO inhibitors from tobacco smoke had not been reported.

In separate lines of enquiry, after the crystal structures of MAO A (De Colibus et al., 2005) and MAO B (Binda et al., 2002) had been solved, many natural products and natural product-based MAO inhibitors have been reviewed as potential therapeutics in the treatment of Parkinson's disease, Alzheimer's disease (AD), and depression (Carradori et al., 2014; Das et al., 2022; Dhiman et al., 2018; Hong et al., 2020; Vina et al., 2012). This work to find new MAO inhibitors for therapeutic purposes has provided a further opportunity to look for reports of MAO inhibitors which are also tobacco smoke components.

The objective of this review is to discuss components in tobacco and tobacco smoke with MAO inhibitory activity especially for their MAO selectivity and reversibility. For this article we used an index of chemical constituents in tobacco and tobacco smoke (Rodgman & Perfetti, 2013) to search for MAO inhibitors in tobacco and tobacco smoke. We crossmatched this index with existing literature reporting on natural compounds and their structural analogs with MAO inhibitory activity.

3.2. Monoamine oxidase

In 1928 Mary Hare characterized an enzyme responsible for the oxidation of tyramine in the liver and named it tyramine oxidase and later Zeller (1938) proposed a more appropriate name for the enzyme to be monoamine oxidase (MAO) to distinguish it from diamine oxidase (DAO; EC 1.4.3.22) (Tipton, 2018).

MAO (EC 1.4.3.4) is a flavin adenine dinucleotide (FAD) dependent enzyme that catalyzes the oxidative deamination of primary, secondary and tertiary amines, including dietary amines, neurotransmitters dopamine, serotonin, norephinephrine, and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin which induces symptoms of Parkinson's disease (Youdim & Bakhle, 2006). MAO is not only important for the modulation of mood, movement, memory, and arousal, but it is also important for the metabolism of exogenous sympathomimetic amines in the diet by breaking them down (Lewis et al., 2007). Monoamine breakdown catalyzed by MAO proceeds *via* amine oxidation and hydrolysis to produce an aldehyde (Ramsay & Albreht, 2018; Ramsay et al., 2011; Yeung et al., 2019) (Fig. 3.1). The MAO-catalyzed reaction produces hydrogen peroxide (H_2O_2), an aldehyde, and ammonia, all of which have neurotoxic potential. In particular, H_2O_2 can generate reactive oxygen species (ROS) and lead to mitochondrial damage and neuronal apoptosis (Bortolato et al., 2008). However, a recent study has shown that dopamine degradation by MAO does not increase the

formation of cytosolic H_2O_2 but gives rise to elevated mitochondrial electron transport chain (ETC) activity (Graves et al., 2020). It has been proposed that the ETC pathway, using ETC complex IV for ATP production from H_2O_2 within the mitochondria, protects dopamine neurons against the H_2O_2 toxicity (Chen & Jonas, 2020).

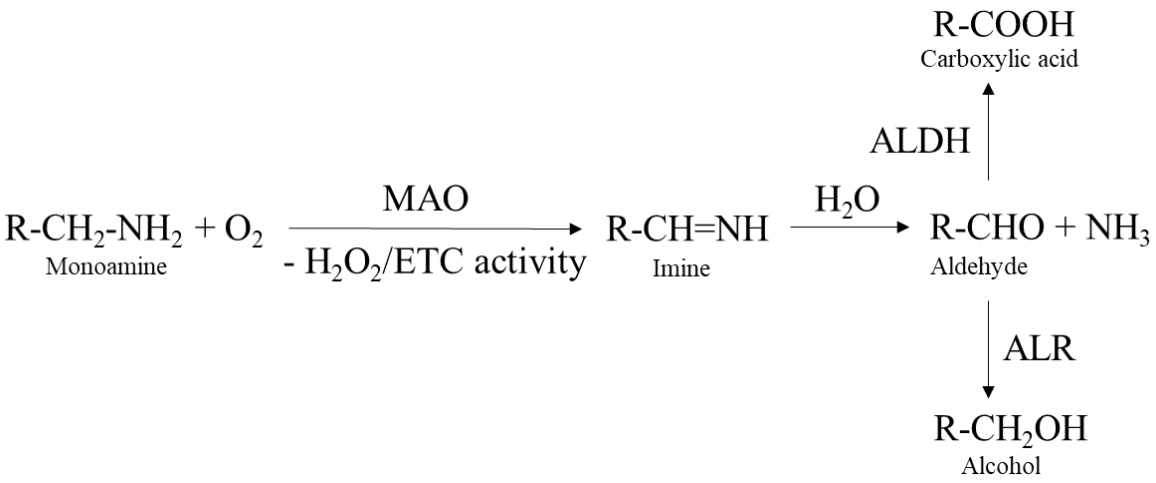

Figure 3.1. Monoamine oxidase (MAO) catalyzed oxidation of monoamines.

MAO oxidation uses an amine and oxygen as substrates and gives an imine and hydrogen peroxide (H_2O_2) as products. The imine product is spontaneously hydrolysed to an aldehyde which is oxidized by aldehyde dehydrogenase (ALDH) into a carboxylic acid or converted into an alcohol by aldehyde reductase (ALR).

Two isoforms, MAO A and MAO B, are located at the outer membrane of mitochondria in most mammalian tissues (Edmondson et al., 2009) and show 70% amino acid identity with similar molecular weights of 59 and 58 kDa, respectively (Shih et al., 1999). These isoforms have different substrate and inhibitor specificities. The preferred substrate for MAO A is serotonin and those for MAO B, beta phenethylamine and benzylamine. Both isoforms catalyze oxidation of dopamine, norepinephrine, epinephrine, tyramine and tryptamine (Youdim et al., 2006).

The first clinically effective monoamine oxidase inhibitor, iproniazid, was originally developed to treat tuberculosis, and then used as an antidepressant. However, its clinical use was discontinued because of hepatotoxicity (Tipton, 2018). MAO A and MAO B are selectively and irreversibly inhibited by the propargyl-containing MAO inhibitors clorgyline and

selegiline (deprenyl), respectively (Youdim et al., 2001). Examples of other MAO inhibitors are shown in Table 3.1, along with their selectivity and whether they are proposed to be reversible or irreversible inhibitors (Entzeroth & Ratty, 2017; Tripathi et al., 2018). Among MAO inhibitors, approved therapeutics for the treatment of Parkinson's disease include selegiline, rasagiline, and safinamide, while MAO inhibitory therapeutics for the treatment of Alzheimer's disease, amyotrophic lateral sclerosis, and cardiovascular diseases, are in development (Duarte et al., 2020). Although the use of MAO inhibitors has been declining for several decades in the treatment of depression, recently, the importance of inhibitor medications (i.e., tranylcypromine, phenelzine, isocarboxazid, and the Selegiline Transdermal System) is emphasized and their continued availability has been recommended (Gillman et al., 2020).

Table 3.1. Examples of MAO inhibitors used or studied in treatment of a variety of conditions.

Class	Inhibitor	Selectivity	Conditions to treat
Irreversible MAO inhibitors			
Hydrazine	iproniazid (Marsilid)	A and B	Depression
	isocarboxazide (Marplan)	A and B	Depression
	phenelzine (Nardil)	A and B	Depression
	nialamide	A and B	Depression
Propargylamine	clorgyline	A	Depression
	selegiline (Emsam)	B	Parkinson's disease
	rasagiline (Azilect)	B	Parkinson's disease
	pargyline	B	Parkinson's disease
	ladostigil	A and B (brain selective)	Depression, Parkinson's disease, and Alzheimer's disease
Cyclopropylamine	tranylcypromine (Parnate)	A and B	Depression
Reversible MAO inhibitors			
Oxazolidinone	befloxatone	A	Depression
	toloxatone	A	Depression
	furazolidone	A and B	Bacterial infection and Depression
	linezolid (Zyvox)	A and B	Bacterial infection and Depression
Benzamide	moclobemide (Aurorix)	A	Depression
α-Aminoamide	safinamide (Xadago)	B	Parkinson's disease

Brand names (shown in brackets) commonly available in the U.S. or other countries (https://www.accessdata.fda.gov/scripts/cder/daf/).

3.2.1. MAO A and MAO B structure

The structures of MAO A and MAO B are shown in Fig. 3.2A and 3.2B. The most distinctive difference between MAO A and MAO B is that MAO A crystallizes as a monomeric form, whilst MAO B crystallizes as dimeric forms. The crystal structure of MAO B in complex with pargyline, an analog of selegiline, was solved at 3.0 Å resolution in 2002 (Binda et al., 2002). Binda and colleagues (2002) were the first to report the MAO crystal structure. The crystal structure shows that the enzyme exists as dimer which is present in orthorhombic and triclinic crystal forms. In the case of human MAO A, a 3.0 Å resolution three-dimensional structure of MAO A with clorgyline was determined in 2005 (De Colibus et al., 2005). It crystallizes as a monomer which is a unique structural feature of MAO A and distinct from the dimeric form of MAO B. Subsequent studies using pulsed EPR (Electron Paramagnetic Resonance) show MAO A and MAO B existing as dimers in their membrane-bound forms, but MAO A is partially (≤50%) dimeric in the detergent (octyl β-D-glucopyranoside, OGP). It has been indicated that the reported monomeric form of MAO A is a consequence of its instability in detergent (Upadhyay et al., 2008).

A B

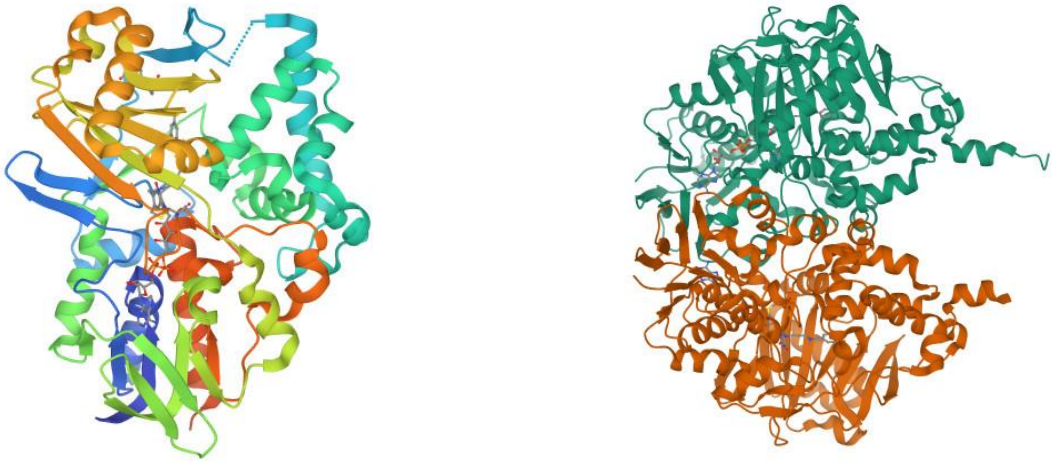

Figure 3.2. Structure of human MAO A (A) and MAO B (B).
(Crystal structures PDB 2BXR and PDB 1GOS - human MAO A and B, respectively).

MAO A and MAO B consists of 527 and 520 amino acids, respectively. An FAD cofactor is covalently bound to the enzyme as 8α-S-cysteinyl-FAD (Figure 3.3A) (Edmondson et al., 2004). MAO A and B have equivalent features in their active sites: the covalently bound FAD and the two tyrosine residues named ‘‘aromatic cage’’. This structural similarity is in agreement with the idea that MAO A and MAO B follow the same mechanisms for MAO catalysis (Figure 3.3B). It has been suggested that the “aromatic cage” plays a steric role in amine binding and access to the flavin and increases the amine nucleophilicity (Li et al., 2006).

A B

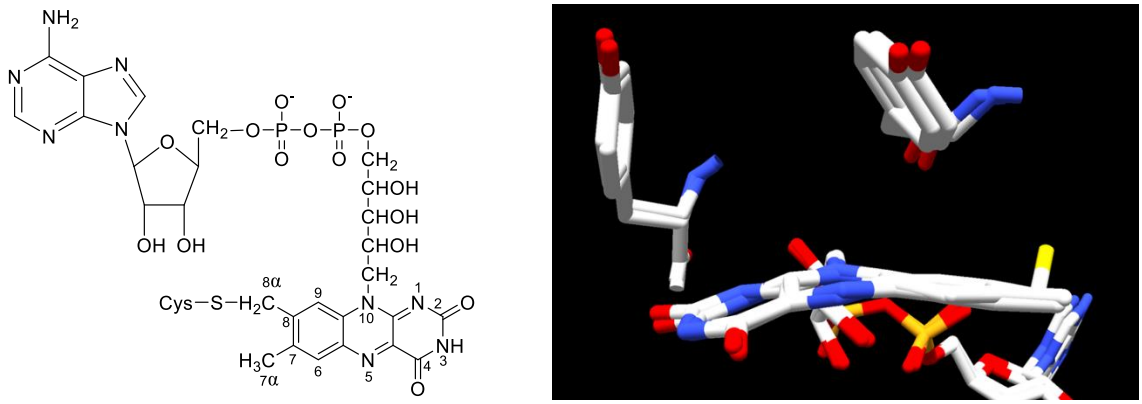

Figure 3.3. Structure of 8α-S-cysteinyl FAD (A) and schematic representation of the “aromatic cage” in MAO B (B).

Carbon atoms are shown in white, nitrogen atoms are in blue, oxygen atoms are in red, phosphorus atoms are in orange, and a sulphur atom is in yellow (Edmondson et al., 2004; crystal structures PDB 2z5x and PDB 1oja - both human MAOs).

The main structural differences between MAO A and MAO B are in their active site geometries. These changes arise from conformational differences relating to MAO A residues 210–216, a region which is called the ‘‘cavity-shaping loop’’ and differences in amino acid residues of MAO A and MAO B in the active sites (Figure 3.4) (Edmondson et al., 2007). The active site structures of MAO A and MAO B are shown in Figure. 2.6. The active site structure of MAO A has a single substrate cavity (~550 $Å^3$), while MAO B includes a dipartite hydrophobic cavity which consists of an entrance cavity (~290 $Å^3$) and a substrate cavity (~ 430 $Å^3$). Rotation of Ile-199 ‘‘gate’’ side chain in MAO B allows the two cavities to be separated or to be fused forming a single cavity (Binda et al., 2003). The MAO A cavity grants binding to larger MAO A substrates such as serotonin and the MAO B entrance cavity allows

access of smaller MAO B substrates such as β-phenethylamine and benzylamine (Finberg, 2014). It has been proposed that different substrate and inhibitor specificities between MAO A and MAO B are primarily caused by the size and shape of the substrate cavity and its steric relationship with the entrance cavity (Binda et al., 2002).

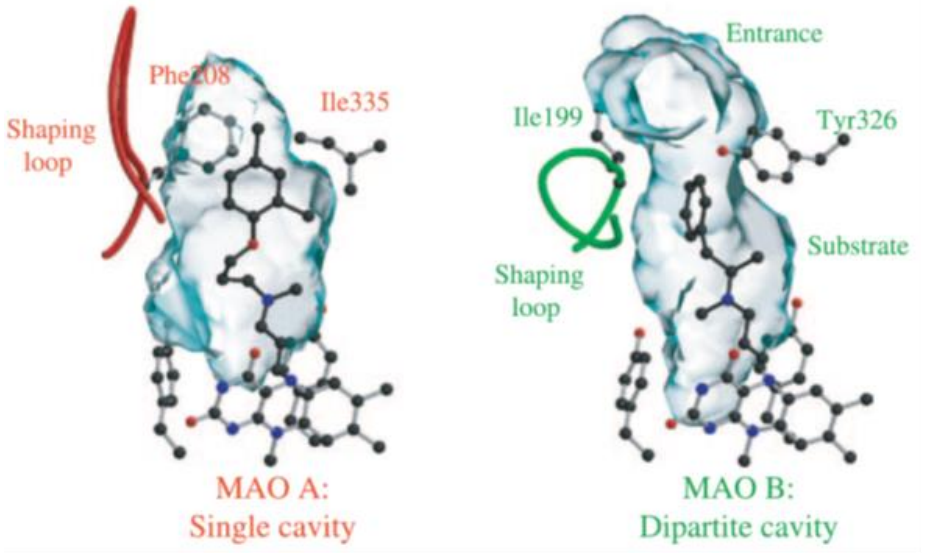

Figure 3.4. Comparison of active site cavities for the clorgyline adduct of human MAO A (left) and for the deprenyl adduct of human MAO B (right).
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3.2.2. Kinetic pathways and mechanisms in MAO catalysis

The proposed pathways catalyzed by MAO are shown in Fig. 3.5. It is possible that MAO catalyzes its reaction by way of a ping-pong (a binary) or an ordered bi–bi (ternary) pathway or a ternary pathway bypassing direct regeneration of oxidised enzyme (E.FADox) (Ramsay & Albreht, 2018; Ramsay et al., 2011).

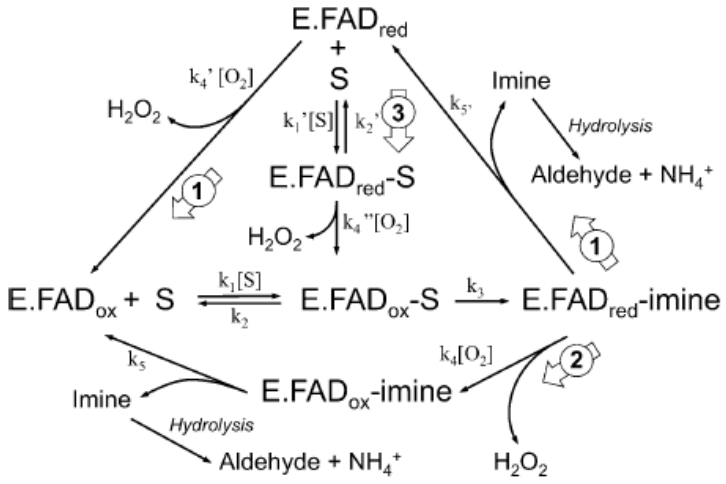


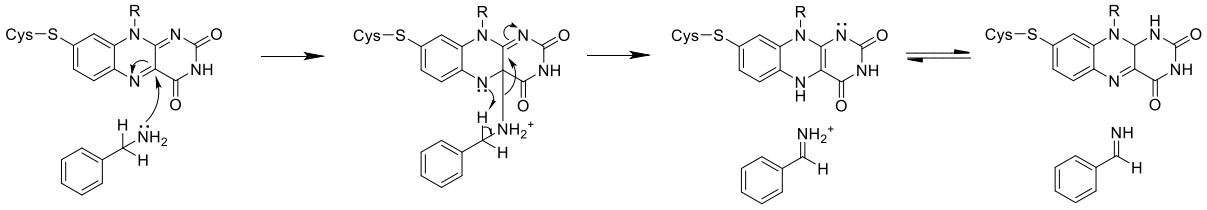
Figure 3.5. Suggested pathways in MAO catalysis.

Three pathways leading to the reoxidation of the reduced FAD. (1) A ping-pong pathway (a binary). (2) An ordered pathway (a ternary). (3) Pathway 3 (Reoxidation of the E.FAD$_{red}$-S species). Reprinted by permission from Springer Nature, Journal of Neural Transmission, An improved approach to steady-state analysis of monoamine oxidases, Ramsay et al., Copyright © (2011).

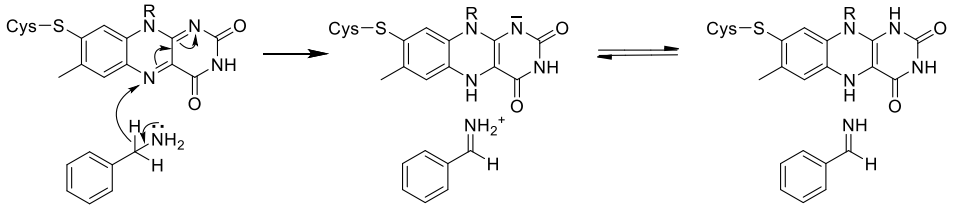
In a ping-pong pathway (Fig. 3.5 (1)), amine substrate binds to the oxidized form of MAO (E.FAD$_{ox}$), giving a complex (E.FAD$_{ox}$-S) that subsequently reacts leading to the E.FAD$_{red}$-imine complex. This is followed by imine product release and reduced enzyme (E.FAD$_{red}$) generation which can be reoxidized by oxygen. Alternatively, reoxidation of the E.FAD$_{red}$-imine complex, via ordered bi–bi pathway (Fig. 3.5 (2)), can lead to the product being released. Lastly, in a ternary pathway (Fig. 3.5 (3)) bypassing E.FAD$_{ox}$, the E.FAD$_{red}$ can bind new substrate, resulting in a ternary complex which is subsequently oxidised.

In addition, the catalytic pathway involves the amine substrate C−H bond cleavage reactions (Edmondson et al., 2009). Several reaction mechanisms have been proposed to explain the catalytic pathway for the C−H bond cleavage step which includes electron transfer from the amine substrate to the FAD cofactor. Three mechanisms for MAO-catalyzed oxidations of amines are shown in Figure 3.6. These mechanisms include polar/nucleophilic, hydride transfer, and single electron transfer (SET) (Fitzpatrick, 2010; Nakamura et al., 2020; Ramsay & Albreht, 2021). In the polar/nucleophilic mechanism, as a nucleophile, the substrate attacks C(4a) position of the flavin moiety to form a covalent adduct, followed by C−N bond cleavage

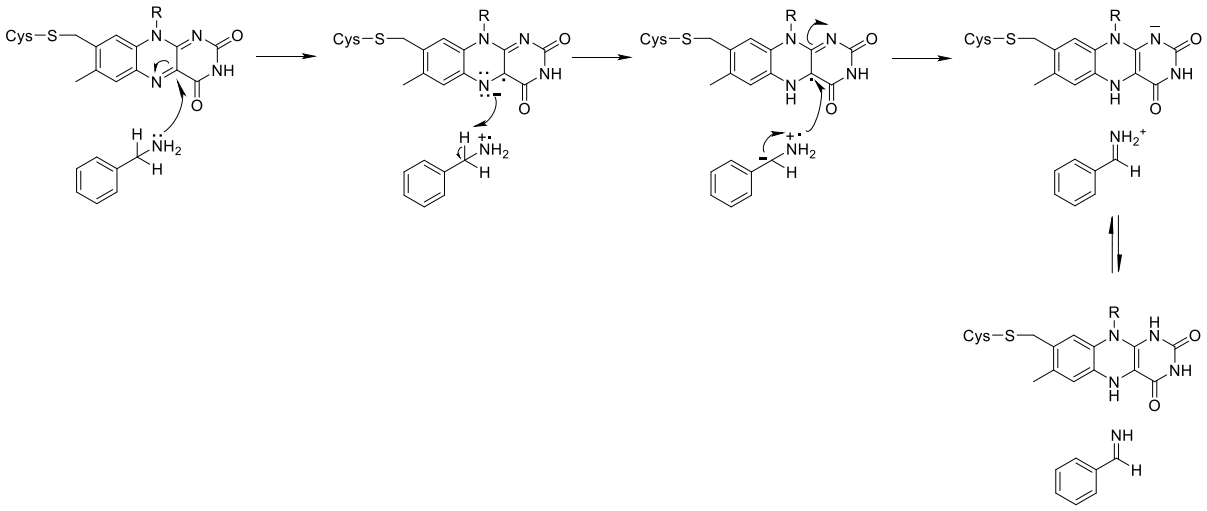
and proton transfer forms the final products (Fig. 3.6a). The hydride transfer mechanism involves hydride (H^-) transfer from the substrate to the flavin N5 atom and proton transfer leading to the final products (Fig. 3.6b). Finally, the single-electron transfer (SET) mechanism proposed, involves the substrate transferring a single electron to the flavin to yield a flavin radical and an amyl radical cation. The final products of SET mechanism are formed by a second electron and proton transfer (Fig. 3.6c).



a) Polar/nucleophilic mechanism.



b) Hydride transfer mechanism.



c) Single-electron transfer (SET) mechanism.

Figure 3.6. Proposed mechanisms for MAO-catalyzed oxidations of amines, illustrated by using benzylamine as a substrate.

a) Polar/nucleophilic mechanism. b) Hydride transfer mechanism. c) Single-electron transfer (SET) mechanism.

The two mechanisms that have been the focus of most studies are the polar/nucleophilic, and/or hydride transfer mechanism (Abad et al., 2013; Orru et al., 2013). There is the general assumption that MAO A and MAO B follow the same mechanisms for MAO catalysis because of the identical structures of the covalent FAD. However, it has been proposed that human MAO A involves C-H bond cleavage step which is formed by H^+ abstraction (via polar/nucleophilic mechanism), while human MAO B includes C-H bond cleavage step which is formed by H^- abstraction (via hydride transfer mechanism) (Fig. 3.7) (Orru et al., 2013). These studies contradict the general assumption and suggest that MAO A and MAO B follow different mechanisms.

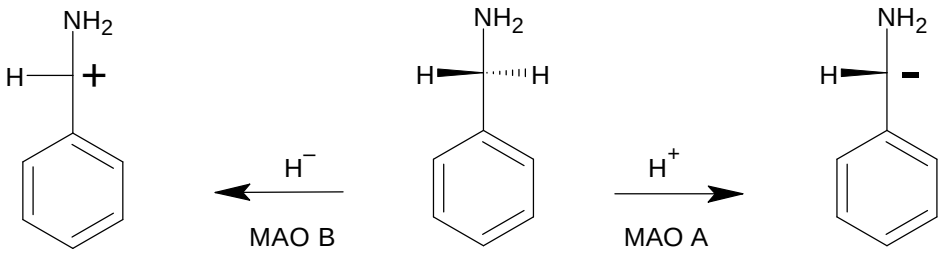


Figure 3.7. Suggested schemes for modes of C–H bond cleavage steps in MAO A and in MAO B.

More recently, a proton-coupled electron transfer (PCET) mechanism has been introduced for MAO catalysis of tertiary amines such as MPTP and its analogue,1-methyl-4-(1-methyl-1H-pyrrol-2-yl)-1,2,3,6-tetrahydropyridine (MMTP) (Fig. 3.8) (Nakamura et al., 2020). This mechanism includes a single-electron transfer (SET) as the first step and the irreversible deprotonation step forming a stable neutral radical. Although tertiary amines are not MAO substrates as a rule, it has been implied that the propargylic amines, pargyline and selegiline may act through the PCET mechanism because they have similar functionality.

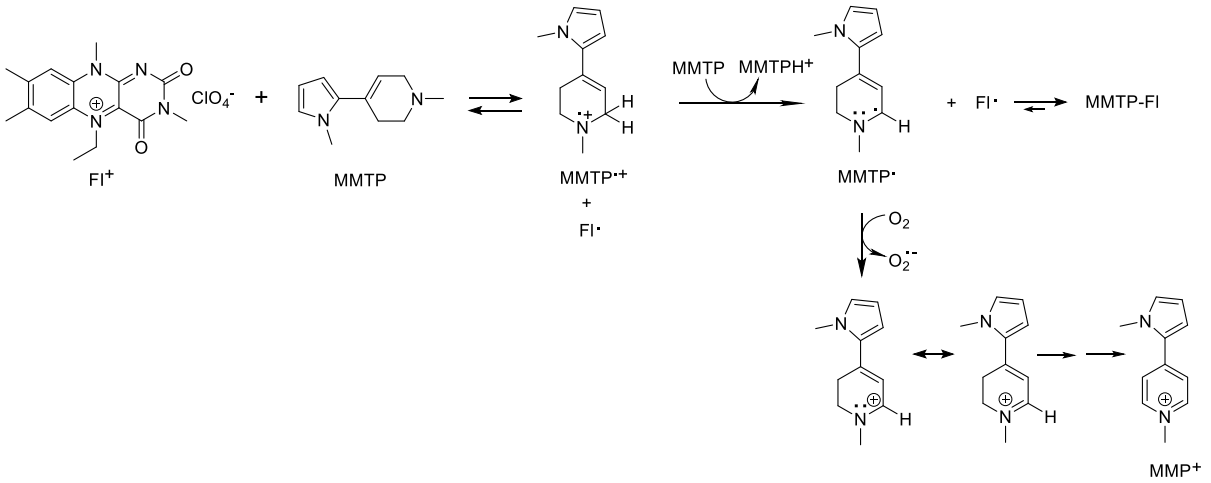


Figure 3.8. Suggested mechanism for the reaction of 5-ethyl-3-methyllumiflavinium (FI^+) with MMTP.

3.2.3. MAO in neurotransmitter metabolism

Monoamine oxidases catalyze the metabolism of neurotransmitters dopamine, norepinephrine, epinephrine and serotonin (Fig. 3.9).

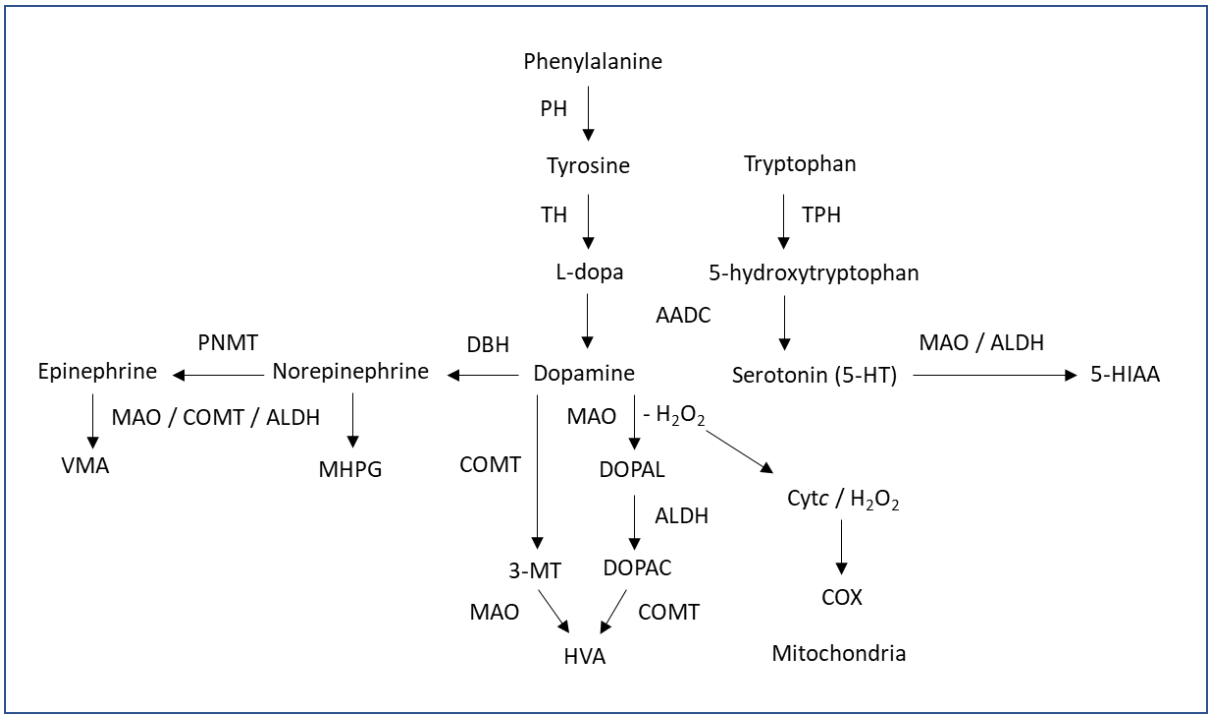


Figure 3.9. Dopamine, norepinephrine, epinephrine and serotonin metabolism in the central nervous system (CNS).

AADC, aromatic L-amino acid decarboxylase; ALDH, aldehyde dehydrogenase; COMT, catechol-*O*-methyltransferase; COX, cytochrome c oxidase; Cyt*c*, cytochrome c; DBH, dopamine β-hydroxylase; DOPAC, dihydroxyphenylacetic acid; L-dopa, L-dihydroxyphenylalanine; DOPAL, 3,4-dihydroxyphenylacetaldehyde; 5-HIAA, 5-hydroxindole acetic acid; 5-HT, 5-hydroxytryptamine; HVA, homovanillic acid; MHPG, 3-methoxy-4-hydroxyphenylglycol; 3-MT, 3-methoxytyramine; PH, phenylalanine hydroxylase; PNMT, phenylethanolamine *N*-methyltransferase; TH, tyrosine hydroxylase; TPH, tryptophan hydroxlase; VMA, vanillylmandelic acid.

The rapid decomposition of brain monoamines like dopamine, norepinephrine and serotonin is crucial for the appropriate functioning of synaptic transmission. Monoaminergic signaling is considered as not only the control of motor, perceptual and cognitive functions, but also one of the basic mechanisms for the mood and emotional modulation (Bortolato et al., 2008). In addition, brain dopamine plays a key role in addictive disorders. Dopamine release in the NAc mediates nicotine-induced pleasurable and rewarding effects, which is assumed to be important mechanism for nicotine dependence (Herman et al., 2014). MAO enzymes are known drug targets and their inhibitors have been used to treat depression, panic disorder, social phobia, bipolar, anxiety, and Parkinson's disease (Duarte et al., 2020; Menkes et al., 2016; Sub Laban & Saadabadi, 2021).

3.3. MAO and tobacco smoking

Each year, tobacco smoking kills more than 8 million people worldwide, of which around 1.2 million are caused by second-hand smoke exposure (World Health Organization, 2021). The main causes of death from smoking are lung cancer, chronic obstructive pulmonary disease (COPD), coronary heart disease, miscarriage, and underdevelopment of the fetus. Smoking also increases the risk of stroke, blindness, deafness, back pain, osteoporosis, and peripheral vascular disease inducing amputation (West, 2017).

Nicotine addiction is influenced by pharmacologic effects of nicotine as well as social and environmental factors such as tobacco product design and pervasiveness of tobacco marketing (Prochaska & Benowitz, 2019). It is also likely that non-nicotinic components in tobacco smoke contribute to nicotine addiction, not just nicotine alone (Brennan et al., 2015). Nicotine

itself does not show any MAO inhibitory activity, but non-nicotinic components in tobacco smoke inhibit MAO, the key enzymes responsible for the degradation of neurotransmitters (Castagnoli et al., 2002; Dome et al., 2010). It has been hypothesized that, since MAO enzymes break down neurotransmitters in the brain, MAO inhibitors in tobacco smoke may increase the lifetime of dopamine and enhance the reinforcing properties of nicotine (Hogg, 2016; Rose, 2006). Evidence that this is a real effect comes from use of MAO inhibitors with nicotine in rats. Work from several studies has found that nicotine plus MAO inhibition was more rewarding than nicotine alone (Guillem et al., 2005; Villegier et al., 2007). Furthermore, it has been reported that the non-selective MAO inhibitor tranylcypromine greatly enhanced nicotine-induced dopamine release in the nucleus accumbens when animals were pretreated with it. This dopamine release is believed to be a neurochemical marker of addictiveness (Dome et al., 2010; Villegier et al., 2007). Thus, MAO inhibition has long been suspected to be an important aspect of the effect of tobacco on smokers, with potential relevance to both tobacco dependence (Hogg, 2016; Rose et al., 2001) and treating other mental health issues (Rose et al., 2001; Villegier et al., 2010).

Smith et al. (2016) found that MAO A inhibition rather than MAO B inhibition is responsible for the increase in nicotine self-administration at low dose, suggesting that cigarette smoke components with MAO A inhibitory activity, have potential to increase the primary reinforcing and reinforcement enhancing effects of nicotine. Nicotine not only acts as a primary reinforcer in tobacco smoke, enhancing the probability of behaviours that lead to nicotine delivery, but also strongly increases behaviours that lead to the delivery of nonpharmacological stimuli (Palmatier et al., 2006).

Although the overall importance of MAO inhibitors as a factor in tobacco dependence has not been fully established, these current findings imply that we need an understanding of the effects of MAO inhibitors on smokers.

3.4. MAO inhibitors in tobacco and tobacco smoke

Tobacco and tobacco smoke are examples of complex mixtures such as wood smoke, plant extracts, coal tar, humus, with many chemical components in inexact proportions (Perfetti & Rodgman, 2011). By 2008, 8430 chemical components in tobacco and tobacco smoke had been identified. A total of 4994 components were identified in tobacco, while 5315 components

were identified in tobacco smoke, of which, 1879 common components were found. These components were categorized into 34 classes of chemicals (Fig. 3.10).

Most markedly, tobacco smoke contains about ten times more hydrocarbons (alkanes, alkenes, alkynes, alicyclics, monocyclic aromatic, polycyclic aromatic) than the tobacco plant. Tobacco smoke also contains more phenols, quinones, ketones, free radicals, halogen-containing and fixed gases, and nitrogen-containing compounds (except for proteins and amines). Among 34 classes of chemicals, a greater number of nitrogen heterocyclic compounds are found in tobacco smoke, whereas the tobacco plant contains a greater number of alcohols.

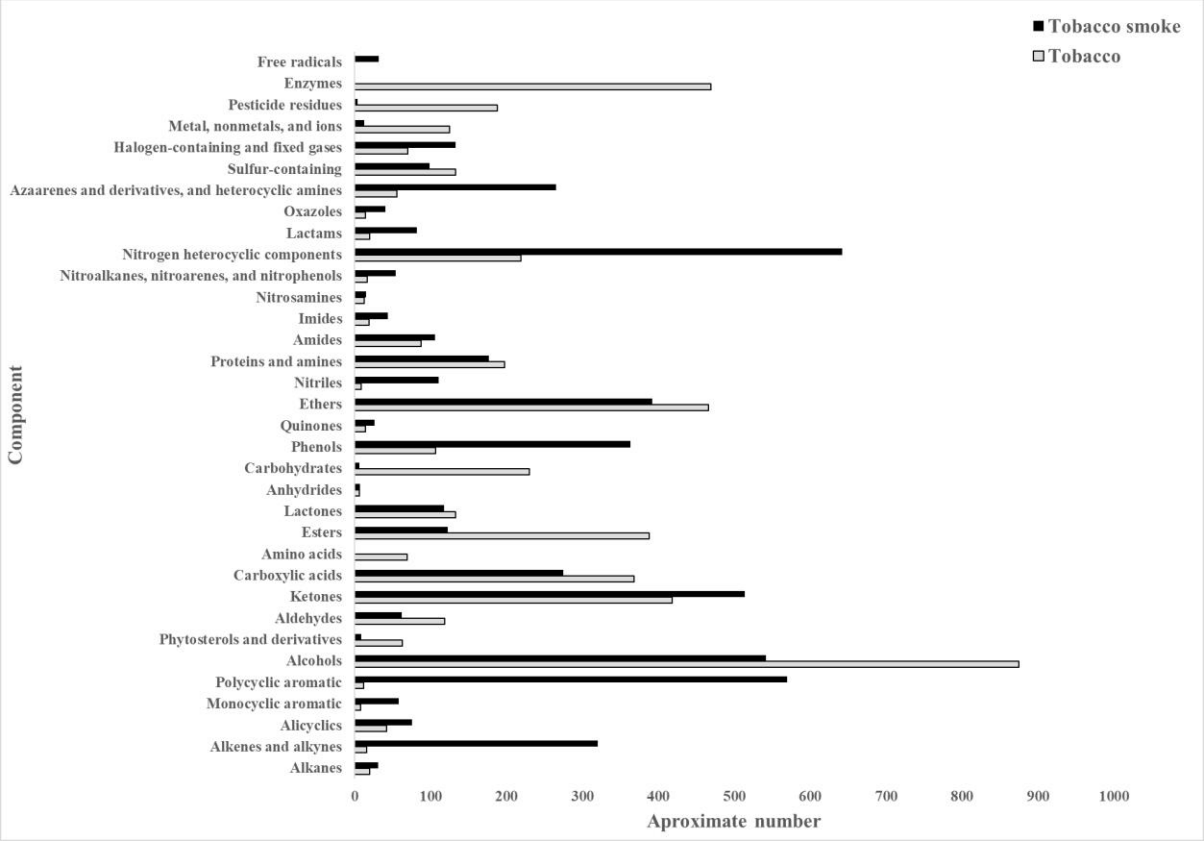


Figure 3.10. Distribution of compounds in tobacco and tobacco smoke according to the chemical classes.
Created using data from Perfetti and Rodgman (2011).

Rodgman and Perfetti (2013) created an alphabetical index of chemical components in tobacco and tobacco smoke. We crossmatched this index with existing literature (Das et al., 2022; Hogg, 2016; Mostert et al., 2017; Sari & Khalil, 2015; Tao et al., 2005; Tripathi et al., 2018; van der

Toorn et al., 2019; Vina et al., 2012) reporting on natural compounds and their structural analogs with MAO inhibitory activity. The IC_{50} values and K_i values for MAO inhibition of compounds so identified are summarized in Table 3.2. The compounds listed above the dotted line are MAO inhibitors identified by studies of tobacco and tobacco smoke. Compounds below the dotted line show tobacco smoke components which are reported in the literature to be MAO inhibitors, however, they have not previously been connected to their potential to contribute to MAO inhibitory activity of tobacco and tobacco smoke.

3.4.1. Nitrogen-containing heterocycles and amines

Fig. 3.11 shows the structures of the heterocycles and amines mentioned in the next three sections.

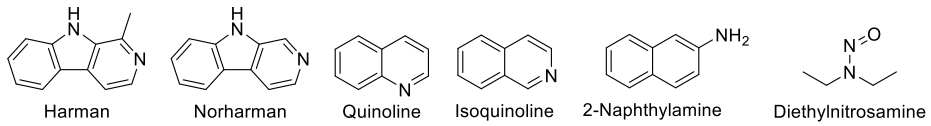


Figure 3.11. Nitrogen-containing heterocycles and amines as MAO inhibitors.

3.4.1.1. β-carbolines: Harman, norharman

Carbolines are a class of pyridoindole compounds and are divided into alpha-, beta-, gamma-, and delta-carbolines according to the location of pyridine nitrogen in relation to the indole (Lin et al., 2016). The condensate of cigarette mainstream smoke contains alpha-carbolines (2.01–10.3 ng/cigarette), beta-carbolines (0.25–2.53 μg/cigarette), and gamma-carbolines (0.29–1.10 ng/cigarette) (Tsuchiya, 2011). Currently, two beta-carboline alkaloids, harman and norharman, have been recognised as potent reversible MAO inhibitors from tobacco smoke (Fig. 3.11) (Herraiz & Chaparro, 2005). Harman inhibits mainly the human MAO A isozyme (K_i 55.54 nM), whereas norharman inhibits both human MAO A (K_i 1.2 μM) and human MAO B (K_i 1.12 μM). The level of harman in the brain of a smoker was estimated to be around 7 nM after smoking 10 cigarettes (Talhout et al., 2007). However, Truman et al. (2017) found that these two known inhibitors together comprise perhaps under 10% of the total MAO A inhibitory activity in cigarette smoke suggesting other inhibitors (either known or unknown) may make significant contributions to total inhibitory activity.

Table 3.2. MAO inhibition shown by components in tobacco (T) and tobacco smoke (S).

Classes	Compound	T or S	IC_{50} or K_i values (μM)		SI[a]	References
			MAO A	MAO B		
Components previously identified as contributing to MAO inhibition by tobacco or tobacco smoke						
β-carbolines	Harman	T, S	IC_{50} 0.34 K_i 0.056	No inhibition up to 25	>73.5	Herraiz and Chaparro (2005)
	Norharman	T, S	IC_{50} 6.47 ± 0.28 K_i 1.2 ± 0.18	IC_{50} 4.68 ± 0.33 K_i 1.12 ± 0.19	0.72 0.93	Herraiz and Chaparro (2005)
Terpenoids	Farnesylacetone	T, S	No inhibition (human placenta)	28% inhibition at 10 (baboon liver)		Castagnoli et al. (2002); Khalil et al. (2006)
	Trans,trans-farnesol	T, S	No inhibition at 10 (human placenta)	K_i 0.80 (human liver)		Khalil et al. (2006)
Amines	2-Naphthylamine	S	K_i 52 (mouse brain)	K_i 40.2 (mouse brain)	0.77	Hauptmann and Shih (2001)
	Diethylnitrosamine	T, S	K_i 202.3 ± 15.5 (rat liver)	K_i 51.5 ± 4.6 (rat liver)	0.25	Obata et al. (1989); van der Toorn et al. (2019)
Quinones	2,3,6-Trimethyl-1,4-naphthoquinone	T, S	K_i 3 (human GI[c])	K_i 6 (human liver)		Khalil et al. (2000)
Crossmatched MAO inhibitors						
Quinones	2-Methyl-1,4-naphthoquinone	S	K_i 26 ± 4	K_i 0.4 ± 0.15	0.02	Cerqueira et al. (2011)
	1,4-Naphthoquinone	S	K_i 7.7 ± 1.2	K_i 1.5 ± 0.4	0.19	Cerqueira et al. (2011)
	1,4-Benzoquinone	S	IC_{50} 4.82 ± 0.12	IC_{50} 10.2 ± 0.42	2.12	Mostert et al. (2017)
Phenols	Guaiacol	T, S	IC_{50} 175 ± 27	IC_{50} >500	>2.86	Tao et al. (2005)
	Eugenol	T, S	IC_{50} 34.4 ± 5.1 K_i 26	IC_{50} 288 ± 47 K_i 211	8.37 8.12	Tao et al. (2005)
	Methyl eugenol	S	IC_{50} 110 ± 13.3	IC_{50} 269 ± 34	2.45	Tao et al. (2005)
	2-Methoxyhydroquinone	S	IC_{50} 13.4 ± 2.4	IC_{50} 8.9 ± 1.4	0.66	Tao et al. (2005)
	3-Methoxyphenol	T, S	IC_{50} 24 ± 2.8	NI[b]		Tao et al. (2005)
	2,6-Dimethoxyphenol	T, S	IC_{50} 62.4 ± 8.6	IC_{50} >500	>8.01	Tao et al. (2005)
	Homovanillyl alcohol	S	IC_{50} 180 ± 24	NI[b]		Tao et al. (2005)
	Guaiacyl acetone	T, S	IC_{50} 30.2 ± 4.7	NI[b]		Tao et al. (2005)
	α-Asarone	T	IC_{50} 124 ± 16	IC_{50} 338 ± 52	2.73	Tao et al. (2005)
	β-Asarone	T	IC_{50} 142 ± 18	IC_{50} 362 ± 43	2.55	Tao et al. (2005)
	Isoeugenol	T, S	IC_{50} 3.72 ± 0.2	IC_{50} 102 ±5	27.4	Zhang et al. (2019)
	Vanillin	T, S	Inhibition at 15 IC_{50} 17	No inhibition at 15 N/A[d]		Zhang et al. (2019) Truman et al. (2019)
Phenolic acids	Ferulic acid	T, S	K_i 7.55 ± 0.49	K_i 24.0 ± 1.98	3.18	Badavath et al. (2016)
	Protocatechuic acid	T, S	IC_{50} 2,411 (rat)	IC_{50} 300 (rat)	0.12	Kim et al. (2012)
	Caffeic acid	T, S	IC_{50} 138.5 ± 1.1	IC_{50} 247.7 ± 3.2	1.79	Andrade et al. (2016)
	Chlorogenic acid	T, S	IC_{50} 79.7 ± 1.8,	IC_{50} 191.4 ± 2.2	2.4	Andrade et al. (2016)
Flavonoids	Kaempferol	T, S	IC_{50} 0.525 ± 0.035 K_i 0.362 ± 0.021	IC_{50} >100	>190	Gidaro et al. (2016)
	Quercetin	T, S	IC_{50} 3.98 ± 0.265 K_i 4.24 ± 0.305	IC_{50} >100	>25.1	Gidaro et al. (2016)
			IC_{50} 15.3, K_i 7.35 (human liver)	N/A[d]		Dixon Clarke and Ramsay (2011)
			IC_{50} 2.8 (mouse brain)	IC_{50} 90 (mouse brain)	32.1	Han et al. (2007)
			IC_{50} 0.01 (bovine brain)	IC_{50} 20 (bovine brain)	2000	Chimenti et al. (2006)
			N/A[d]	IC_{50} 10.89, K_i 7.95		Lee et al. (2001)
	Quercitrin	T, S	N/A[d]	IC_{50} 19.06, K_i 21.01		Lee et al. (2001)
	Isoquercitrin	T	N/A[d]	IC_{50} 11.64, K_i 2.72		Lee et al. (2001)
	Rutin	T, S	N/A[d]	IC_{50} 3.89, K_i 1.83		Lee et al. (2001)
	Naringenin	T	IC_{50} 955 ± 129 (rat liver)	IC_{50} 288 ± 18 (rat liver)	0.3	Olsen et al. (2008)
	Naringin	T	IC_{50} 33.3 ± 7.05	IC_{50} 44.6 ± 11.2	1.34	Carradori et al. (2016)
	Diosmetin	T	IC_{50} 5.74 ± 0.571	IC_{50} 1.58 ± 0.887	0.28	Carradori et al. (2016)
Coumarins	Scopoletin	T, S	K_i 82.18 K_i 75.12 (rat)	K_i 20.7 K_i 22.6 (rat)	0.25 0.3	Basu et al. (2016)
	Esculetin	T, S	IC_{50} 30.1 (mouse brain MAO)			Lee et al. (2000)
Azaarenes	Quinoline	T, S	K_i 28.4 ± 1.3 (human placenta)			Naoi and Nagatsu (1987)
			K_i 31.7 ± 1.5 (human synaptosomes)	K_i 1360 ± 181 (human synaptosomes)	42.9	
	Isoquinoline	T, S	K_i 35.7 ± 2.0 (human placenta)			Naoi and Nagatsu (1987)
			K_i 62.2 ± 4.3 (human synaptosomes)	K_i 60.7 ± 4.9 (human synaptosomes)	0.98	

[a] SI: selectivity index is defined as the ratio of [IC_{50} (MAO B)]/[IC_{50} (MAO A)] or [K_i (MAO B)]/[K_i (MAO A)]. [b] NI: no significant inhibition observed up to 500 μM. [c] GI: gastrointestinal. [d] N/A: not available. The reported IC_{50} and K_i values for the inhibition of MAO are not directly comparable because they have been measured in various tissues under different experimental settings. The enzyme source is always the human recombinant enzyme if the species is not specified in the Table.

3.4.1.2. Azaarenes

Azaarenes in the smoke are thermolysis products of proteins and other nitrogenous leaf substances (Dong et al., 1978). Quinoline is the most abundant azaarene found in the tobacco smoke. Isoquinoline is a constitutional isomer of quinoline, present in the smoke at relatively low concentration. Quinoline competitively and selectively inhibited MAO A in human brain synaptosomes (K_i 31.7 μM) with a selectivity index of 42.9 over MAO B, while isoquinoline showed noncompetitive MAO A and MAO B inhibition (Naoi & Nagatsu, 1987).

3.4.1.3. Amines

2-Naphthylamine is an established human bladder carcinogen present in tobacco smoke. Levels of 2-naphthylamine hemoglobin adduct were higher in smokers than in nonsmokers (Bryant et al., 1988), and determination of hemoglobin adducts can possibly be used for evaluating exposure to this and other tobacco constituents (Bukowska, 2015). 2-Naphthylamine was found to be a mixed type (competitive and non-competitive) inhibitor of mouse brain MAO with K_i values of 52 μM for MAO A and 40.2 μM for MAO B (Hauptmann & Shih, 2001). Diethylnitrosoamine is also a carcinogen widely used in animal model systems and has been detected in various products such as cigarette smoke, meat, and whiskey (Kang et al., 2007). It inhibited rat liver MAO A and B competitively and displayed selective MAO B inhibitory activity with a K_i value of 51.5 μM (Obata et al., 1989).

3.4.2. Phenolics and polyphenolics

Fig. 3.12 shows the structures of phenols and phenolic acids mentioned in the next two sections.

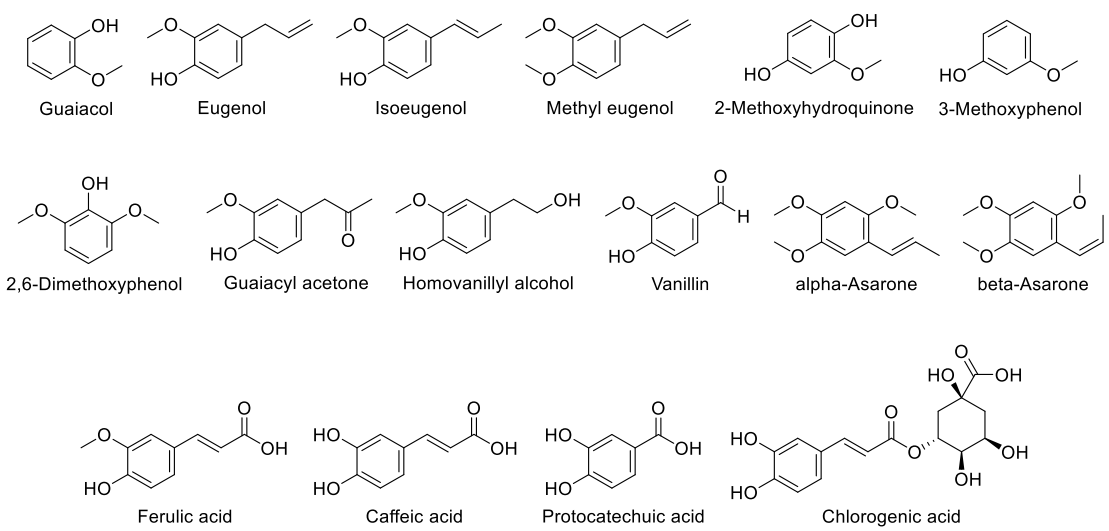


Figure 3.12. Phenols and phenolic acids as MAO inhibitors.

3.4.2.1. Phenols

Plant phenolics consist of coumarins, flavonoids, lignans, lignins, phenolic acids, simple phenols, some stilbene derivatives, and tannins (Kumar & Goel, 2019) and have received considerable attention because of their antioxidant properties and potential beneficial health effects as an essential part of the human diet (Shahidi & Ambigaipalan, 2015). Many phenolics, such as those found in cigarette smoke, also exist in foodstuffs. Examples of those compounds include eugenol and isoeugenol (clove and cinnamon), vanillin (vanilla), protocatechuic acid (wheat), and caffeic acid (coffee bean) (Smith et al., 2002), of which isoeugenol is reported as the most selective and potent MAO A inhibitor (Table 3.2).

Eugenol (4-allyl-2-methoxyphenol), is a flavor additive found in tobacco products such as clove cigarettes (Lisko et al., 2014). α-Asarone, β-asarone, methyl eugenol, 2-methoxyhydroquinone, 3-methoxyphenol, 2,6-dimethoxyphenol, homovanillyl alcohol, guaiacol, and guaiacyl acetone are eugenol analogs that have also been detected in tobacco plants and/or tobacco smoke and showed inhibitory activity toward human recombinant MAO (Table 3.2 and Fig. 3.12). Eugenol exhibited reversible and competitive inhibition for MAO A and MAO B and showed MAO A selectivity over MAO B, with a K_i value of 26 μM for MAO A. Replacing the 4-allyl group of eugenol with a hydroxyl group as in 2-methoxyhydroquinone increases potency towards MAO A and MAO B (Table 3.2), while replacing it with hydrogen (guaiacol) or a 2-hydroxyethyl (homovanillyl alcohol) group decreases potency for MAO A and MAO B. Substituting the free hydroxyl group of eugenol with a methoxy group as in methyl eugenol decreases inhibition for MAO A.

Eugenol has been shown to protect rat pheochromocytoma (PC12) cells against beta-amyloid peptide (Aβ)-induced cell death (Irie & Keung, 2003). Furthermore, eugenol and its structural analogs that exhibit inhibitory activity on human MAO A also possess antidepressant-like activity suggesting that a potential relationship between the antidepressant-like action of eugenol and its inhibitory activity for MAO A (Tao et al., 2005).

As noted in Section 2, MAO inhibitors are used for treatment of depression, and have the potential for treating Alzheimer's disease. Depression is among the most prevalent neuropsychiatric comorbidities of Alzheimer's disease (Lee & Lyketsos, 2003). It is intriguing that eugenol, a major active principle, from *Rhizoma acori graminei* has antidepressant-like activity because *Rhizoma acori graminei* has been used as a herbal medicine to treat stroke, Alzheimer's disease, and vascular dementia (Kang et al., 2006). As such, eugenol and related

compounds are candidate therapeutic agents for treatment of depression and Alzheimer's disease.

3.4.2.2. Phenolic acids

An ester of caffeic acid and quinic acid, chlorogenic acid (3-O-caffeoylquinic acid), is the major polyphenolic constituent in tobacco and comprises about 2–3% of tobacco (Sharma et al., 2002; Wang et al., 2013). Chlorogenic acid and caffeic acid (Fig. 3.12) exhibited IC_{50} values for MAO A of 79.7 μM and 138.5 μM, respectively (Andrade et al., 2016).

Ferulic acid, protocatechuic acid and other phenolic acids have been found in the cigarette smoke prepared without flavorings or other additives (Yang & Wender, 1962). Ferulic acid inhibited MAO A selectively with a K_i value of 7.55 μM (Badavath et al., 2016), while protocatechuic acid exhibited preferential but weak inhibition of rat MAO B inhibition (IC_{50} 300 μM) (Kim et al., 2012).

3.4.2.3. Flavonoids

Fig. 3.13 shows the structures of the flavonoids, and coumarins mentioned in the next two sections.

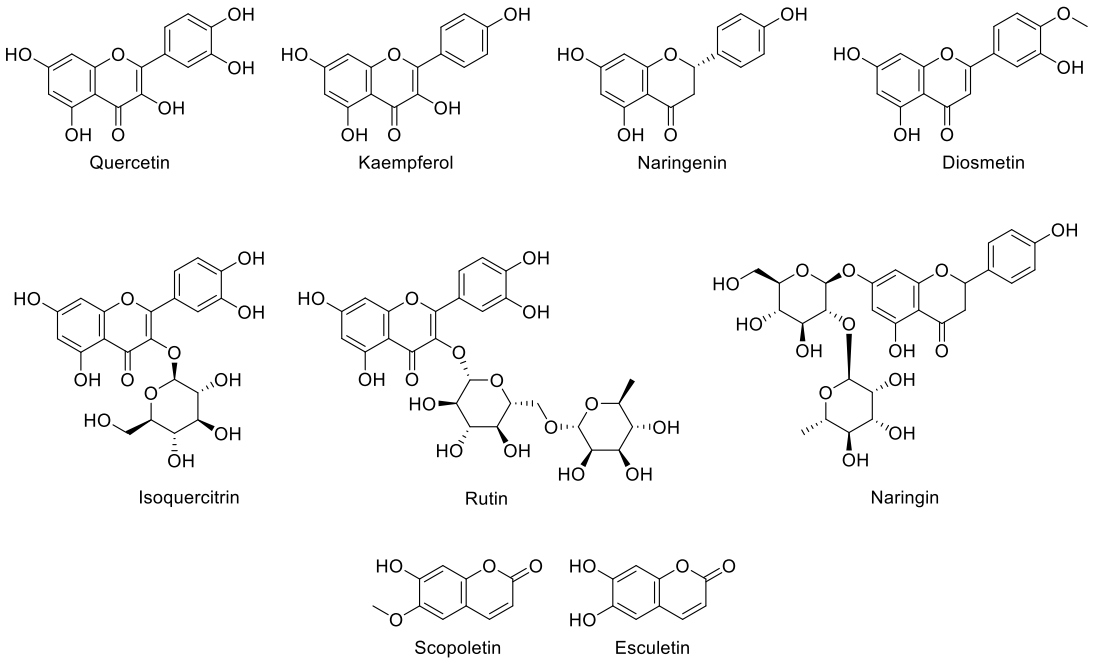


Figure 3.13. Flavonoids and coumarins as MAO inhibitors**.**

It has been reported that five subclasses of natural flavonoids including flavanols, flavanones, flavones, isoflavones, and flavonols show MAO inhibitory activity (Vina et al., 2012). Tobacco flavonoids which inhibit MAO are kaempferol, quercetin, rutin, quercitrin, isoquecitrin, naringenin, naringin, and diosmetin (Table 3.2 and Fig. 3.13). *In vitro* human MAO A and MAO B inhibition, and molecular modeling studies were carried out for kaempferol along with quercetin (Gidaro et al., 2016). Kaempferol and quercetin are both potent, reversible, and selective MAO A inhibitors with IC_{50} values of 0.525 and 3.98 μM, respectively, but they have no effect on MAO B up to 100 μM. In addition, four natural flavonoids, including quercetin, quercitrin, isoquercitrin, and rutin have been evaluated for MAO B inhibitory and free radical scavenging activities (Lee et al., 2001). They displayed inhibitory activities against MAO B and the most potent flavonoid was rutin (Table 3.2). The mode of inhibition of quercetin, quercitrin, isoquercitrin, and rutin towards MAO B is mixed type in relation to the substrate benzylamine, with K_i values in the low micromolar range (Table 3.2). MAO A inhibition of three of these flavonoids has not been described. There are many reported different IC_{50} and K_i values for quercetin (Table 3.2), measured in various tissues under different experimental conditions. Quercetin displayed potent MAO A inhibitory activity, while some MAO B inhibitory activity of lower potency was detected or no MAO B inhibitory activity was observed up to 100 μM (Chimenti et al., 2006; Gidaro et al., 2016; Han et al., 2007) (Table 3.2). Thus quercetin is a selective MAO A inhibitor based on the combined data from several authors. Recently, eight natural flavonoids including naringin, and diosmetin were selected to assess the human MAO inhibitory properties (Carradori et al., 2016). Diosmetin is a potent MAO inhibitor with IC_{50} values in the low micromolar range (Table 3.2), whereas naringin showed only moderate MAO inhibitory activity.

3.4.2.4. Coumarins

Coumarins (2H-1-benzopyran-2-one) are secondary metabolites found in a broad range of vascular plants, animal species, and some microorganisms (Stringlis et al., 2019). Coumarins are named for the plant *Coumarouna odorata,* which contains basic coumarin. The two simple coumarins scopoletin and esculetin (also known as aescultin) exist in all parts of the tobacco plant (Nugroho & Verpoorte, 2002). Scopoletin and esculetin have been shown to have mouse brain MAO inhibitory activity (Lee et al., 2000). A further study on scopoletin, reported that it has antidepressant-like effects in both the tail suspension test and forced swimming test in mice, using fluoxetine, as a positive control (Capra et al., 2010). Furthermore, *in vitro* MAO inhibitory activity, molecular docking, and the effect on brain amine metabolism were explored.

Scopoletin inhibited the MAO B isoform reversibly and selectively with a K_i value of 20.7 µM (human) and 22.6 µM (rat), respectively. Moreover, an *in vivo* study has shown that scopoletin treatment elevated striatal dopamine level significantly in mice, while dopamine's metabolite, striatal 3,4-dihydroxyphenylacetic acid (DOPAC), was significantly decreased. The authors proposed that scopoletin is not only involved in brain amine metabolism, but also crosses the blood brain barrier (Basu et al., 2016).

3.4.3. Terpenoids

Fig. 3.14 shows the structures of the terpenoids and quinones mentioned in the next two sections.

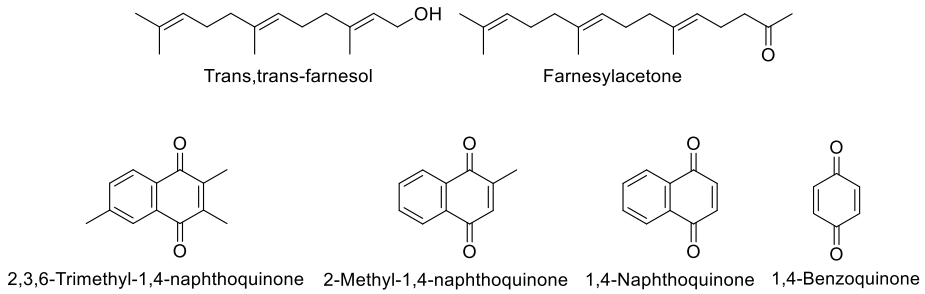


Figure 3.14. Terpenoids and quinones as MAO inhibitors.

Farnesylacetone and trans, trans-farnesol along with other compounds with aroma have been detected and quantified in cigarette smoke condensates (Zhou et al., 2013). A selective MAO B inhibitor, farnesylacetone has been isolated from tobacco leaf and showed inhibitory activity on baboon liver MAO B (Castagnoli et al., 2002). Trans, trans-farnesol is a selective and potent MAO B inhibitor with a K_i value of 800 nM (human liver), whereas it has no effect on MAO A at 10 µM (human placenta) (Khalil et al., 2006).

3.4.4. Quinones

Cigarette smoke contains 2,3,6-trimethyl-1,4-naphthoquinone (TMN), other naphthoquinones, 9,10-anthraquinone, 2-methyl-9,10-anthraquinone, and 1,4-benzoquinones (Schmeltz et al., 1977). Among them, TMN displayed a K_i value of 3 µM for human gastrointestinal MAO A and K_i value of 6 µM for human liver MAO B (Khalil et al., 2000). The MPTP mouse model of Parkinson's disease has been utilized to study the possible neuroprotective properties of TMN. The results of these studies showed that TMN is neuroprotective, which supports the

possibility that tobacco components may have neuroprotective functions (Castagnoli & Murugesan, 2004). Two other naphthoquinones found in tobacco smoke as MAO inhibitors are 2-methyl-1,4-naphthoquinone (menadione) and 1,4-naphthoquinone. Menadione and 1,4-naphthoquinone are potent, reversible, and selective MAO B inhibitors with K_i values for human MAO B of 0.4 μM and 1.5 μM, respectively (Cerqueira et al., 2011). Quinones are easily formed from cigarette smoke components capable of autooxidation. For instance, 1,4-benzoquinone is formed either by hydroquinone autoxidation in aqueous extracts of cigarette tar or by *in vivo* oxidation in living organisms (Wooten et al., 2006).

In smokers' lungs, 1,4-benzoquinone is produced from significant amounts of semiquinone (100–200 μg/cigarette), contained in cigarette smoke (Ghosh et al., 2012). A previous study showed that 1,4-benzoquinone reversibly inhibited MAO A and MAO B in human brain synaptosomes (MAO A K_i 9.62 μM, MAO B K_i 20.3 μM) (Naoi et al., 1987). However, it is also reported that 1,4-benzoquinone irreversibly inactivated human MAO A (IC_{50} 4.82 μM) (Mostert et al., 2017). The reasons for these differences are not obvious, but Mostert and coworkers found that near complete inhibition occurred at an inhibitor concentration of 2 × IC_{50} and recovery of MAO A activity was not restored by dialysis, showing a similar result as pargyline, an irreversible inhibitor. An earlier study had found that MAO is irreversibly inhibited by some components of cigarette smoke (Yu & Boulton, 1987). To our knowledge, the findings of Mostert and coworkers may provide the first explanation for the irreversible inhibitory activity of cigarette smoke. Other irreversible activities may remain to be discovered. Furthermore, Castagnoli and Murugesan (2004) reviewed the literature on MAO inhibition by components of cigarette smoke and stated that cigarette smoke solution and tobacco extract were found to inhibit MAO-catalyzed oxidation of amines such as serotonin, tyramine and beta phenethylamine. When serotonin, the preferred substrate for MAO A, was used the effect was twice greater, indicating that cigarette smoke has a greater inhibitory effect on MAO A activity than MAO B activity *in vitro*. We suggest that an irreversible MAO A inhibitor, 1,4-benzoquinone, plays a role in the irreversible inhibition of MAO A by cigarette smoke.

3.5. Conclusion

The objective of this literature review is to discuss components in tobacco and tobacco smoke with MAO inhibitory activity especially for their MAO selectivity and reversibility. For this purpose we used an index of chemical constituents in tobacco and tobacco smoke (Rodgman & Perfetti, 2013) to search for MAO inhibitors in tobacco and tobacco smoke. We

crossmatched this index with existing literature reporting on natural compounds and their structural analogs with MAO inhibitory activity.

This review reports the currently known non-nicotinic components found in tobacco and tobacco smoke which are MAO inhibitors. They are divided into nine chemical classes containing thirty eight compounds. The five classes of MAO inhibitors, not previously linked to tobacco, discussed as MAO inhibitors in tobacco and tobacco smoke, include phenols, phenolic acids, flavonoids, coumarins, and azaarenes. We also suggest that 1,4-benzoquinone may play a part in the irreversible inhibition of MAO A by cigarette smoke.

Among non-nicotinic components of tobacco and tobacco smoke, harman, trans,trans-farnesol, menadione (2-methyl-1,4-naphthoquinone), and kaempferol showed most potent inhibitory activity on human MAO with IC_{50} or K_i values in the nanomolar range. Most phenols and phenolic acids with MAO inhibitory activity are reversible and selective inhibitors primarily of MAO A, while trans,trans-farnesol, menadione, 1,4-naphthoquinone, scopoletin, and diosmetin with MAO inhibitory activity are reversible and selective inhibitors primarily of MAO B (Table 2). These combined findings suggest that MAO inhibitors from tobacco and tobacco smoke could provide leads for the development of effective therapeutics for depression, and Parkinson's disease. As a result of selective inhibition of MAO A, neurotransmitter levels in noradrenergic and serotonergic neurons of the central nervous system (CNS) may be increased, leading to clinical antidepressant action. By virtue of selective inhibition of MAO B, in the Parkinsonian brain, the MAO B inhibitors should increase dopamine levels where there is partial depletion of dopaminergic neurons of the substantia nigra pars compacta, resulting potential treatment for Parkinson's disease (Finberg & Rabey, 2016). On the other hand, non-selective MAO inhibitors have the potential for interactions with various tyramine containing foods and sympathomimetics and other drugs. These interactions can lead to hypertensive reactions. So people need to follow the food and drug restrictions allocated to use of non-selective MAO inhibitors. Even though selective MAO inhibitors are safer to use (Livingston & Livingston, 1996), it will be important to examine potential drug and food interactions of any new therapeutic MAO inhibitors under investigation.

The Food and Drug Administration (FDA) recently authorized the marketing of very low nicotine cigarettes (Food and Drug Administration, 2021), but these will still contain MAO inhibitors. Since MAO A inhibition is responsible for increasing nicotine self-administration at low doses (Smith et al., 2016), further research on the MAO A inhibitors in tobacco smoke,

should be carried out in case there is also a need for regulation of levels of these inhibitors. It is unknown what level of MAO A inhibitors could shift the threshold dose for reinforcement enhancement or whether this is dependent on the tobacco type.

Future studies could explore the reinforcement effects of flavoring compounds in tobacco products or alternative tobacco products such as electronic cigarettes (e-cigarettes). Recently, Truman et al. (2019) found that flavoured e-liquids showed a wide range of MAO inhibitory activity and identified vanillin and ethyl vanillin as active compounds. It is possible that other flavoring compounds in e-liquids may have MAO inhibitory activities and have potential to increase the reinforcing properties of nicotine. This aspect of e-cigarettes may require monitoring.

Further studies of the MAO inhibitors in tobacco smoke are required, in order to assess their contribution to MAO inhibition in smokers, and their potential behavioural effects. If specific MAO inhibitors are proven to promote nicotine addiction, they could perhaps be used with nicotine to produce a more effective form of nicotine replacement therapy, with the potential to help many smokers cease smoking.

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Chapter 4

Characterisation of irreversible monoamine oxidase (MAO) inhibitors in tobacco smoke

This chapter contains the article "Characterisation of irreversible monoamine oxidase (MAO) inhibitors in tobacco smoke", co-authored by Sa Weon Hong (SH), Ali Heydari (AH), Paris Wilson (PW), Paul Teesdale-Spittle (PT-S), Rachel Page (RP), Peter T. Northcote (PTN), Robert A. Keyzers (RAK), and Penelope Truman (PT). This article will be submitted to Neurotoxicology.

Author contributions: For this PhD study, designed by SH and PT, SH performed the MAO assay (IC_{50}), reversibility test, and time-dependent assay. Preliminary studies to identify the inhibitors (which contribute to this manuscript) were conducted by SH, PT, RAK, PTN, AH, and PW. SH wrote the manuscript. PT, RP, and PT-S contributed to the final shape of the manuscript.

This chapter discusses the following: (1) Isolation of irreversible monoamine oxidase (MAO) inhibitors in tobacco smoke; (2) Biological activities of irreversible MAO inhibitors from tobacco smoke; and (3) Reversibility and time-dependent inhibition by the new phenolic MAO inhibitors.

This article addresses research questions 2 and 3 (Sections 1.4.2 and 1.4.3) for four of the six novel MAOIs identified in tobacco smoke by P Truman and colleagues. These 4 novel MAOIs are phenolic compounds.

4.1. Abstract

Smoking is a main cause of premature death and preventable disease in the world and nicotine is the major addictive constituent in cigarette smoke. Interestingly, animal studies indicate that inhibition of monoamine oxidase (MAO), the key enzyme for the degradation of neurotransmitters, increased self-administration of nicotine and that MAO A inhibition, but not MAO B inhibition, increased self-administration of nicotine at low doses. It has been suggested that identification of irreversible inhibitors from tobacco smoke is critical to fully identifying the mechanisms responsible for tobacco addiction. In this study, the identification of irreversible phenolic compounds in tobacco smoke and their MAO inhibitory activities are described. Three MAO inhibitors, hydroquinone, catechol, and 4-methylcatechol have been isolated from tobacco smoke and two other tobacco catechols, 4-ethylcatechol and 4-vinylcatechol, were included as well, to test for their inhibitory potencies because they have similar structures and have been founded in cigarette smoke condensate. A centrifugation-ultrafiltration method was used to test for the reversibility of MAO A binding, while time-dependent assays for enzyme inhibition were used to analyse the binding of inhibitors for MAO A. We show that MAO inhibition by phenolic compounds from tobacco smoke is irreversible for human MAO A. Two alkylcatechols, 4-methylcatechol and 4-ethylcatechol, inhibited MAO A with IC_{50} values of 14.11 and 12.56 μM, respectively. Inhibition of MAO enzymes were found to show significant time dependence. After 1h preincubation, 4-methylcatechol displayed the highest inhibitory activity for MAO A with an IC_{50} value of 0.267 μM and showed potent MAO B inhibition with higher potency compared to norharman. These results suggest that irreversible MAO inhibition by the phenolic compounds and others from tobacco smoke may not only play a role in contribution to the addictive effects of nicotine in human but also may play a role in the low incidence of Parkinson's disease in smokers.

4.2. Introduction

Smoking is still a major cause of premature death and preventable disease in the world (Gowing et al., 2015; West, 2017). Nicotine addiction occurs when smokers become dependent on smoking for modulation of mood and arousal, relief of withdrawal symptoms, or both (Benowitz, 2010). Like other abuse drugs, dopamine release in the nucleus accumbens (NAc) mediates nicotine-induced pleasure and reward, which is presumed to be important mechanism for the onset and maintenance of nicotine addiction (Herman et al., 2014). However, animal

studies suggested that nicotine had relatively weak reinforcing properties as opposed to other drugs of abuse including amphetamine, cocaine or morphine (Balfour, 2009). It has been proposed that monoamine oxidase (MAO) inhibitors in tobacco smoke decrease the breakdown of neurotransmitters linked to the reward pathway, and act synergistically with nicotine to increase the addictive effects of nicotine (Hogg, 2016).

MAO catalyzes the degradation of a variety of monoamines, including neurotransmitters such as dopamine, serotonin, norepinephrine and epinephrine. Two isoforms, MAO A and MAO B exist in most mammalian tissues and have different inhibitor sensitivities and substrate specificities (Youdim et al., 2006). MAO A is selectively inactivated by clorgyline at low doses and favourably oxidizes serotonin and norephinephrine, while MAO B is inactivated by selegiline (l-deprenyl) at low doses and oxidizes beta phenethylamine and benzylamine. Both isoforms catalyze dopamine, tyramine, and tryptamine (Lewis et al., 2007). MAO inhibitors are currently used in medicine. Selective MAO A inhibitors are effective treatment for depression, consistent with their effectiveness in decreasing the metabolism of serotonin and norephinephrine, while selective MAO B inhibitors are an effective treatment for Parkinson's disease (PD) (Finberg, 2014).

Surprisingly, positron emission tomography (PET) studies show that smokers have lower levels of brain MAO A (28%; $p<0.0003$) and MAO B (40%; $p<0.0002$) activity compared to non-smokers (Fowler et al., 1996a; Fowler et al., 1996b). The mechanism of MAO inhibition in smokers is unknown. However, it is conceivable that several tobacco-derived substances may induce MAO inhibition in smokers, either due to additive or synergistic effects (Hogg, 2016). Animal studies have shown that MAO inhibition increased self-administration of nicotine (Guillem et al., 2005; Villegier et al., 2007) and MAO A inhibition, but not MAO B inhibition, increased self-administration of nicotine at low doses (Smith et al., 2016). Therefore, it may be important to characterize unidentified MAO inhibitors in tobacco smoke to elucidate the mechanism of MAO inhibition and know their contribution to the addictive effects of nicotine in human.

Previously, a number of reversible MAO A and/or MAO B inhibitors in tobacco smoke have been studied (Herraiz & Chaparro, 2005; Khalil et al., 2006; Khalil et al., 2000). The beta-carboline alkaloids harman and norharman are the most well-known and potent reversible MAO inhibitors in tobacco smoke, but these inhibitors comprise less than 10% of the overall MAO A inhibitory activity of tobacco smoke implying other inhibitors may contribute

significantly to overall inhibitory activity (Truman et al., 2017). On the other hand, for irreversible MAO inhibitors, Yu and Boulton (1987) reported that aqueous extracts of cigarette smoke inhibited rat lung mitochondrial MAO irreversibly suggesting that chronic use of cigarettes may conduce to prevent a neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or MPTP-like compounds from MAO-catalyzed oxidation. Castagnoli and Murugesan (2004) also found that cigarette smoke extracts reversibly and irreversibly inhibited MAO when the samples were analysed for MAO activity after incubation for 1h. Recently, we crossmatched an index of the chemical constituents of tobacco and tobacco smoke with existing publications in search for MAO inhibitors in tobacco and tobacco smoke and reported that 1,4-benzoquinone is the first irreversible MAO A inhibitor identified in tobacco smoke (Hong et al., 2022). Although many studies were conducted to isolate MAO inhibitors from tobacco smoke, until now experimental studies on the identification of irreversible inhibitors in tobacco smoke remained unexplored. Furthermore, since MAO inhibitors have been used clinically in the treatment of anxiety, depression, and Parkinson's disease, MAO inhibitors found in tobacco smoke may have therapeutic potential for the treatment of anxiety, depression and Parkinson's disease or in effective smoking cessation strategies.

In this article, we report on the bioassay-guided fractionation and characterisation of several irreversible MAO inhibitors identified in tobacco smoke.

4.3. Materials and Methods

4.3.1. Chemicals and general experimental procedures

All reagents were obtained from Sigma Aldrich (St Louis, MO). NMR spectra were acquired on a 600 MHz Varian Direct Drive spectrometer equipped with a triple-resonance HCN cryogenic probe operating at 25 K. The ^{1}H chemical shifts (δ) were internally referenced to the residual solvent peak (Gottlieb et al., 1997). Normal-phase column chromatography was achieved using diol-functionalized silica gel (DIOL). HP20 poly(styrene-divinylbenzene) (PSDVB) chromatographic resin was used for reversed-phase column chromatography. All solvents used for liquid chromatography were of analytical or HPLC grade. Unless otherwise stated, solvent mixtures are reported as percent v/v.

4.3.2. HPLC-DAD-ELSD analysis

HPLC was carried out using an Agilent Technologies 1260 Infinity HPLC coupled with a diode array detector and an Agilent 380 evaporative light scattering detector (ELSD). The separation

was conducted on an Alltima C18 column (250 mm × 4.6 mm, 5 µm, Alltech, USA). Mobile phases are made up of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The following elution was applied: 0–25 min (linear gradient, 5–20% B); 25–30 min (isocratic, 20% B). The flow rate was 1 mL/min and the column temperature was 25 °C. The DAD wavelengths were set at 210, 254, and 280 nm. The ELSD parameters were as follow: evaporator temperature 40 °C; nebuliser temperature 40 °C; gas flow 1.6 L/min.

4.3.3. Bioassay-guided fractionation of TPM

Bioassay-guided fractionation was conducted to identify MAO inhibitors from tobacco particulate matter (TPM). We were following the purification of activity which was higher after a 1 h preincubation than it was without preincubation. TPM was prepared as previously described (Sheehan et al., 2019) with the modifications that the puff interval was minimised, so as to increase the amount of particulate matter collected, and no drying step was included. Briefly, 1kg of roll-your-own (RYO) tobacco was hand-rolled into cigarettes that each contain approximately 1 g of tobacco. Cigarettes were individually smoked using a smoking machine and TPM collected onto filters, using 5 cigarettes per filter. The TPM was stored on the filters at -80 until extraction. TPM (126.5 g) was extracted three times with MeOH (3 × 1.5 L) for 1 h. The extracts were loaded onto HP20 beads (1 L) by successive dilution with H_2O to 25% MeOH in H_2O (cyclic loading). For inhibition screening, an aliquot of each dried fraction (50 µg) was dissolved in EtOH (50 µL), using 4 µL/100 µL reaction volume (in triplicate) to test MAO activity. The most active fraction (25% MeOH/H_2O fraction, 12.2 g), which had not stuck to the column, was diluted further with water, loaded back onto fresh HP20 beads and this column was eluted with Me_2CO. The resulting fraction contained the highest specific MAO inhibitory activity. A portion (470.6 mg) of the fraction was then loaded onto a DIOL column (30 mL), and eluted with 50% CH_2Cl_2/*n*-hexane, CH_2Cl_2, 10% EtOAc/CH_2Cl_2, 25% EtOAc/CH_2Cl_2, MeOH, and 50% MeOH/H_2O. The 10% EtOAc/CH_2Cl_2 fraction (27.4 mg, containing the highest specific activity for MAO A inhibition) was subjected to reversed-phase HPLC, with a H_2O (A) /ACN (B) gradient (5–20% B at 0–25 min, 20% B at 25–30 min, and 20–5% B at 30–35 min), which afforded hydroquinone (t_R = 9.6 min, 1.1 mg), catechol (t_R = 18.2 min, 9.7 mg) and 4-methylcatechol (t_R = 29.3 min, 0.6 mg).

4.3.4. Monoamine oxidase inhibition assay

The kynuramine assay as modified from previously described methods (Lewis et al., 2012; Truman et al., 2017) was used to determine MAO inhibitory activity. Human recombinant

MAO A and MAO B (MAO A specific activity: 0.33 U/mg; MAO B specific activity: 0.4 U/mg; 1 U will deaminate 1 nmol of kynuramine per min at pH 7.4 at 37 °C) were purchased from Sigma Aldrich and stored at -80°C. The reactions (final volume, 100 µL) contained phosphate buffer (50 mM, pH 7.2), kynuramine substrate (100 µM), and the test inhibitors (0.001–300 µM). The inhibitors were dissolved in EtOH (final concentration 4% EtOH (v/v)). Controls with inhibitor and buffer only were included to remove the intrinsic fluorescence of the sample itself. Control reactions with no EtOH or inhibitor were also included. The reaction is initiated by addition of MAO (0.00625 mg protein/mL) and incubated in a black microplate for 15 min (MAO A) or 30 min (MAO B) at 37 °C in an incubator. After incubation, the reaction was stopped by addition of 2.5 N NaOH (50 µL) and production of 4-hydroxyquinoline (the product of MAO-catalyzed kynuramine oxidation) monitored using a microplate reader (FLUOstar Omega, BMG Labtech) with excitation and emission at 320 and 380 nm, respectively. GraphPad Prism 9 (GraphPad Software Inc.) was used for fitting a sigmoidal dose-response curve to determine the IC_{50} values. All assays were carried out in triplicate and the IC_{50} values reported as the mean ± SEM (n = 3). In studies of the dependence of inhibition on incubation time, MAO A and MAO B were preincubated at 37 °C with a range of concentrations of compounds for 1h prior to kynuramine substrate addition. MAO enzyme activity was measured as described above. We used norharman, a potent inhibitor of MAO A and MAO B found in tobacco smoke, as the positive control (Herraiz & Chaparro, 2005).

During the bioassay-directed purification, the activity monitored was the MAO A inhibitory activity measured after a 1 h preincubation of 4 µg of each fraction with MAO A, less the same activity measured with no preincubation.

4.3.5. Reversibility test

A centrifugation-ultrafiltration method was used as the primary test of reversibility (Park et al., 2019). First, the recombinant human MAO A enzymes (0.03 mg protein/mL) were incubated with the novel inhibitors for 1h at 37 °C. Next, following an incubation, an aliquot (200 µl) is placed in an Amicon Ultra centrifugal filter (3 kDa cutoff, Millipore) and centrifuged at 16,000 x*g* at 4°C for 10 min, while another aliquot (200 µl) was stored in the fridge at 4 °C for the following MAO A enzyme assay. Then, the enzyme retained on the filter (3 kDa) was resuspended in phosphate buffer (50 mM, pH 7.2) (400 µl), centrifuged, and the same process repeated five times. Finally, the enzyme was resuspended in buffer (400 µl), and then tested for MAO A activity. Control experiments were carried out to define 100% MAO A activity.

Percent (%) MAO A inhibition was separately calculated for test samples before and after washout.

4.3.6. Time-dependent inhibition

To investigate the time-dependence of inactivation, recombinant human MAO A enzyme was preincubated with hydroquinone (1.0 μM), 4-methylcatechol (1.0 μM), 4-ethylcatechol (1.0 μM), catechol (1.0 μM), norharman (1.0 μM), and clorgyline (1.0 nM) for 0–3 h at 37 ℃ before kynuramine addition. After preincubation time, the reaction was initiated by addition of kynuramine (100 μM) to each sample and MAO A enzyme activity was measured as described above. The inhibitors were dissolved in EtOH (final concentration 4% EtOH (v/v)) and control with EtOH was included. Control reactions with no EtOH or inhibitor were also performed. The MAO A activity (% activity) remaining was plotted against the preincubation time to show the time-dependence of MAO inactivation by the inhibitors. This analysis describes a variation of the inhibition assay described in 4.3.4.

4.3.7. ADME prediction

The ADME (absorption, distribution, metabolism and excretion) prediction was performed using the SwissADME (http://www.swissadme.ch) (Daina et al., 2017) for the novel MAO inhibitors identified from tobacco smoke. Molecular weight (MW), number of hydrogen bond acceptors (HBA) and donors (HBD), topological polar surface area (TPSA), logarithm of partial coefficient (iLogP) (Daina et al., 2014), gastrointestinal (GI) absorption, blood-brain barrier (BBB), P-glycoprotein (Pgp) substrate, and Lipinski #violations are parameters that have been used in this study. PreADMET (https://preadmet.webservice.bmdrc.org) web tool was also used to predict ADME data and to build drug-like library.

4.4. Results

4.4.1. Extraction and isolation

Phenols were isolated from the MeOH extract of TPM by bioassay-guided fractionation. The MeOH extracts were purified using reversed-phase (PSDVB, MeOH/H_2O gradient) chromatography. The 25% MeOH/H_2O fraction was purified using a normal-phase (DIOL, EtOAc/CH_2Cl_2) chromatography. Final purification of the 10% EtOAc/CH_2Cl_2 fraction using reversed-phase HPLC afforded the isolation of hydroquinone, catechol, and 4-methylcatechol. The structures of the compounds were identified by NMR spectroscopy such as ^{1}H NMR and

^{1}H-^{1}H correlation spectroscopy (COSY). The amounts in the final fractions were becoming too small for further fractionation, but this NMR analysis of the fractions allowed identification of major components. These were confirmed as the major contributors by testing of commercially purchased compounds for MAO inhibitory activity. The scheme for bioassay-guided fractionation processes is shown in Fig. 4.1.

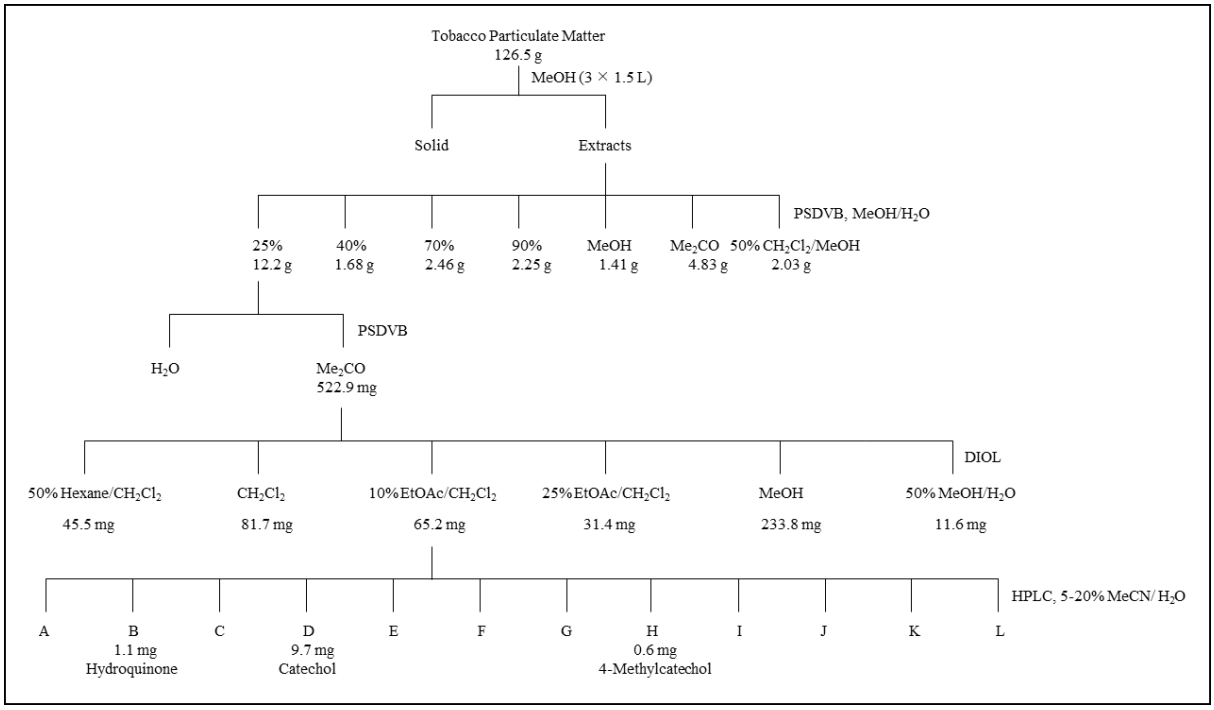
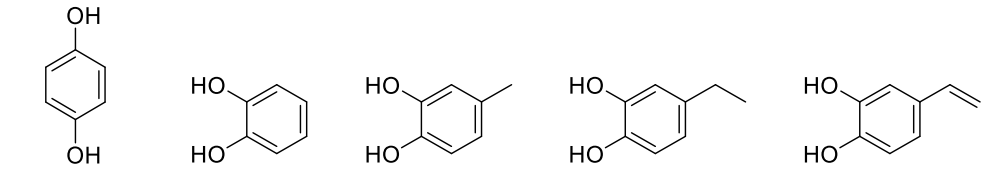


Figure 4.1. Isolation of hydroquinone and catechols from tobacco particulate matter.



Hydroquinone Catechol 4-Methylcatechol 4-Ethylcatechol 4-Vinylcatechol

Figure 4.2. Chemical structures of the hydroquinone and catechols.

Further, we have included 4-ethylcatechol and 4-vinylcatechol to test for their MAO inhibitory activity because they have been detected in cigarette smoke condensate (Park, 1982; Schlotzhauer et al., 1978) (Fig. 4.2) and are structurally related to catechol. Four of the five compounds are commercially available (Sigma Aldrich). 4-Vinylcatechol was successfully synthesized from caffeic acid by thermal decarboxylation (Jia et al., 2015; Terpinc et al., 2011)

but showed weak MAO inhibitory activity compared to other catechols. It was also expected to be unstable (Bernini et al., 2007) and difficult to obtain reliable results from further research. Therefore 4-vinylcatechol was not studied further.

4.4.2. Monoamine oxidase inhibition assay

The IC_{50} values for inhibition of human MAO A and MAO B by the four novel MAO inhibitors identified in tobacco smoke that are phenolic compounds are shown in table 4.1. The major phenolic compounds of cigarette tar, catechol (1,2-benzenediol) and hydroquinone (1,4-benzenediol), both displayed potent MAO inhibition. Substitution with alkyl groups at the 4-position on the catechol led to improved MAO A and MAO B inhibitory activities. The IC_{50} values for 4-methylcatechol and 4-ethylcatechol were found to be 14.11 and 12.56 μM for MAO A, respectively. Inhibition of MAO A and MAO B were found to display significant time dependence. Among these, 4-methylcatechol showed the highest activity against MAO A with an IC_{50} value of 0.267 μM after 1h preincubation.

Table 4.1. The IC_{50} values for inhibition of human MAO A and MAO B by catechols and hydroquinone.

Compound	IC_{50} (μM), no preincubation		SI [a]	IC_{50} (μM), 1h preincubation		SI [a]
	MAO A	MAO B		MAO A	MAO B	
Hydroquinone	15.2 ± 2.6	20.5 ± 4.2	1.35	4.00 ± 0.6	3.07 ± 0.16	0.77
Catechol	34.3 ± 6.5	46.4 ± 8.2	1.35	6.69 ± 1.03	5.40 ± 2.29	0.81
4-Methylcatechol	14.1 ± 2.6	32.1 ± 5.5	2.28	0.27 ± 0.02	2.64 ± 0.19	9.78
4-Ethylcatechol	12.6 ± 3.6	21.9 ± 6.1	1.74	0.43 ± 0.03	30.7 ± 6.05	71.4
Norharman	4.23 ± 1.16	5.17 ± 0.35	1.22	3.16 ± 0.44	5.54 ± 0.32	1.75

[a] SI: selectivity index is defined as the ratio of [IC_{50} (MAO B)]/[IC_{50} (MAO A)]. The inhibition data are given as the mean ± SEM (triplicate). Control: norharman (reversible MAO inhibitor) found in tobacco smoke.

4.4.3. Reversibility test

To evaluate the reversibility of human MAO A binding by phenolic compounds in tobacco smoke, a centrifugation-ultrafiltration method was used. We measured the MAO A inhibitory activities of hydroquinone, 4-methylcatechol, 4-ethylcatechol, catechol, norharman, a reversible MAO inhibitor, and clorgyline, an irreversible MAO A inhibitor (Table 4.2). All compounds tested showed about 60–90% inhibition of MAO A activity. After repeated washout, we found that phenolic compounds displayed no recovery of MAO A activity, indicating that they are irreversible inhibitors of MAO A. MAO A activity was also not

recovered in the assay using clorgyline, as a positive control. However, norharman showed recovery of MAO A activity under identical conditions (Table 4.2).

Table 4.2. Reversibility of MAO A binding of catechols and hydroquinone.

Compound	MAO A inhibition (%)	
	before washing	after repeated washing
Hydroquinone (10 μM)	61.30 ± 0.32	63.33 ± 0.30
4-Methylcatechol (10 μM)	58.78 ± 0.63	60.94 ± 1.80
4-Ethylcatechol (20 μM)	68.83 ± 1.72	68.35 ± 0.76
Catechol (300 μM)	66.57 ± 0.66	68.92 ± 0.97
Norharman (10 μM)	87.90 ± 0.21	23.78 ± 1.73
Clorgyline (5 nM)	56.06 ± 0.92	58.15 ± 0.10

The results are given as the mean ± SEM (triplicate). Controls: clorgyline (irreversible MAO A inhibitor), norharman (reversible MAO inhibitor) found in tobacco smoke.

4.4.4. Time-dependent inhibition

Human MAO A inhibition by hydroquinone (1.0 μM), 4-methylcatechol (1.0 μM), 4-ethylcatechol (1.0 μM), and catechol (1.0 μM) was time-dependent (Figure 4.3). Among them, 4-methylcatechol showed 100% inhibition of MAO A activity after 3h preincubation. The results of this study suggested that phenolic compounds in tobacco smoke are irreversible MAO A inhibitors. MAO A inhibition with clorgyline a positive control was also time-dependent. On the contrary, norharman a reversible inhibitor was not dependent on incubation time.

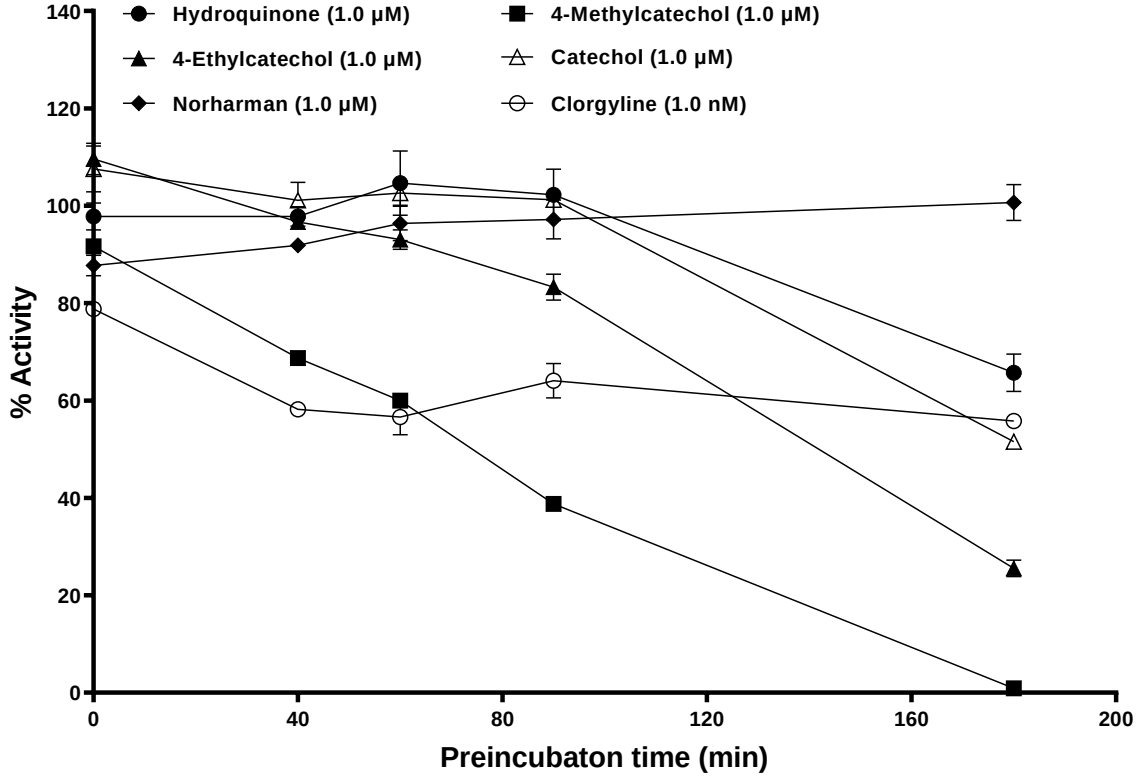


Figure 4.3. Time-dependent inhibition of human recombinant MAO A by catechols and hydroquinone.

The MAO A activity remaining is expressed as a percentage of activity in controls. The results are given as the mean ± SD (triplicate).

4.4.5. ADME prediction

All tested phenolic compounds are predicted to display high gastrointestinal absorption and cross the blood brain barrier by passive penetration (Table 4.3). However, SwissADME predicts harman and norharman, two potent MAO inhibitors from tobacco smoke, as substrates for Pgp (P-glycoprotein), a drug efflux pump. On the other hand, all phenolic compounds, clorgyline, and selegiline are predicted as non-substrates for Pgp. Further, SwissADME and PreADMET web tools were applied to predict the absorption, distribution, metabolism, excretion, and toxicity (ADMET) data (Appendix 4.3 and 5.2).

Table 4.3. Computed parameter values of catechols and hydroquinone in tobacco smoke.

Compound	MW	HBA	HBD	TPSA	iLogP	GI Absorption	BBB	Pgp substrate	Lipinski violations
Catechol	110.11	2	2	40.46	1.13	High	Yes	No	0
4-Methylcatechol	124.14	2	2	40.46	1.39	High	Yes	No	0
4-Ethylcatechol	138.16	2	2	40.46	1.64	High	Yes	No	0
4-Vinylcatechol	136.15	2	2	40.46	1.49	High	Yes	No	0
Hydroquinone	110.11	2	2	40.46	0.92	High	Yes	No	0
Harman	182.22	1	1	28.68	1.75	High	Yes	Yes	0
Norharman	168.19	1	1	28.68	1.43	High	Yes	Yes	0
Selegiline	187.28	1	0	3.24	2.8	High	Yes	No	0
Clorgyline	272.17	2	0	12.47	3.43	High	Yes	No	0

MW: molecular weight; HBA: hydrogen bond acceptor; HBD: hydrogen bond donor; TPSA: topological polar surface area (TPSA needs to be between 20–130$Å^2$); iLogP: for implicit log P (the logarithm of the partition coefficient between n-octanol and water) (iLogP should be between -3.93–6.46); GI absorption: gastrointestinal absorption; BBB: blood-brain barrier; Pgp substrate: P-glycoprotein substrate (a drug efflux pump); Lipinski: number of Lipinski's rule of five violations (drug-likeness evaluation) (maximum value: 4).

4.5. Discussion

In the present study, the major phenolic compounds of cigarette tar, catechol (1,2-benzenediol) and hydroquinone (1,4-benzenediol), along with alkyl catechols such as 4-methylcatechol and 4-ethylcatechol displayed potent human MAO A and MAO B inhibition. Our results suggest that irreversible MAO inhibition by catechols and hydroquinone from tobacco smoke may play a role in contribution to the addictive effects of nicotine in smokers. The condensate of cigarette mainstream smoke contains significant amounts of catechol (195 µg/cigarette), hydroquinone (121.5 µg/cigarette), 4-methylcatechol (38 µg/cigarette), 4-ethylcatechol (28 µg/cigarette), and 4-vinylcatechol (84 µg/cigarette) (Smith et al., 2002) compared to norharman (2.19 µg/cigarette) and harman (0.85 µg/cigarette) (Herraiz & Chaparro, 2005), two potent MAO inhibitors in tobacco smoke, suggesting the phenolic compounds may make significant contributions to MAO inhibitory effect of cigarette smoke.

An earlier report stated that methylcatechol and hydroquinone from the phenolic fraction of cigarette smoke condensates displayed strong inhibitory activity on rat brain MAO B (Lim et al., 1997). However, to our knowledge, MAO inhibition by the phenolic compounds that we tested on the human enzyme source have not been reported. Previously, phenolic compounds

have been reported as reversible MAO inhibitors (Zhang et al., 2019). Phenolic compounds are known as different kinds of reversible MAO inhibitors including competitive, non-competitive, or mixed-type MAO inhibitors (Badavath et al., 2016; Gidaro et al., 2016; Kong et al., 2004; Lee et al., 2001; Tao et al., 2005). Examples of these compounds include competitive inhibitors of MAO A (kaempferol and quercetin), MAO B (paeonol), or MAO A and MAO B (eugenol and ferulic acid), non-competitive inhibitors of MAO A (paeonol) or mixed-type inhibitors of MAO B (quercetin, quercitrin, isoquercitrin, and rutin). To our knowledge, we report for the first time that the phenolic compounds from tobacco smoke are irreversible MAO inhibitors and the findings may explain the irreversible inhibitory activity of cigarette smoke.

The phenolic compounds from tobacco smoke showed irreversible MAO inhibition when analysed using time-dependent assay for enzyme inhibition and using a centrifugation-ultrafiltration method. Irreversible MAO inhibitors are known as mechanism-based inhibitors which have seven criteria: time dependence of inactivation, irreversibility, saturation kinetics, substrate protection, fixed stoichiometry, involvement of an enzyme-catalyzed reaction, and inactivation before release of active species (Ramsay & Albreht, 2018; Silverman, 1995). Although the phenolic compounds meet the two criteria, time dependence of inactivation and irreversibility, further studies are needed to confirm whether they meet the other criteria.

It is possible that catechols and hydroquinone from tobacco smoke may be converted to benzoquinones and that it is these which form a covalent adduct with MAO enzymes. A range of catechols from natural products produce 1,2-benzoquinones (*o*-quinones) which may be responsible for the cytotoxic, genotoxic and chemopreventive effects of the catechols (Bolton et al., 2018). 1,4-benzoquinone (*p*-quinone) is also produced via autooxidation of hydroquinone in aqueous cigarette tar extracts or via *in vivo* oxidation in living organisms (Wooten et al., 2006). Mostert et al. (2017) found that human MAO A is irreversibly inactivated by 1,4-benzoquinone and other 1,4-benzoquinones and suggested that 1,4-benzoquinones may react with the reduced FAD (flavin adenine dinucleotide) cofactor to form covalent adducts. Further structural studies are needed to describe the reactive sites of 1,4-benzoquinone and the potential mechanism of 1,4-benzoquinone.

Our findings also suggest that catechols may play a role in the low incidence of Parkinson's disease in smokers. Lim et al. (1997) proposed that both the suppression of MPTP-induced neurotoxicity and the low incidence of Parkinson's disease in smoker relative to nonsmokers could result from MAO B inhibition by cigarette smoke components. Also, catechol and catechol derivatives are catechol-*O*-methyl transferase (COMT) inhibitors and catechol was

found to be both a competitive substrate of COMT and a COMT inhibitor *in vivo* (Guldberg & CA, 1975). Both MAO B inhibitors and COMT inhibitors such as nitrocatechol derivatives are used as adjuvant drugs to levodopa-associated motor complications in PD patients (Jankovic & Stacy, 2007). As such, our findings suggest that phenolic compounds tested in this study and other MAO B and/or COMT inhibitors (either known or unknown) in tobacco smoke may play a part in the low incidence of Parkinson's disease in smokers. Due to the fact that MAO B and COMT are responsible for dopamine and levodopa metabolism, it is hypothesized that dual MAO B/COMT inhibitors may synergistically enhance dopamine neurotransmission (de Beer et al., 2021). Therefore, it is also possible that, since catechols show MAO B inhibition and COMT inhibition together, catechols may be potential leads for dual MAO B/COMT inhibitors in the treatment of Parkinson's disease.

Tobacco addiction includes not only pharmacologic effects of nicotine, but also genetics, and social and environmental factors (Benowitz, 2010). In addition, research shows that tobacco smoking has been linked with MAO A inhibition in the brain of smokers (Fowler et al., 1996b) and that MAO A inhibition increased self-administration of nicotine at low doses in animal studies (Smith et al., 2016). Thus, our findings suggest that MAO A inhibition by catechols and hydroquinone found in tobacco smoke may contribute to enhance the primary reinforcing and reinforcement enhancing effects of nicotine in human smokers.

4.6. Conclusion

It has long been suggested that tobacco smoke results in irreversible MAO inhibition. We earlier suggested that 1,4-benzoquinone may play a role in the irreversible inhibition of MAO A (Hong et al., 2022). Although many studies have been conducted to isolate MAO inhibitors from tobacco smoke, experimental studies on the identification of irreversible inhibitors in tobacco smoke has until now remained unsuccessful. To determine reversibility of the MAO inhibitors identified, a time-dependent assay and a reversibility test were used. The findings from the reversibility test, using a centrifugation-ultrafiltration method, showed that MAO A activities were not recovered by repeated washout, indicating that the phenolic compounds are irreversible inhibitors of MAO A. The results from this study suggest that irreversible MAO inhibition by the phenolic compounds and others from tobacco smoke may play a role in contribution to the addictive effects of nicotine and the low incidence of Parkinson's disease in smokers.

Further studies of the phenolic MAO inhibitors in tobacco smoke are needed to examine their contribution to self-administration of nicotine. Future studies of the phenolic MAO inhibitors will be required to elucidate their mechanism of MAO inactivation. In conclusion, these findings suggest that MAO inhibitors from tobacco smoke may have effective therapeutic potential, in the treatment of anxiety, depression and Parkinson's disease or in effective smoking cessation therapies.

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Chapter 5

Alpha-linolenic and linoleic acid: Bioassay-guided isolation and biological activities as MAO (Monoamine Oxidase) inhibitors from tobacco smoke

This chapter contains the article "Alpha-linoleic and linolenic acids: Bioassay-guided isolation and biological activities as MAO (Monoamine Oxidase) inhibitors from tobacco smoke", co-authored by Sa Weon Hong (SH), Ali Heydari (AH), Paris Wilson (PW), Paul Teesdale-Spittle (PT-S), Rachel Page (RP), Peter T. Northcote (PTN), Robert A. Keyzers (RAK), and Penelope Truman (PT). This article will be submitted to Neurotoxicology.

Author contributions: For this PhD study, designed by SH and PT, SH performed the MAO assay (IC_{50}), kinetic studies (K_i), and molecular docking. PT-S advised on the molecular docking studies. Preliminary studies contributing to this manuscript were conducted by SH, PT, RAK, PTN, AH, and PW. SH wrote the manuscript. PT, RP, and PT-S contributed to the final shape of the manuscript.

This chapter discusses the following: (1) Isolation of reversible monoamine oxidase (MAO) inhibitors in tobacco smoke; (2) Biological activities of reversible MAO inhibitors from tobacco smoke; and (3) Kinetics and the potential mode of binding and interactions of the two PUFAs.

This article addresses research questions 2, 3, and 4 (Sections 1.4.2 – 1.4.4) for two of the six novel MAOIs identified in tobacco smoke by P Truman and colleagues. These 2 novel MAOIs are polyunsaturated fatty acids (PUFAs)

5.1. Abstract

A bioassay-guided isolation from an extract of tobacco smoke showed that both α-linolenic acid and linoleic acid displayed monoamine oxidase (MAO) inhibitory activity. Purification procedures, including reversed-phase and normal-phase chromatography, failed to separate significant peaks of MAO inhibitory activity from the fatty acids. Those fatty acids in the highly active fractions were identified by gas chromatography–mass spectrometry (GS-MS) and the most prominent were tested for MAO inhibitory activity. Two polyunsaturated fatty acids (PUFAs), α-linolenic acid and linoleic acid displayed a selective inhibitory effect for human MAO A with IC_{50} values of 15.74 and 23.8 μM, respectively. Kinetic analysis showed that α-linolenic acid is a competitive inhibitor of MAO A and MAO B. Kinetics analysis also revealed that linoleic acid is a competitive inhibitor of MAO A and MAO B and the mode of MAO inhibition is parabolic competitive. Molecular docking studies suggests that ternary complexes [MAO-linoleic $acid_2$ species (EII)] may be formed. These findings suggest that reversible MAO inhibition by α-linolenic acid and linoleic acid and other compounds from tobacco smoke may play a role in the lower MAO levels or activity seen in smokers.

5.2. Introduction

Monoamine oxidase (MAO; EC 1.4.3.4) is a flavin adenine dinucleotide (FAD) dependent enzyme catalyzing the oxidative deamination of a variety of primary, secondary and tertiary amines, including the neurotransmitters dopamine, norephinephrine, ephinephrine, serotonin, and dietary amines such as tyramine and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin which causes Parkinson's disease-like symptoms (Youdim & Bakhle, 2006). Two isoenzymes, MAO A and MAO B can be differentiated by their substrate selectivity and inhibitor sensitivity (Shih et al., 1999). MAO A oxidizes serotonin and is inactivated by clorgyline at low concentrations, whereas MAO B oxidizes benzylamine and 2-phenylethylamine and is inactivated by selegiline (l-deprenyl) at low concentrations (Youdim et al., 2006). Both MAO A and MAO B metabolize dopamine, noradrenaline, adrenaline, tryptamine, and tyramine in most species.

Interestingly, it is reported that different factors that affect different MAO levels in the brain involve smoking status, age, depressive mood, impulsive and aggressive personality, alcohol dependence, and DNA methylation state (Fowler et al., 2015). Hogg (2016) reported that smoking is related not only to the lower density of binding sites of MAO in human brain, but

also to the lower MAO B activity in human platelets and proposed that additional/synergistic effects of some tobacco-derived chemicals may result in the lower MAO levels or activity seen in smokers. On the other hand, PET studies using carbon 11–labelled harmine revealed that brain MAO A binding density was elevated during acute withdrawal in heavy smoker and this change in MAO A binding covaried with the decrease in plasma levels of harman ($P = 0.01$), a potent MAO A inhibitor found in tobacco smoke (Bacher et al., 2011). The mechanism of MAO A elevation is unknown, but future studies of tobacco MAO inhibitors such as harman or norharman are required to know whether they affect MAO A synthesis or removal.

MAO inhibitors have been used to treat depression, panic disorder, social phobia, bipolar, anxiety, and Parkinson's disease. Likewise, MAO inhibitors in tobacco and tobacco smoke may have pharmaceutical possibilities, in new smoking cessation strategies, or in relief of anxiety or depression or in treatment of Parkinson's disease. In this article, we report on the characterisation and biological activities of linoleic and α-linolenic acid from tobacco smoke as MAO inhibitors.

5.3. Materials and Methods

5.3.1. General experimental procedures

NMR spectra were recorded using a 600 MHz Varian Direct Drive spectrometer equipped with a triple-resonance HCN cryogenic probe operating at 25 K. The ^{1}H chemical shifts (δ) were internally referenced to the residual solvent peak (Gottlieb et al., 1997). Normal-phase column chromatography was performed using silica gel and diol-functionalized silica gel (DIOL). Reversed-phase column chromatography was performed using HP20 poly(styrene-divinylbenzene) (PSDVB) chromatographic resin. All solvents used for liquid chromatography were of analytical or HPLC grade. Solvent mixtures are reported as percent v/v unless otherwise noted.

5.3.2. Bioassay-guided fractionation of TPM

Anti-MAO bioassay-directed fractionation was conducted to identify MAO inhibitors from tobacco particular matter (TPM). 1kg of tobacco (Drum roll-your-own) was hand-rolled into cigarettes each containing approximately 1 g of tobacco. Cigarettes were individually smoked using a smoking machine and TPM collected onto filters (Sheehan et al., 2019), using 5 cigarettes per filter. The TPM was stored on the filters at -80 until use. Tobacco particulate matter (126.5 g) was extracted three times with MeOH (3 × 1.5 L) for 1 h. The extracts were

loaded onto HP20 beads (1 L) by successive dilution with H_2O to 25% MeOH in H_2O (cyclic loading). The column was then eluted with 40% MeOH/H_2O, 70% MeOH/H_2O, 90% MeOH/H_2O, and MeOH. For inhibition screening, each dried fraction (50 μg) was dissolved in 50 μL of EtOH, using 4 μL/100 μL reaction volume (in triplicate) to test activity. The most active fraction (MeOH fraction, 1.41 g) was then loaded onto a DIOL column (80 mL), and eluted with increasing concentrations of CH_2Cl_2 in n-hexane (12.5%–70%), followed by CH_2Cl_2, EtOAc, and MeOH. Among them, the 70% CH_2Cl_2/n-hexane and CH_2Cl_2 fractions showed the most potent MAO A inhibitory activities. They were combined, loaded onto silica gel (5 mL), and eluted with 50% CH_2Cl_2/n-hexane, CH_2Cl_2, and EtOAc in n-hexane (1%–100%). Finally, the two active fractions (1% EtOAc/n-hexane and 10% EtOAc/n-hexane) were concentrated to dryness for chemical analysis.

5.3.3. Esterification of Polyunsaturated Fatty Acids (PUFAs)

To analyse polyunsaturated fatty acids, synthesis of fatty acid methyl esters was conducted using acid catalyzed transesterification (Eder, 1995) with slight modifications. The 1% EtOAc/n-hexane fraction (100 μg) was added to 1 mL of a solution of boron trifluoride (BF_3) in MeOH (14%). This mixture was heated at 90 °C for 10 min, followed by liquid-liquid extraction with H_2O (0.5 mL) and CH_2Cl_2 (0.5 mL) three times. The combined organic phases were concentrated to dryness. EtOAc (1.5 mL) was added to the sample and analysis was conducted on a Shimadzu GC-2Db, using a RXI-5Sil-MS column. This same procedure was repeated with the 10% EtOAc/n-hexane fraction (100 μg).

5.3.4. Monoamine oxidase inhibition assay

To determine MAO inhibitory activity, the kynuramine assay was conducted with slight modifications of the previously described methods (Lewis et al., 2012; Truman et al., 2017). Human recombinant MAO enzymes (MAO A specific activity: 0.33 U/mg; MAO B specific activity: 0.4 U/mg; 1 U will deaminate 1 nmol of kynuramine per min at pH 7.4 at 37 °C) were purchased from Sigma Aldrich and stored at -80°C. Briefly, the reaction mixture (final volume, 100 μL) contained 50 mM phosphate buffer (pH 7.2), 100 μM kynuramine substrate, and the test inhibitors (0.001–300 μM). The test inhibitors were dissolved in EtOH (final concentration 4% EtOH (v/v)). Controls with inhibitor and buffer only were included to remove the intrinsic fluorescence of the sample itself. Control reactions with no EtOH or inhibitor were also included. The reaction is initiated by addition of MAO (0.00625 mg protein/mL) and incubated for 15 min (MAO A) or 30 min (MAO B) at 37 °C in a black 96-well microplate. After

incubation time, the reaction was stopped by addition of 2.5 N NaOH (50 μL) and production of 4-hydroxyquinoline, the fluorescent product of MAO-catalyzed kynuramine oxidation, was monitored using a microplate reader (FLUOstar Omega, BMG Labtech) with excitation at 320 nm and emission at 380 nm. GraphPad Prism 9 (GraphPad Software Inc.) was used for fitting a sigmoidal dose-response curve to obtain the IC_{50} values. All assays were carried out in triplicate and the IC_{50} values reported as the mean ± SEM (n = 3). In the study, norharman, a potent inhibitor of MAO A and MAO B present in tobacco smoke, was used as the positive control (Herraiz & Chaparro, 2005).

5.3.5. MAO enzyme kinetics

Sets of Lineweaver-Burke (LB) plots were prepared to understand the kinetics and mechanism of inhibition of recombinant human MAO by α-linolenic acid and linoleic acid. MAO A (0.00625 mg protein/mL) was incubated at 37 °C for 15 min with kynuramine (20–300 μM) in the presence of α-linolenic acid (0, 10, 20, or 30 μM) or linoleic acid (0, 15, 20, or 25 μM). MAO B (0.00625 mg protein/mL or 0.0094 mg protein/mL) was also incubated at 37 °C for 30 min with kynuramine (20–300 μM) in the presence of α-linolenic acid (0, 10, or 30 μM) or linoleic acid (0, 60, 80, or 100 μM). MAO enzyme activities were measured as described for the monoamine oxidase inhibition assay. Enzyme kinetic parameters and mode of inhibition were determined using nonlinear regression and visualised using classical enzyme kinetic plots (Antunes et al., 2003; Baici, 1981). Each were characterised as to its K_i (concentration of inhibitor needed to inhibit half of the enzyme activity, at low substrate concentrations) whether it is a competitive inhibitor and whether or not it is reversible. K_i is the dissociation constant determined for the initial non-covalent interaction with the inhibitor (Strelow, 2017).

5.3.6. Molecular docking

Docking simulations were conducted with Autodock Vina (v1.1.2) which is an open-source program (Eberhardt et al., 2021; Trott & Olson, 2010). To generate the protein coordinate files, X-ray crystal structure of hMAO B in complex with safinamide (PDB ID: 2V5Z) was downloaded from the Protein Data Bank (PDB; www.rcsb.org/pdb). Water molecules, ligands and the B chain of MAO B were deleted and polar hydrogens were added by using Discovery Studio 2021 Client (https://discover.3ds.com/discovery-studio-visualizer-download). AutoDockTools (ADT) was also used for preparation of coordinate files which includes merging non polar hydrogens and adding Kollman charges. To prepare the ligands coordinate files, experimental coordinates were downloaded from PDB or PubChem

(https://pubchem.ncbi.nlm.nih.gov) and ADT computed Gasteiger charges, merged nonpolar hydrogens and assigned atom types to each atom. A configuration file for AutoDock Vina was created, which include the receptor, ligand file name, and the docking parameters. The grid box for MAO B was centered on the ligand (x = 52.148, y = 156.183, z = 28.029). The size of the search space was x = 26, y = 26, z = 26 with grid point spacing of 1 Å.

Molecular docking was performed to study the potential formation of the ternary MAO B–linoleic $acid_2$ species (EII). To generate a protein-ligand complex in PDB format, we used Cygwin (https://www.cygwin.com/), an open-source software providing useful Unix utilities to Windows environments (Racine, 2000). Docking simulations were carried out for MAO B–linoleic acid complex to linoleic acid as described above.

5.3.7. ADME prediction

In silico ADME (absorption, distribution, metabolism and excretion) prediction were conducted on the novel MAO inhibitors identified from tobacco smoke. This work included assessing physicochemical properties, pharmacokinetics, drug-likeness and medicinal chemistry friendliness using the SwissADME web tool (http://www.swissadme.ch) (Daina et al., 2017). We also performed the predictions of gastrointestinal absorption and brain access of the MAO inhibitors using the BOILED-Egg (Brain Or IntestinaL EstimateD permeation method) model (Daina & Zoete, 2016) from SwissADME. We also used PreADMET (https://preadmet.webservice.bmdrc.org) web tool to evaluate ADME, druglikeness, and toxicity of the novel MAO inhibitors.

5.4. Results

5.4.1. Extraction and isolation

Polyunsaturated Fatty Acids (PUFAs) were isolated from the MeOH extract of TPM by Anti-MAO bioassay-directed fractionation. The MeOH extracts were purified using reversed-phase (PSDVB, MeOH/H_2O gradient) chromatography. The extracts were loaded onto HP20 beads by successive dilution with H_2O to 25% MeOH in H_2O. We used this “cyclic loading” process which increases the polarity of the mobile phase, and retains compounds of slightly higher polarity on the beads (Singh et al., 2014). The MeOH fraction, which was taken further because it showed high MAO inhibitory activity at the concentration of 40 µg/mL in the reaction mixture, was fractionated using a sequence of normal-phase (DIOL, CH_2Cl_2/n-hexane, silica gel, EtOAc/n-hexane) chromatography. The structures of the fatty acids in the high activity

fractions were identified by GS-MS. The scheme for bioassay-guided fractionation processes is shown in Fig. 5.1.

On testing for MAO A inhibitory activity, stearic and palmitic acids were found to be inactive, but linoleic and α-linolenic acids displayed strong inhibitory activity.

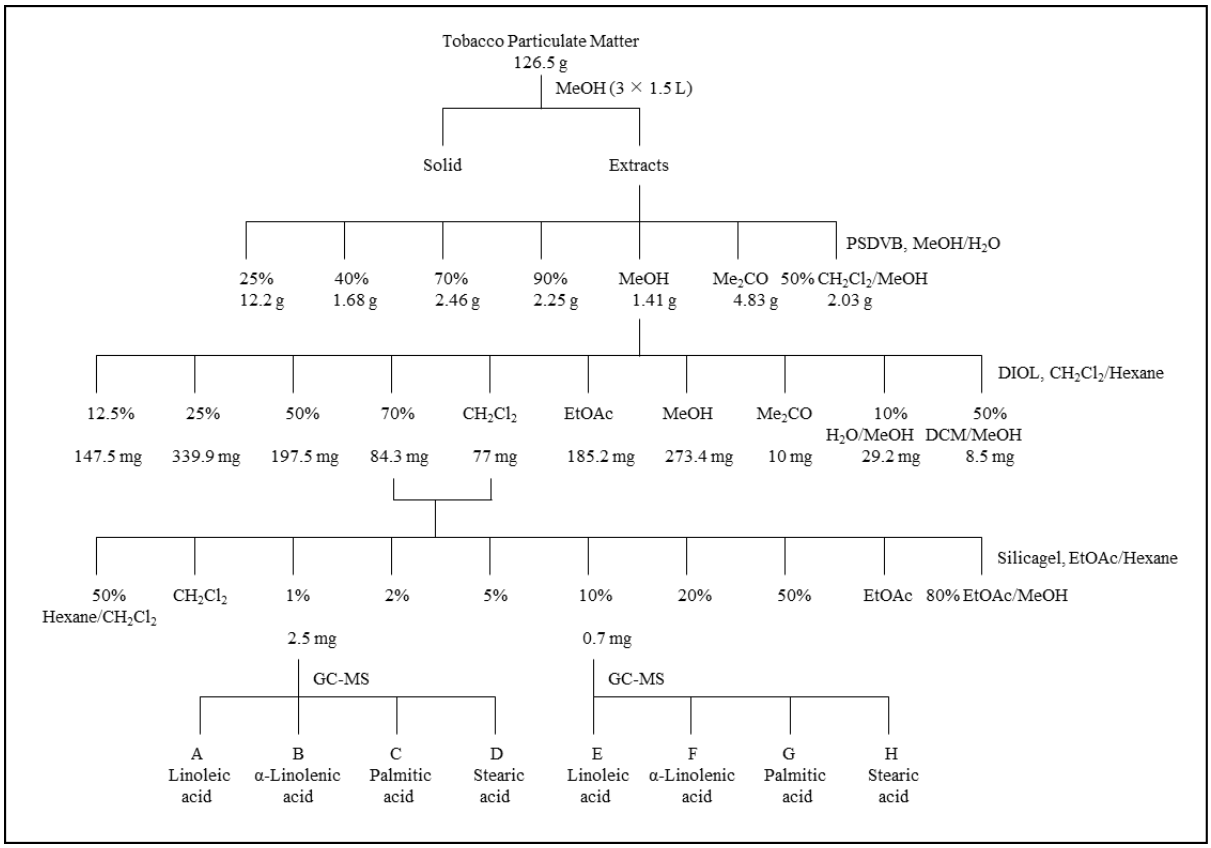
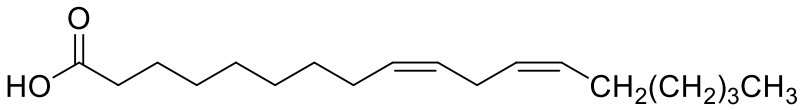
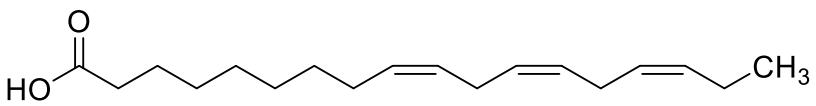


Figure 5.1. Isolation of the linoleic and α-linolenic acid from tobacco particulate matter.



Linoleic acid, 18:2 (n-6)



α-Linolenic acid, 18:3 (n-3)

Figure 5.2. Chemical structures of the linoleic and α-linolenic acid.

5.4.2. Monoamine oxidase inhibition assay

The IC_{50} (half maximal inhibition) values for inhibition of human MAO A and MAO B by the two novel MAOIs identified in tobacco smoke that are fatty acids are shown in Table 5.1. In studies of the dependence of inhibition on incubation time, MAO A and MAO B were preincubated at 37 °C with a range of concentrations of compounds for 1 prior to kynuramine substrate addition. Saturated fatty acids such as palmitic acid (C16:0) and stearic acid (C18:0) displayed no MAO A inhibition (data not shown). The inhibition potency of MAO A by cis unsaturated fatty acids with two or three double bonds were more potent than that of monounsaturated fatty acid (oleic acid) (data not shown). α-Linolenic acid and linoleic acid are both reversible and selective MAO A inhibitors with IC_{50} values of 15.74 and 23.8 μM, respectively. They showed inhibitory activities against MAO B with IC_{50} values of 31.94 and 71.74 μM, respectively. Inhibition of MAO B by linoleic acid was found to show time dependence. This suggest that linoleic acid may be a tight-binding inhibitor of MAO B. Some tight-binding inhibitors display slow binding kinetics and their inhibition is time-dependent (Copeland et al., 1995).

Table 5.1. The IC_{50} values for inhibition of human MAO A and MAO B by α-linolenic acid and linoleic acid.

Compound	IC_{50} (μM), no preincubation		SI [a]	IC_{50} (μM), 1h preincubation		SI [a]
	MAO A	MAO B		MAO A	MAO B	
Linoleic acid	23.8 ± 8.7	71.7 ± 11.0	3.01	18.7 ± 2.6	16.8 ± 6.3	0.9
α-Linolenic acid	15.7 ± 1.5	31.9 ± 5.2	2.03	16.4 ± 8.1	29.7 ± 13.0	1.81
Norharman	4.23 ± 1.16	5.17 ± 0.35	1.22	3.16 ± 0.44	5.54 ± 0.32	1.75

[a] SI: selectivity index is defined as the ratio of [IC_{50} (MAO B)]/[IC_{50} (MAO A)]. The inhibition data are given as the mean ± SEM (triplicate). Positive control; norharman: reversible MAO inhibitor found in tobacco smoke.

5.4.3. MAO enzyme kinetics

Lineweaver-Burke (LB) plots were prepared to understand the kinetics and mechanism of inhibition of recombinant human MAO by α-linolenic acid and linoleic acid. LB plots for the inhibition of MAO A and MAO B by α-linolenic acid were linear and intersected on the y-axis, which suggests α-linolenic acid is a competitive inhibitor of MAO A and MAO B (Fig. 5.3a and 5.3c). Dixon plots of the inhibition of MAO A and MAO B by α-linolenic acid were also linear (Fig. 5.3b and 5.3d). LB plots for the inhibition of MAO A and MAO B by linoleic acid were linear and intersected on the y-axis, which suggests linoleic acid is a competitive inhibitor of MAO A and MAO B (Fig. 5.4a and 5.4c). However, Dixon plots indicated that the inhibition

of MAO A and MAO B by linoleic acid was parabolic (Fig. 5.4b and 5.4d). Because LB slope replots (data not shown) and Dixon plots are nonlinear, these nonlinearities might correspond to the formation of the ternary MAO–linoleic $acid_2$ species (EII).

The K_i values for inhibition of human MAO A and MAO B by the two fatty acids are shown in Table 5.2. Kinetic analysis of inhibition by α-linolenic acid showed competitive inhibition towards for MAO A and MAO B, with K_i values of 10.5 μM for MAO A and 17.98 μM for MAO B. Linoleic acid showed parabolic competitive inhibition towards for MAO A and MAO B, with K_i values of 11.65 μM for MAO A and 61.51 μM for MAO B.

In addition, to characterize the inhibition of human MAO A activity by fatty acids, Graphad Prism 9 was used for fitting a sigmoidal dose-response curve to obtain the Hill coefficient (Hill slope). The Hill coefficient of α-linolenic acid for MAO A inhibition was 1.05, while the Hill coefficient for linoleic acid for MAO A inhibition was 2.83, suggesting positive cooperativity (Table 5.2).

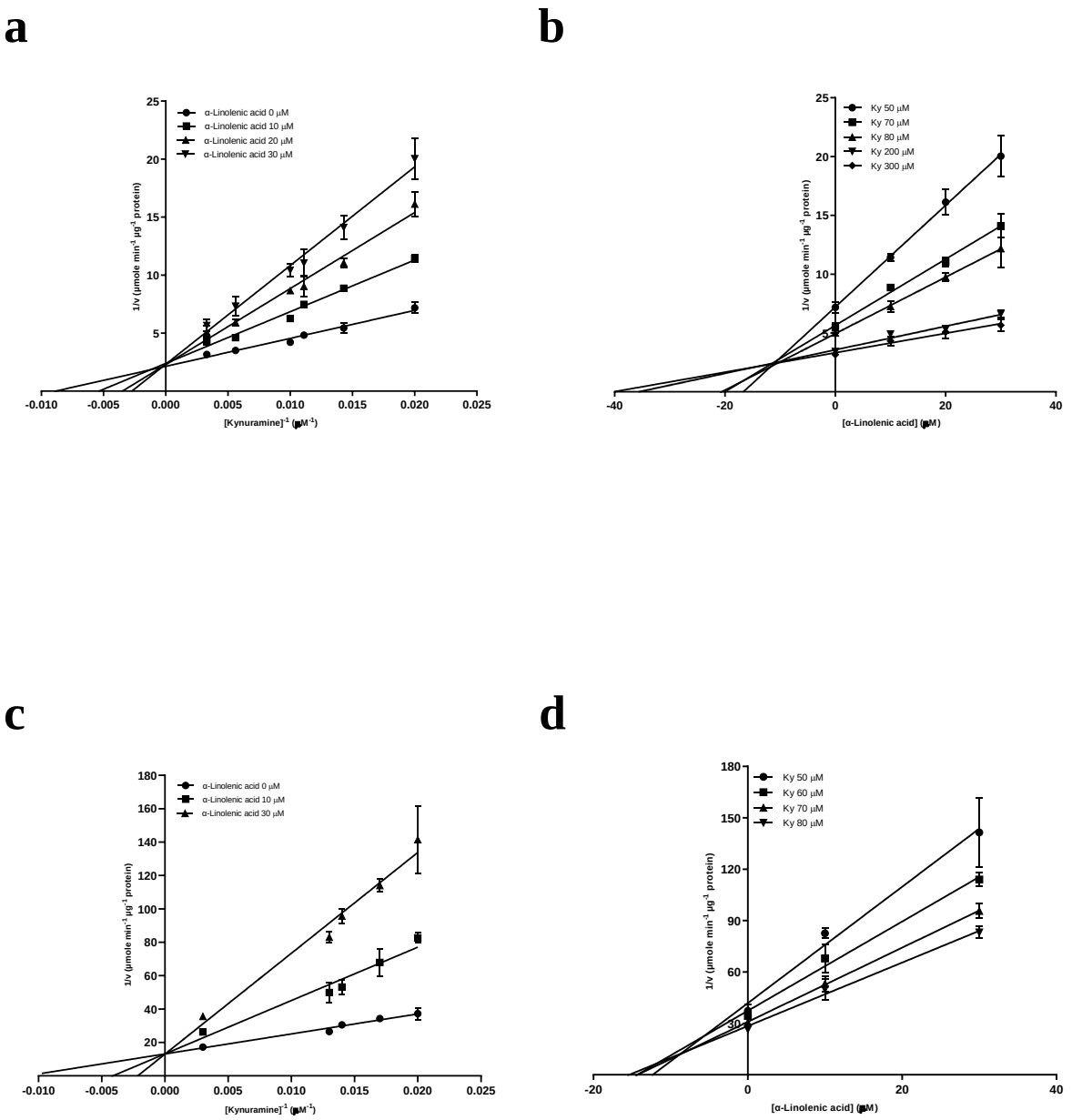


Figure 5.3. LB and Dixon plots of the inhibition of MAO A and MAO B by α-linolenic acid. For MAO A, (a) and (b); and MAO B, (c) and (d).

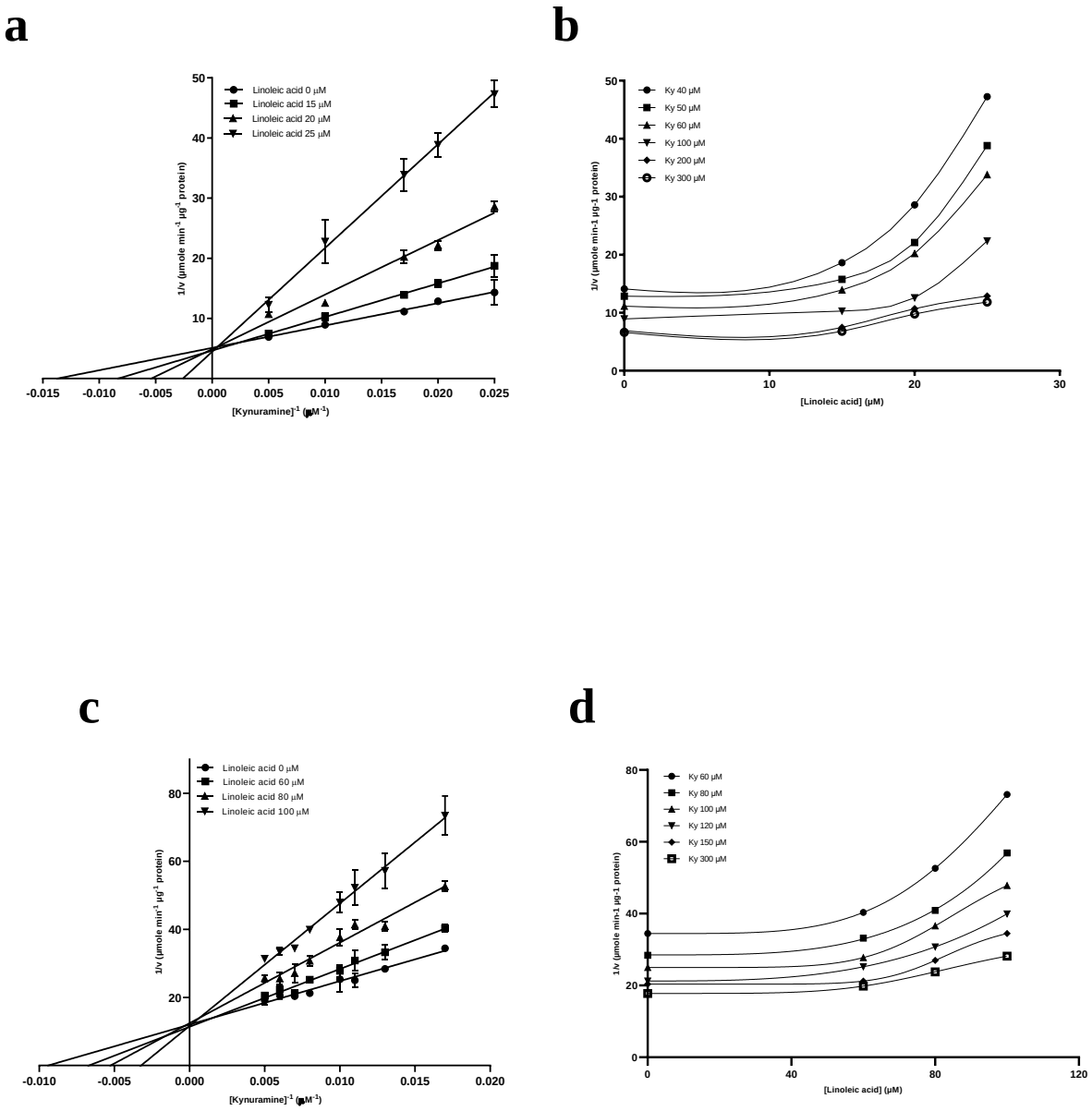


Figure 5.4. LB and Dixon plots of the inhibition of MAO A and MAO B by linoleic acid. For MAO A, (a) and (b); and MAO B, (c) and (d).

Table 5.2. Inhibition of human MAO A activity by α-linolenic acid and linoleic acid.

Compound	MAO A		Mechanism of inhibition
	K_i (μM)	Hill coefficient	
Linoleic acid	11.65 ± 1.22	2.83 ± 1.13	Parabolic competitive
α-Linolenic acid	10.50 ± 0.99	1.05 ± 0.16	Competitive
Norharman	0.95 ± 0.07	0.96 ± 0.20	Competitive

Hill coefficient: the cooperativity of ligand binding. Competitive parabolic: inhibitor (*I*) binding to a second site to form an EII complex. K_i is the dissociation constant of the enzyme inhibitor (EI) complex, while IC_{50} value represents the concentration of inhibitor that halves the rate of an enzyme-catalyzed reaction (Burlingham & Widlanski, 2003).

5.4.4. Molecular docking

Molecular docking studies were conducted to characterize the potential binding sites of α-linolenic and linoleic acid to hMAO B. The compounds showed the same binding affinity (-8.1 kcal/mol) to MAO B, whereas safinamide showed binding affinity of -10.3 kcal/mol for MAO B (Table 5.3). Docking interactions of α-linolenate and linoleate with MAO B are shown in Fig. 5.5 and 5.6. Docking simulations exhibited that the compounds were well located at the active site of MAO B. The carboxylate moiety of α-linolenate forms hydrogen bond with Tyr 435. Pi-alkyl interactions can be seen between Tyr60, Phe103, Trp119, Tyr326, Phe343, Tyr398 with α-linolenate. Other interactions such as alkyl and electrostatic interactions (Gln206, FAD 1502) were observed as well. On the other hand, linoleate has pi-sigma interaction with FAD 1502. Also, the carboxylate moiety of linoleate has pi-anion interaction with Tyr 435. Similarly, pi-alkyl interactions can be seen between Trp119, Tyr326, Tyr398 with linoleate, but linoleate did not form hydrogen bond with the MAO B.

As enzyme kinetics indicated that linoleic acid showed competitive parabolic inhibition, Autodock vina was used to carry out the molecular docking simulation for MAO B–linoleic acid complex to linoleic acid (Figure 5.7). The binding energy data shows that EI_2 binding is less energetically favourable (Table 5.3). The calculated K_i value for the ternary EI_2 complex increased the K_i value from 1.147 to 28.38 μM (about 25 times) for linoleic acid (Table 5.3). Molecular docking simulation for MAO B–α-linolenic acid complex to α-linolenic acid was also performed (Figure 5.8). Calculated K_i value for the ternary EI_2 complex increased from 1.147 to 593.148 μM (about 500-fold) for α-linolenic acid (Table 5.3). These docking studies support the formation of the ternary MAO B–linoleic $acid_2$ species (EII) as being more energetically favourable than the formation of similar ternary structures with α-linolenic acid.

Table 5.3. Docking calculations of α-linolenic acid and linoleic acid against hMAO B.

Compound	ΔG (kcal mol^{-1})	Calculated K_i (μM)
α-Linolenic acid (α-Linolenate)	-8.1	1.147
α-Linolenic $acid_2$	-4.4	593.148
Linoleic acid (Linoleate)	-8.1	1.147
Linoleic $acid_2$	-6.2	28.38
Safinamide	-10.3	0.279

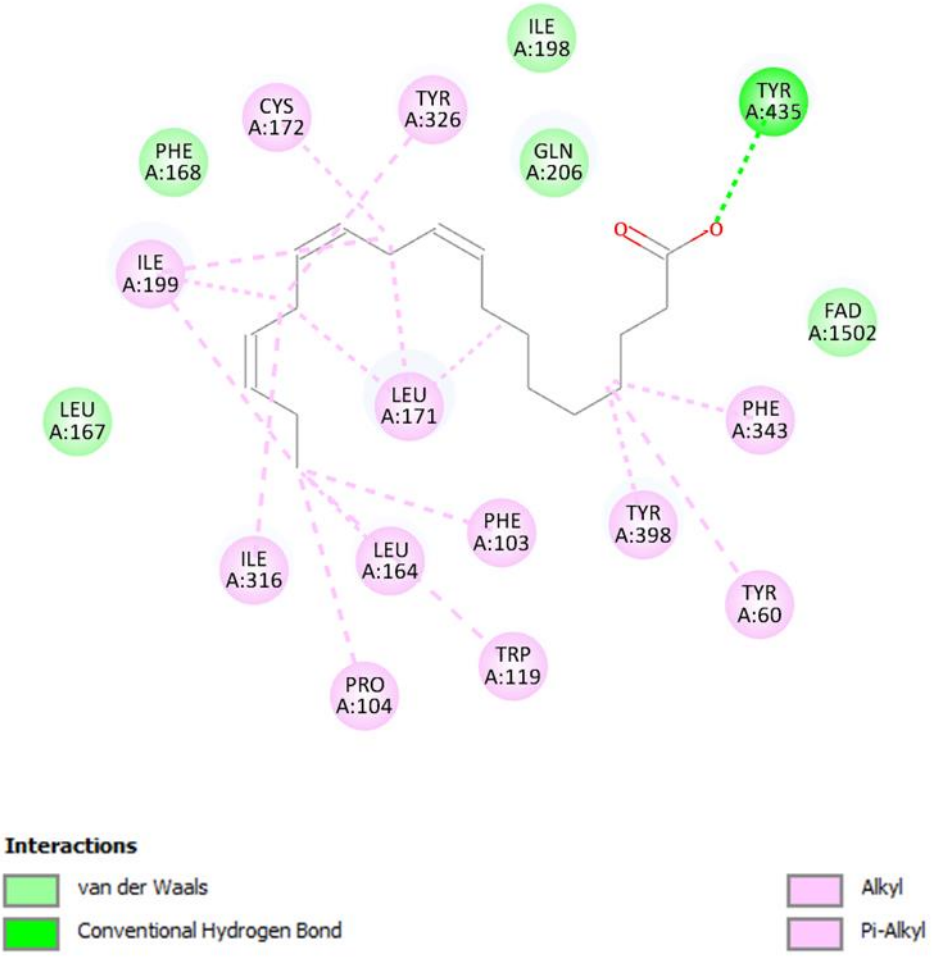


Figure 5.5. Docking interactions of α-linolenate with hMAO B.

The results were visualized and analysed using Discovery Studio.

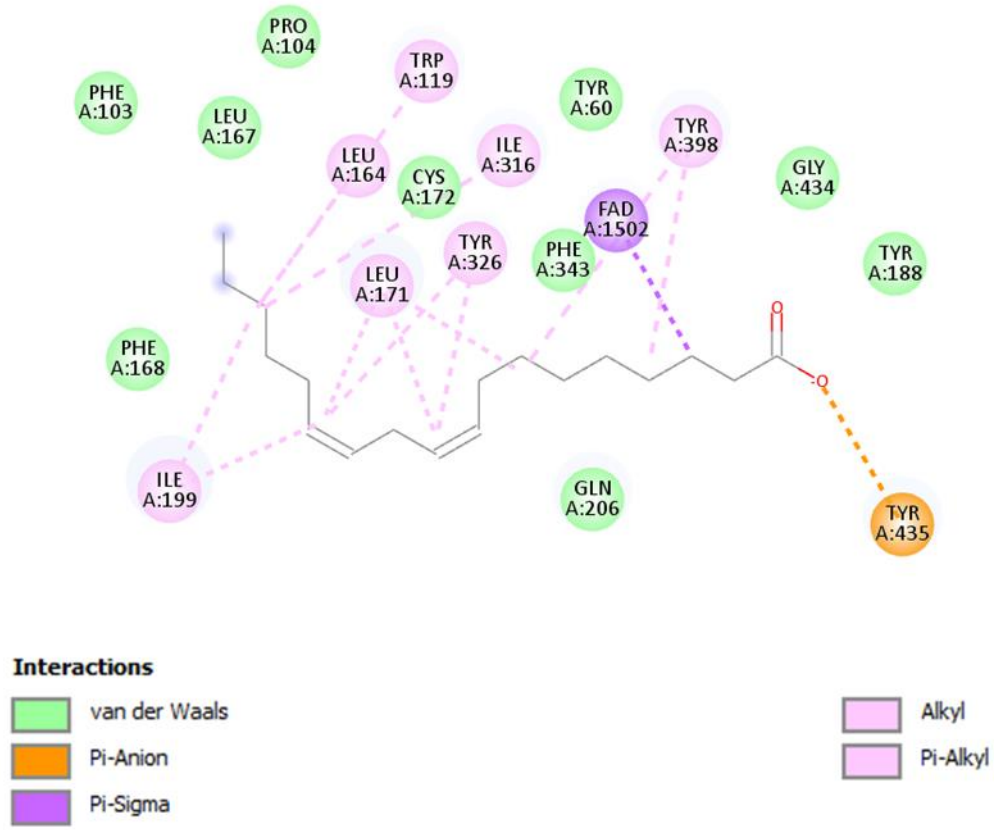


Figure 5.6. Docking interactions of linoleate with hMAO B.

The results were visualized and analysed using Discovery Studio.

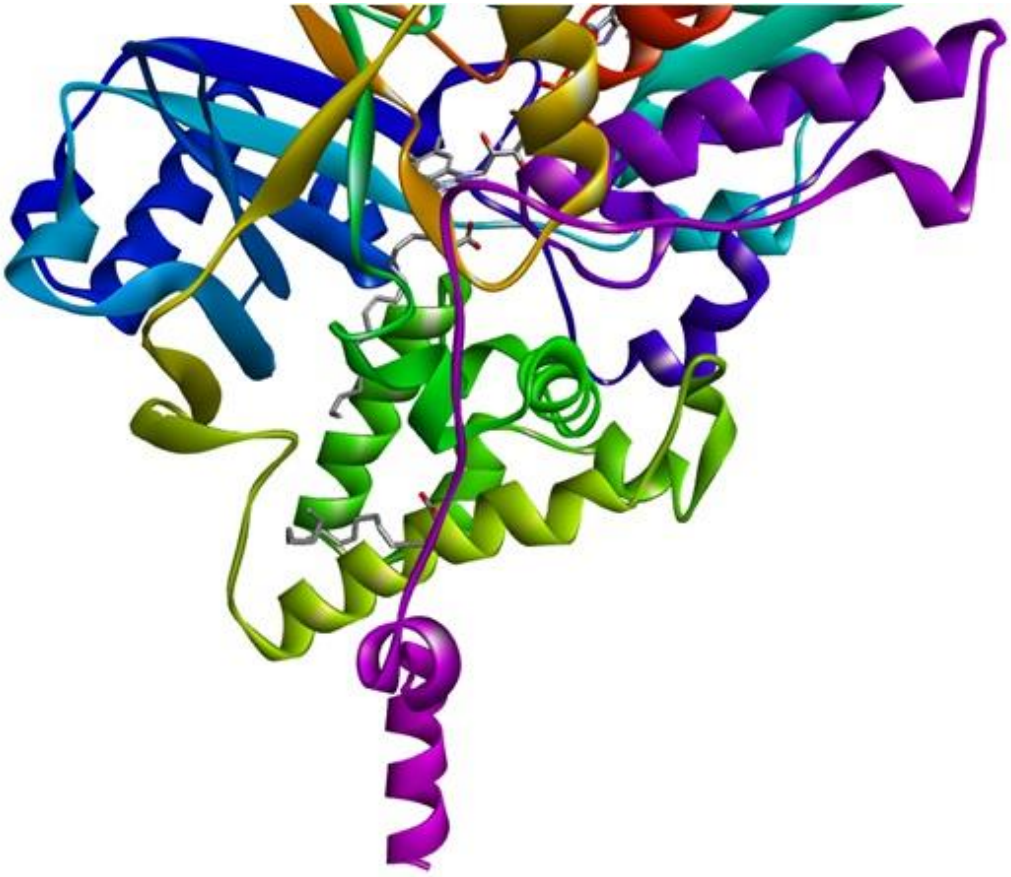


Figure 5.7. Docking model of binding interaction of MAO B–linoleic $acid_2$ species (2v5z_linoleate-$linoleate_2$)

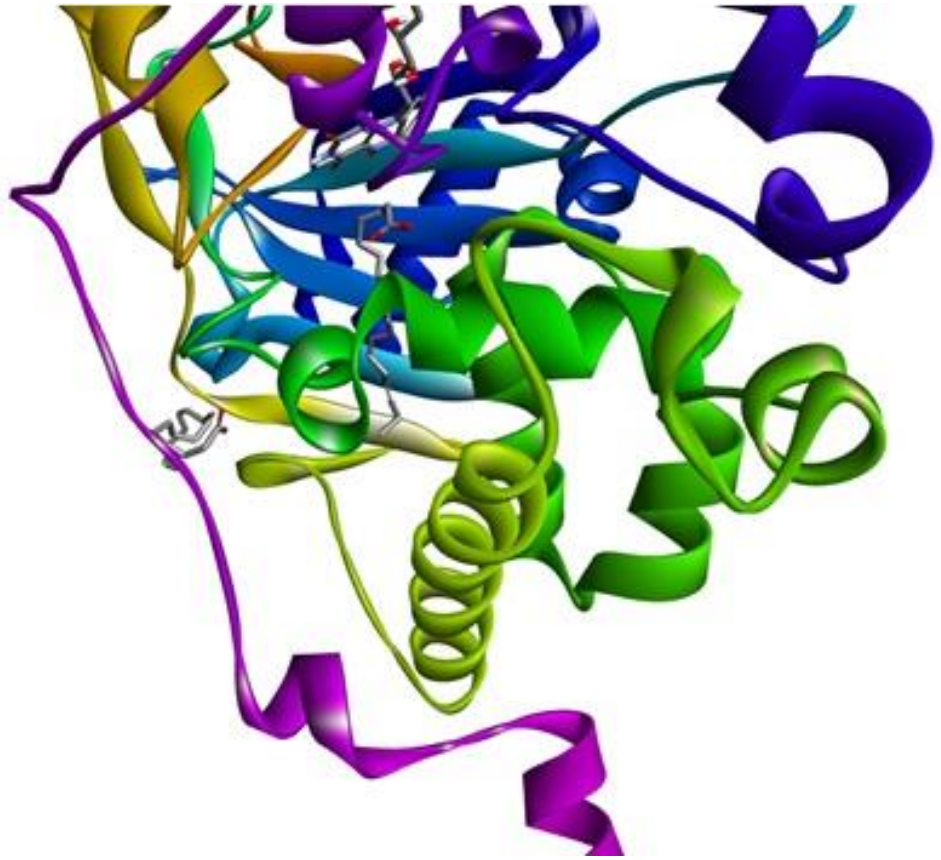


Figure 5.8. Docking model of binding interaction of MAO B–α-linolenic acid$_2$ species. (2v5z_alpha-linolenate-alpha-linolenate$_2$).

5.4.5. ADME prediction

To predict gastrointestinal absorption and brain access of the MAO inhibitors, the BOILED-Egg model from SwissADME was used (Figure 5.9). Blood-brain barrier (BBB), P-glycoprotein (a drug efflux pump), topological polar surface area (TPSA), passive human gastrointestinal absorption (HIA), WLOGP (a log *P* method) (Wildman & Crippen, 1999) are parameters that have been used in this study. All tested compounds are predicted to show high gastrointestinal absorption (white area) and cross the blood brain barrier (yolk area) by passive penetration. In addition, α-linolenic acid and linoleic acid are predicted as non-Pgp substrate (PGP−), while harman and norharman, two potent MAO inhibitors from tobacco smoke, are predicted to be pumped out by Pgp (PGP+). The reference inhibitors, clorgyline and selegiline are predicted as non-substrates for Pgp (PGP−). Further, in silico studies using SwissADME and PreADMET web tools were performed to predict the absorption, distribution, metabolism, excretion, and toxicity (ADMET) data (Appendix 4.3 and 5.2).

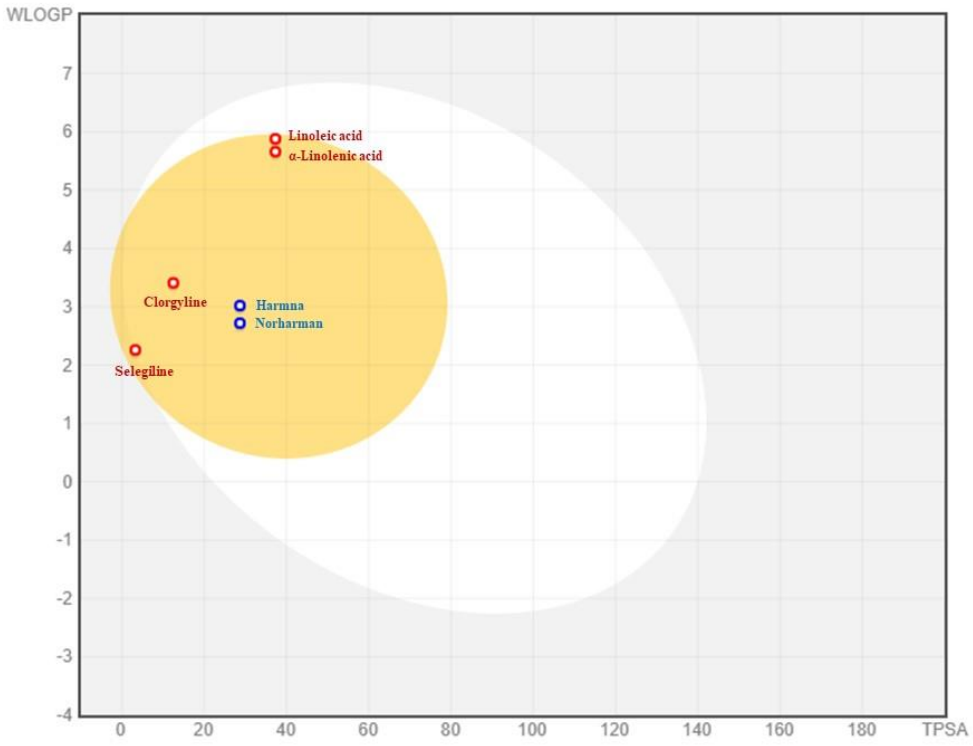


Figure 5.9. Predicted BOILED-Egg representation from Swiss ADME for α-linolenic acid and linoleic acid.

The white area is more likely to be passively absorbed by the gastrointestinal tract, and the yellow area is more likely to penetrate the blood-brain barrier. The two areas are not mutually exclusive. The blue dot indicates P-gp substrate (PGP+) and the red dot indicates P-gp non-substrate (PGP−). WLOGP and TPSA are physicochemical descriptors for lipophilicity and

apparent polarity, respectively. WLOGP is an implementation of the atomistic method from Wildman and Crippen. TPSA needs to be between 20–130Å^2.

5.5. Discussion

In the present study, we found that α-linolenic acid and linoleic acid from tobacco smoke showed potent human MAO A and MAO B inhibition. Our findings suggest that reversible MAO inhibition by α-linolenic acid and linoleic acid from tobacco smoke may play a role in the lower MAO levels or activity in smokers. Two PUFAs, α-linolenic acid and linoleic acid selectively inhibited MAO A with IC_{50} values of 15.74 and 23.8 μM, respectively. The mainstream smoke of different types of cigarettes contains large amounts of α-linolenic acid (52–329 μg/1g cigarette) and linoleic acid (50–146 μg/1g cigarette) (Hoffmann & Woziwodzki, 1968), suggesting these two PUFAs may make substantial contributions to total MAO inhibitory activity of cigarette smoke. Truman et al. (2017) reported that harman and norharman, two potent MAO inhibitors in tobacco smoke, only moderately contribute to the total MAO A inhibitory activity (less than 10%). Even though the PUFAs are only moderately potent as inhibitors compared to harman/norharman, they may be significant contributors because there is so much more of them than there is of harman (0.85 μg/cigarette)/norharman (2.19 μg/cigarette) (Herraiz & Chaparro, 2005). Further studies are required to investigate the contribution to the total MAO inhibitory activity of cigarette smoke.

PUFAs are involved in the regulation of the structure and the function of neurons and non-neuronal cells such as glial cells and endothelial cells in the brain (Bazinet & Layé, 2014). The PUFAs linoleic acid and α-linolenic acid, which are essential fatty acids, produce arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), and contribute significantly to the regulation of body homeostasis of inflammation/anti-inflammation, platelet aggregation/antiaggregation, vasodilatation/vasoconstriction, and bronchoconstriction/ bronchodilation (Saini & Keum, 2018). Since n–3 PUFAs including α-linolenic acid and DHA are able to cross the blood brain barrier via major facilitator superfamily domain-containing protein 2a (Mfsd2a) (Nguyen et al., 2022), α-linolenic acid from tobacco smoke may be involved in lowering MAO levels or activity in the brains of smokers.

Selective MAO A inhibition from α-linolenic acid, an n–3 PUFA from tobacco smoke, may lead to antidepressant action in the brains of smokers. As a consequence of selective MAO A inhibition, levels of serotonine and norepinephrine may be increased, causing clinical

antidepressant action (Finberg & Rabey, 2016). Interestingly, it has been suggested that n–3 PUFA intake may have a positive effect on depressive status through the potential interaction between n–3 PUFA and the serotonin and dopamine neurotransmission which includes metabolism, release, absorption and receptor functions (Grosso et al., 2014). Our findings suggest that future studies should be carried out to show whether or not other n–3 PUFAs and n–6 PUFAs act as inhibitors of MAO enzymes.

Kinetic analysis revealed that linoleic acid is a competitive inhibitor of MAO A and MAO B and the mode of MAO inhibition is parabolic competitive. Competitive parabolic inhibition is observed when inhibitor (I) binds to a second inhibition site to form an EII complex (Antunes et al., 2003). Parabolic inhibition can be parabolic competitive, parabolic uncompetitive, or parabolic noncompetitive (Cleland, 1963). Because linoleic acid is a competitive inhibitor of MAO and LB slope replots and Dixon plots of linoleic acid are parabolic, these findings suggest that linoleic acid may bind to a second inhibition site to form the ternary MAO–linoleic $acid_2$ species (EII). The Hill coefficient of fatty acids for MAO A inhibition were also obtained. Hill coefficient is not only linked with the stoichiometry of enzyme–inhibitor interactions but also indicates the steepness of the dose–response relationship (Copeland, 2013). In the sigmoidal curve of linoleic acid for MAO A inhibition, the Hill coefficient of linoleic acid for MAO A is greater than 1, proposing positive cooperativity. Positive cooperativity means the ligand binding to one active site can increase the ligand binding affinity to the other active sites. This result suggests that mechanism of MAO A inhibition by linoleic acid does not follow 1-to-1 binding (stoichiometric binding of one inhibitor to one enzyme).

Docking simulations exhibited that α-linolenic acid and linoleic acid were well located at the active site of MAO B showing the same binding affinity (-8.1 kcal/mol). A previous docking study provided comparable evidence suggesting omega PUFAs from fish oil inhibit MAO B and could give protection against neurological disorders, but further biochemical investigations are required (Masroor et al., 2019). Also, we conducted molecular docking simulation of the binding of a PUFA to a pre-formed MAO B–PUFA complex. Docking studies suggest that ternary MAO B-linoleic $acid_2$ species (EII) may be formed and that formation of an analogous ternary complex is less likely with α-linolenic acid, in agreement with kinetic results.

Here, we report that α-linolenic acid and linoleic acid are reversible and potent MAO inhibitors. The results suggest that α-linolenic acid and linoleic acid from tobacco smoke may play a role in reducing the MAO levels or activity in smokers. Although a previous study suggested that

an unsaturated aldehyde, (Z)-9,17-octadecadienal and n-hexadecanoic acid (palmitic acid) may be potential leads as selective MAO A inhibitors for the treatment of psychiatric disorders (Margret et al., 2015), to our knowledge, these are the first n–3 and n–6 PUFAs with MAO A inhibitory activity to be reported.

5.6. Conclusion

It has been reported that non-nicotinic compounds in tobacco are commonly reversible inhibitors of MAO and show competitive inhibition, non-competitive, or mixed-type inhibition (Hong 2022). Kinetic analysis of the MAO inhibition by the newly identified PUFA inhibitors was explored to determine the mode of inhibition. LB plots for the inhibition of MAO A by α-linolenic acid suggests α-linolenic acid is a competitive inhibitor of MAO A. Kinetic analysis revealed that linoleic acid is a competitive inhibitor of MAO A and MAO B and the mode of MAO inhibition is parabolic competitive. Molecular docking studies were conducted to characterize the potential binding sites of the inhibitors to MAO. Molecular docking studies suggests that ternary complexes may be formed particularly for linoleic acid. The results from this study suggest that reversible MAO inhibition by α-linolenic acid and linoleic acid and others from tobacco smoke may play a role in the lower MAO levels or activity in smokers. Overall, these findings suggest that MAO inhibitors from tobacco smoke may have pharmaceutical possibilities, in effective smoking cessation, or in relief of anxiety or depression or in treatment of Parkinson’s disease and further work to examine these possibilities should be done.

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Chapter 6

General Discussion and Conclusions

This chapter provides a discussion of the key findings and presents the overall conclusions of this PhD study. Additionally, it includes future research directions.

6.1. Summary of Key Findings

A summary of the key findings related to the four research questions established for this PhD study (Chapter 1, Section 1.4) are outlined below:

1. **What already known non-nicotinic components in tobacco and tobacco smoke could contribute to the total MAO inhibitory activity in cigarette smoke?**
   A review on the monoamine oxidase (MAO) inhibitors in tobacco and tobacco smoke was conducted (Hong et al., 2022). MAO inhibitors were crossmatched with known tobacco and tobacco smoke components. The most potent inhibitors found in this review included harman, kaempferol, and quercetin for MAO A and farnesol, menadione, and norharman for MAO B.
2. **Do the novel MAO inhibitors identified show potent inhibition for human MAO A and MAO B isoenzymes?**
   The findings from the kynuramine assay showed that 4-methylcatechol and 4-ethylcatechol are potent MAO A inhibitors in tobacco smoke with IC_{50} values in the low micromolar range (Table 4.1). Among the phenolic compounds, 4-methylcatechol showed the highest activity for MAO A with an IC_{50} value of 0.267 μM after 1h preincubation. 4-Methylcatechol also showed potent MAO B inhibition with higher potency compared to norharman (Table 4.1). α-Linolenic acid and linoleic acid also inhibited MAO A with K_i values in the low micromolar range (Table 5.1).
3. **Do the novel MAO inhibitors identified show reversible inhibition or irreversible inhibition against human MAO A and MAO B?**
   The findings from the reversibility test using a centrifugation-ultrafiltration method showed that MAO A activities were not recovered by repeated washout, indicating that the phenolic compounds are irreversible inhibitors of MAO A. MAO A activity inhibited by clorgyline, as a positive control was also not recovered, while norharman showed recovery of MAO A activity under identical conditions. On the other hand, kinetics analysis showed that α-linolenic acid and linoleic are potent, reversible, and selective MAO A inhibitors (Table 5.2).
4. **What are the mode of inhibition and potential binding sites of the novel MAO inhibitors to human MAO enzymes?**
   LB plots for the inhibition of MAO A by α-linolenic acid suggests α-linolenic acid is a competitive inhibitor of MAO A. Kinetics analysis revealed that linoleic acid is also a

competitive inhibitor of MAO A and MAO B, but the mode of MAO inhibition is parabolic competitive. The Hill coefficient for linoleic acid for MAO A inhibition proposes positive cooperativity. Molecular docking studies suggest that ternary complexes [MAO B-linoleic $acid_2$ species (EII)] may be formed.

6.2. Discussion of main findings

The main findings of the MAO inhibitory compounds in tobacco and tobacco smoke are discussed in relation to the four research questions for this study and are outlined below.

6.2.1. MAO inhibitors in tobacco and tobacco smoke

The literature review (Chapter 3) reports the MAO inhibitors identified from tobacco and tobacco smoke which are divided into nine chemical classes containing thirty eight compounds. The five classes of MAO inhibitors, not previously related to tobacco, discussed as MAO inhibitors in tobacco and tobacco smoke. The most potent inhibitors found in this review included harman, kaempferol, and quercetin for MAO A and farnesol, menadione, and norharman for MAO B. In addition, we suggest that 1,4-benzoquinone may play a part in the irreversible inhibition of MAO A by cigarette smoke and other irreversible activities may remain to be discovered. To our knowledge, 1,4-benzoquinone is the first irreversible inhibitor reported in tobacco smoke. Further *in vivo* studies on the tobacco MAO inhibitors are required to assess their contribution to MAO inhibition.

6.2.2. Inhibitors responsible for MAO inhibition

The findings in Chapter 4 show that MAO inhibition by phenolic compounds from tobacco smoke is irreversible for human MAO A. Among the phenolic compounds, 4-methylcatechol showed the highest activity for MAO A (IC_{50} 0.267 μM) after 1h preincubation and displayed potent MAO B inhibition with higher potency compared to norharman. Since it has been suggested that identification of irreversible inhibitors from tobacco smoke is critical to fully identifying the mechanisms responsible for tobacco addiction (Villegier et al., 2007), it may be possible that irreversible MAO inhibition by the phenolic compounds from tobacco smoke may play a role in contribution to the addictive effects of nicotine in smokers.

On the other hand, harman and norharman, have been recognised as major and potent MAO inhibitors in tobacco smoke. Moreover, PET study revealed that MAO A binding density was elevated in the prefrontal cortex and anterior cingulate cortex during acute withdrawal in heavy

smokers and this change in MAO A binding covaried with the decrease in plasma levels of harman (P = 0.01), as well as severity of depression (P = 0.006) (Bacher et al., 2011). The mechanism of MAO A elevation during withdrawal from heavy smoking is unknown, but it is possible that either known or unidentified components in tobacco smoke may contribute to MAO A level changes in smokers.

6.2.3. MAO reversibility

The findings in Chapters 4 and 5 show that phenolic compounds from tobacco smoke are irreversible MAO inhibitors, while two PUFAs from tobacco smoke are reversible MAO inhibitors.

Previously, phenolic compounds have been reported as reversible MAO inhibitors (Zhang et al., 2019). A recent review of MAO inhibitors in tobacco or tobacco smoke have described that phenols and phenolic acids with MAO inhibitory activity are reversible and selective inhibitors primarily of MAO A (Hong et al., 2022). To our knowledge, we report for the first time that hydroquinone and catechols from tobacco smoke are irreversible MAO inhibitors and the findings may explain the irreversible inhibitory activity of cigarette smoke.

6.2.4. Mode of MAO inhibition

Phenolic compounds are known as different types of reversible MAO inhibitors including competitive, non-competitive, or mixed-type MAO inhibitors (Badavath et al., 2016; Gidaro et al., 2016; Kong et al., 2004; Lee et al., 2001; Tao et al., 2005). The findings in Chapters 4 revealed that phenolic compounds such as catechols and hydroquinone from tobacco smoke are irreversible MAO inhibitors. Catechols and hydroquinone from tobacco smoke may be converted to benzoquinones and form a covalent adduct with MAO enzymes. 1,2-Benzoquinones (o-quinones) is produced from a range of catechols (Bolton et al., 2018), while 1,4-benzoquinone (*p*-quinone) is also produced via autooxidation of hydroquinone in aqueous cigarette tar extracts or via *in vivo* oxidation in living organisms (Wooten et al., 2006). 1,4-Benzoquinones may react with the reduced FAD cofactor to form covalent adducts (Mostert et al., 2017), but further structural studies are needed to define the MAO sites at which 1,4-benzoquinones react and the potential mechanism by 1,4-benzoquinones.

On the other hand, α-linolenic acid and linoleic are potent and reversible MAO inhibitors in tobacco smoke. The Hill coefficient for linoleic acid for MAO A inhibition proposes positive cooperativity which means the ligand binding to one active site can increase the ligand binding

affinity to the other active sites. Moreover, LB slope replots and Dixon plots are nonlinear, these nonlinearities might correspond to the formation of the ternary MAO–linoleic $acid_2$ species (EII). Molecular docking was performed to study the potential formation of the ternary MAO B–linoleic $acid_2$ species (EII). Molecular simulation for MAO B–linoleic acid complex to linoleic acid suggests that ternary complexes [MAO B–linoleic $acid_2$ species (EII)] may be formed. This study helps to elucidate the binding mode and inhibitory potency of linoleic acid towards MAO enzymes. In addition, the second inhibitor-binding site may be an attractive target for drug discovery of new and potent MAO inhibitors.

6.3. Limitations

The limitations for the research conducted in this PhD study are discussed in detail in each relevant chapter. The main limitations are as follows:

- The main limitation for the review (Chapter 3) was the small number of studies investigating several compounds that display MAO inhibition. Therefore, MAO inhibitors were crossmatched with known tobacco and tobacco smoke components. This review focused on components in tobacco and tobacco smoke with MAO inhibitory activity related to nicotine addiction and does not discuss the environmental factors such as smoking cues, smoking friends, stress, and product marketing or other factors that affect susceptibility involve age, sex, genetic preposition, psychiatric disorder and substance abuse.
- One of the challenges encountered in the MAO inhibition study (Chapter 4 and 5) was the intrinsic fluorescence of the sample itself. Fluorescent signals of 4-hydroxyquinoline, the product of kynuramine deamination, can be disturbed by phenolic compounds with fluorescence when measured by the microplate reader. We found that phenolic compounds showed strong fluorescence at concentration ranging from 100–300 μM. To remove the intrinsic fluorescence of the sample, we included controls with inhibitor and buffer only in the MAO assay. Furthermore, HPLC-DAD analysis can be used to avoid this interferences with 4-hydroxyquinoline (Herraiz et al., 2018).
- One of the challenges encountered in the Docking study with Autodock Vina (Chapter 5) was a rigid receptor. The use of a rigid receptor decreases the size of the conformational space (Forli et al., 2016). Future research should use flexible side-chain docking with AutoDock Vina. In addition, another challenge for AutoDock Vina is a

lack of support for modeling explicit water molecules. Water molecules frequently mediate interactions between biological molecules. AutoDock Vina 1.2.0, a new version, has been suggested (Eberhardt et al., 2021).

6.3. Future Directions

The important issues or queries addressed in the current thesis are summarized as follows, which should be explored in further studies.

6.3.1. Studies on the MAO inhibitors

Further studies on the MAO inhibitors in tobacco smoke are needed to evaluate their contribution to MAO inhibition in smokers, and their behavioural effects. If specific MAO inhibitors are proven to promote addiction to smoking, they could be used with nicotine to develop a more effective form of nicotine replacement therapy for smoking cessation and help many smokers quit smoking.

6.3.2. Studies on the low nicotine cigarettes

The FDA authorized the marketing of very low nicotine cigarettes (Food and Drug Administration, 2021), but these tobacco products will still include MAO inhibitors. Since MAO A inhibition, but not MAO B, is responsible for increasing nicotine self-administration at low doses (Smith et al., 2016), further research on the MAO A inhibitors in tobacco smoke, should be conducted in case there is also a need for regulating the levels of these inhibitors. It is not known what level of MAO A inhibitors could shift the threshold nicotine dose for reinforcement enhancement or whether this depends on the tobacco type.

6.3.3. Studies on the flavoring chemicals in e-liquids

Future studies to explore the reinforcement effects of flavoring compounds in tobacco products or alternative tobacco products such as electronic cigarettes (e-cigarettes) are required. Truman et al. (2019) found that flavoured e-liquids contained a broad range of MAO inhibitory activity and identified vanillin and ethyl vanillin as active compounds. It is likely that other flavoring chemicals in e-liquids may have MAO inhibitory activities and have potential to increase the reinforcing effects of nicotine. This aspect of e-cigarettes may require ongoing monitoring.

6.3.4. In silico studies on the MAO inhibitors from tobacco smoke

MAO inhibition by components in tobacco and tobacco smoke were reported in this thesis (Chapter 3). Further in silico studies may be performed using on-line software such as SwissADME (Daina et al., 2017) and PreADMET (Lee et al., 2003) (Appendices 4-5)

6.3.5. Studies on the MAO-adduct characterisation

The findings from reversibility test (Chapter 4) showed that human MAO A is irreversibly inactivated by the phenolic compounds from tobacco smoke. It is possible that 1,2-benzoquinones (*o*-quinones) from catechols may form covalent adducts with the FAD cofactor of MAO. Future studies could explore potential mechanism of MAO inactivation using mass spectrometry. It is reported that mass spectrometry enables the characterisation of catechol quinone-derived protein adducts (Chen & Li, 2019). Further studies may include MAO-adduct characterisation, MAO-adduct structure elucidation, and chemical mechanism of MAO-irreversible inhibition by new inhibitors.

6.3.6. Therapeutic effects of novel MAOIs in Parkinson's disease

The findings in Chapter 4 suggest that catechols may play a role in the low incidence of Parkinson's disease in people who smoke. We also suggested that catechols may be potential leads for dual MAO B/COMT inhibitors which are used as adjuvant drugs to levodopa-associated motor complications in patients with Parkinson's disease (Jankovic & Stacy, 2007). Future research could evaluate catechols as MAO B inhibitors whether they prevent onset of Parkinsonian symptoms in animal models, and whether they show potent inhibition for COMT.

6.3.7. Therapeutic effects of novel MAOIs in helping smoking cessation

Further studies of the novel MAOIs are needed to examine their contribution to nicotine self-administration at low doses of nicotine. It has been suggested that the reinforcing value of low doses of nicotine may be increased by cigarette smoke components that inhibit MAO A (Smith et al., 2016). If the novel MAOIs are proven to promote addiction to smoking, they could be used as a more effective form of nicotine replacement therapy for smoking cessation and help people who smoke stop smoking.

6.3.8. Studies on the n–3 PUFAs and n–6 PUFAs

The findings in Chapter 5 show that α-linolenic acid and linoleic acid from tobacco smoke are reversible and selective MAO A inhibitors. Investigations should be explored to establish

whether or not other n–3 PUFAs and n–6 PUFAs act as MAO inhibitors. In addition, future research is required to determine a possible link between MAO A inhibitory activity of n–3 PUFAs and antidepressant activity. This research will contribute to a better understanding of the pathophysiology of depression. There is growing evidence indicating that n–3 PUFAs play a part in depression (Deacon et al., 2017). n–3 PUFAs are considered to have a positive effect on depression due to the changes to cell membrane structure and function that specifically affect cell communication, neurotransmitter activities and inflammatory processes (Appleton et al., 2021).

6.4. Concluding Remarks

The overall research aim for this thesis was to characterise six novel MAOIs identified from tobacco smoke and examine their interaction with MAO A and MAO B. The results from this PhD project showed that six novel MAOIs demonstrated potent inhibition of human MAO A and MAO B. It has long been suggested that tobacco smoke results in irreversible MAO inhibition. The catechols and hydroquinone are irreversible inhibitors and to the best of our knowledge, these are the first irreversible phenolic MAO inhibitors to be reported. These results suggest that irreversible MAO inhibition by catechols and hydroquinone from tobacco smoke may play a role in contribution to the addictive effects of nicotine and the low incidence of Parkinson's disease in smokers. On the other hand, two PUFAs, α-linolenic acid and linoleic acid are reversible and selective MAO inhibitors. To our knowledge, this is the first PUFA MAO A inhibitors to be reported. The results suggest that reversible MAO inhibition by α-linolenic acid and linoleic acid from tobacco smoke may plays a role in the lower MAO levels or activity in smokers. Further studies of six novel MAOIs are needed to assess their contribution to nicotine self-administration of nicotine at low doses. Further research is required in case there is a need for regulating the levels of these inhibitors. It is unknown what level of these inhibitors could shift the threshold nicotine dose for reinforcement enhancement. In conclusion, these findings provide a foundation from which to investigate whether MAO inhibitors from tobacco smoke may influence tobacco dependence directly or indirectly and have pharmaceutical possibilities, perhaps in smoking cessation, or in relief of anxiety or depression or treatment of Parkinson's and Alzheimer disease.

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Appendices

Appendix 1 – List of research outputs

Peer-reviewed Journal (Review)
1. Hong, S.W., Teesdale-Spittle, P., Page, R., Truman, P., 2022. A review of monoamine oxidase (MAO) inhibitors in tobacco or tobacco smoke. Neurotoxicology, 93, 163-172. doi: 10.1016/j.neuro.2022.09.008. 2. Hong, S.W., Teesdale-Spittle, P., Page, R., Ellenbroek B, Truman, P., 2022. Biologically active compounds present in tobacco smoke: Potential interactions between smoking and mental health. Front Neurosci. 16:885489. doi: 10.3389/fnins.2022.885489.
Peer-reviewed Journal (Research)
1. Hong, S.W., Heydari, A., Wilson P., Teesdale-Spittle, P., Page, R., Northcote, P.T., Keyzers, A.K., Truman, P., Characterisation of irreversible monoamine oxidase (MAO) inhibitors in tobacco smoke. Neurotoxicology (in prep.). 2. Hong, S.W., Heydari, A., Wilson P., Teesdale-Spittle, P., Page, R., Northcote, P.T., Keyzers, A.K., Truman, P., Alpha-linoleic and linolenic acids : Bioassay-guided isolation and biological activities as MAO (Monoamine Oxidase) inhibitors from tobacco smoke. Neurotoxicology (in prep.).
Awards
1. Recipient of the Massey University Foundation Grant, 2022/2023.

Appendix 2 – Statement of contribution doctorate with publications/manuscripts

Appendix 2.1 – Chapter 2 published article

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MINI REVIEW
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Biologically Active Compounds Present in Tobacco Smoke: Potential Interactions Between Smoking and Mental Health

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Tobacco dependence remains one of the major preventable causes of premature morbidity and mortality worldwide. There are well over 8,000 compounds present in tobacco and tobacco smoke, but we do not know what effect, if any, many of them have on smokers. Major interest has been on nicotine, as well as on toxic and carcinogenic effects and several major and minor components of tobacco smoke responsible for the negative health effects of smoking have been elucidated. Smokers themselves report a variety of positive effects from smoking, including effects on depression, anxiety and mental acuity. Smoking has also been shown to have protective effects in Parkinson's Disease. Are the subjective reports of a positive effect of smoking due to nicotine, of some other components of tobacco smoke, or are they a manifestation of the relief from nicotine withdrawal symptoms that smoking provides? This mini-review summarises what is currently known about the components of tobacco smoke with potential to have positive effects on smokers.

Keywords: tobacco dependence, nicotine, tobacco smoke components, monoamine oxidase inhibition, mental health, Parkinson's Disease

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INTRODUCTION

Smoking is a major cause of preventable premature death and disability, believed to cause six million deaths worldwide each year with smokers, on average, losing 10 years of their lives (West, 2017). The negative health effects of prolonged smoking are well established. Smoking primarily damages lung and cardiovascular health, as well as impacting negatively on every organ of the body (U.S. Department of Health and Human Services., 2004). The reason people keep smoking, despite knowing that this habit is likely to eventually kill them, is that smoking is addictive (Henningfield and Fant, 1999). The major addictive component of tobacco smoke is nicotine, but it is increasingly evident that tobacco dependence is multi-faceted (West and Cox, 2021). Nicotine in laboratory tests is much less addictive than the lived experience of smokers would suggest (Jain, 2003; Balfour, 2009). The explanations are varied, ranging from societal and behavioural influences (Moolchan et al., 2003), to strong cue

association and cognitive effects (Sacco et al., 2004; Chiamulera, 2005) and to the influence of tobacco companies (Hoek et al., 2012).

Smokers themselves report smoking relieves stress and anxiety, and aids concentration (Benowitz, 2010) and smokers with a variety of mental health conditions report using smoking as a form of self-medication (Leonard et al., 2001; Aubin et al., 2012). Unsurprisingly, the interpretation of these reports varies widely. However, smoking is proven to have protective effects in Parkinson's Disease (Castagnoli and Murugesan, 2004; Gigante et al., 2017), with smoking having a well-established neuroprotective effect against this disease (Veljkovic et al., 2018).

A strong theme coming through the literature is that components in tobacco smoke other than nicotine, possibly monoamine oxidase (MAO) inhibitors, may enhance tobacco dependence, or may have positive effects on mood (Fowler et al., 2003; Rose, 2006; Hogg, 2016; Harris et al., 2020). Thus, apart from the effects of nicotine, the observed difficulty that people have in stopping smoking could be influenced by other chemical components of the tobacco smoke. Such interpretations are controversial. For instance, the high rate of relapse in smokers attempting to stop smoking is attributed to relief from nicotine withdrawal, rather than thinking of a positive effect on mood or concentration from smoking as reinforcing nicotine dependence (Moylan et al., 2013).

It is completely accepted that tobacco smoking is a harmful habit, due to the many toxic and carcinogenic components of the smoke. However, this review aims to focus attention on areas of the literature suggesting pharmacological drivers behind tobacco dependence other than the immediate effects of nicotine in inducing dependence, and our current understanding of the short-term effects of tobacco smoke components on smokers. Are some of these effects positive, as in neuroprotection against Parkinson's Disease? Are there known tobacco components which could have a positive effect?

NICOTINE

Nicotine acts at the nicotinic acetylcholine receptors (nAChRs) to cause flow-on effects in specific areas of the brain. These nAChRs are believed to be important in coordinating brain responses, by action of the natural transmitter, acetylcholine (ACh). Nicotine binds to nAChRs mimicking the action of ACh and altering responses within the brain (Balfour, 2009).

The key addictive response is stimulation of nAChRs in the ventral tegmental area of the brain, which causes the release of dopamine in the nucleus accumbens, believed to be central to the development of all addictive responses (Di Chiara and Imperato, 1988). Nicotine's pharmacology has been well reviewed by others (Benowitz, 1996; Laviolette and van der Kooy, 2004) as have the complexities of the nicotinic receptors in the brain (Albuquerque et al., 2009; Wu and Lukas, 2011). The nAChRs belong to the ionotropic family of receptors, with five proteins forming a channel. Seventeen different proteins have been identified, leading to a wide diversity of nicotinic receptor subtypes. Thus the diversity of nicotinic receptor types and their varied localisation within the brain allows for much more nuanced and complex responses to nicotine than simple dopamine release.

Several milligrams of nicotine are present in each gram of tobacco, and nicotine reaches around 0.2 micromolar concentrations in the blood of smokers, sufficient to cause nAChR responses (Alkondon et al., 2000). Nicotine concentrations rise rapidly when tobacco smoke is inhaled, reaching the brain in under two minutes. It then dissipates slowly, having a half-life of around 2 h (Benowitz, 2009). As nicotine brain concentrations fall, in the addicted smoker, cravings for nicotine begin, leading to smokers repeating the experience. Many of the effects of smoking tobacco (dopamine release and dependence, and withdrawal effects and relapse back to smoking) can be related back to these key effects of nicotine on the brain.

As the major pharmacologically active component of tobacco smoke, nicotine has also been investigated to see whether it can cause other effects, reported from smoking, such as relief from anxiety and depression, improved concentration and symptom control in Schizophrenia, Parkinson's Disease, Attention Deficit Hyperactivity Disorder, and Alzheimer's Disease (Mihailescu and Drucker-Colin, 2000; Newhouse et al., 2004a; Veljkovic et al., 2018).

Nicotine and Cognition/Concentration

In utero exposure and exposure of children to tobacco smoke are both believed to interfere with cognitive development, causing deleterious effects on attention span and ability to concentrate, in children (Alhowail, 2021; Hajdusianek et al., 2021). With some caveats as to the strength of the evidence (Chan et al., 2020) it is generally accepted that smoking in pregnancy is bad for the unborn child, although there is some doubt as to whether nicotine causes all of the problems noted (Baler et al., 2008; Chan et al., 2020).

In adults, however, nicotine is believed to have a positive effect on mental acuity (Conley et al., 2021; Nop et al., 2021). Again, this has been suggested by studies in animals and in humans (Kumari et al., 2003; Newhouse et al., 2004b), and trials of the effect of nicotine in older adults have produced some evidence of benefit. A recent meta-analysis by Majdi and coworkers suggested that nicotine has a moderate but positive effect on attentional ability in healthy non-smoking adults (Majdi et al., 2021) and it has been suggested that nicotine could be used to treat late-life depression, with part of this action being mediated by effects on cognition (Gandelman et al., 2018).

Smoking as a long-term enhancer of cognition is not recommended, however, since smoking is a risk factor for vascular dementia (Lopez-Arrieta et al., 2001) as well as for many other health conditions.

Nicotine and Anxiety/Depression

The suggestion that nicotine can relieve stress and help with anxiety and depression is controversial. The key argument is whether the positive effects noted by smokers are real and act as a reinforcer of tobacco dependence by improving mood (Pomerleau et al., 1984; Choi et al., 2015) or whether nicotine dependence causes depression and/or anxiety over time, with

relief of cravings being misread by smokers as relief from the related mood disorders (Moylan et al., 2013; Molas et al., 2017). A systematic review by Fluharty and co-workers (Fluharty et al., 2017) found evidence for causation in both directions and suggested the need for more studies. More recent work tends to look at effects of transdermal nicotine, and has found potential for nicotine to be used in cases of major depressive disorder (Janes et al., 2018), and for relief of later life depression (Conley et al., 2021).

Nicotine and Schizophrenia

Schizophrenia is also strongly associated with tobacco smoking, however, the direction of causation is controversial. Scott and coworkers examined the evidence that smoking might cause schizophrenia and suggested nicotine as the causative agent (Scott et al., 2018). Others have suggested that nicotine's effects within the brain might give relief from the symptoms of schizophrenia, providing motivation to continue smoking (Postma et al., 2006). It is possible that nicotine's positive effect on cognition is the mediating mechanism for this (Waterhouse et al., 2018).

Nicotine and Alzheimer's Disease

The possibility that nicotine might be useful in treating early stages of Alzheimer's Disease has been studied for some years. As with Parkinson's Disease, smokers are under-represented among those with Alzheimer's Disease. Impaired cholinergic function is an early feature of Alzheimer's Disease and nAChR agonists have been suggested as potentially helpful in ameliorating symptoms (Albuquerque et al., 2001). Transdermal nicotine has been used in trials with positive effects on attention and other cognitive measures (Newhouse et al., 2004b). Nicotine has also been shown to be effective in reducing behavioural and synaptic plasticity deficits in a rat model of Alzheimer's disease (Esteves et al., 2017). In a separate line of enquiry nicotine is also thought to delay formation of amyloid plaque (Zhang et al., 2006), giving another mechanism by which smoking (nicotine) could be helpful in delaying Alzheimer's Disease onset.

Nicotine and Parkinson's Disease

While smoking is well known to be protective against Parkinson's Disease (Gigante et al., 2017), the causative agents in this case are likely to include both nicotine, though its action on dopaminergic pathways (Thiriez et al., 2011), and monoamine oxidase inhibitors (Castagnoli and Murugesan, 2004). Additionally, Kardani et al. (2017) have demonstrated that nicotine can slow the formation of α-synuclein fibrils. However, although nicotine appeared to be neuroprotective in Parkinsonian animals it had no effect in restoring damage in the same experimental system (Huang et al., 2009) and was not significantly effective in Phase II clinical trials (Villafane et al., 2018).

MINOR TOBACCO ALKALOIDS

Like nicotine, the other nicotine analogues found in tobacco smoke have also been found to act on the nicotinic receptors. The "minor alkaloids" in tobacco smoke other than nicotine include nornicotine, myosmine, cotinine, anabasine and anatabine. Where nicotine is found at around 10 milligrams per cigarette in tobacco, these minor alkaloids are found in microgram per cigarette amounts (Smith et al., 2015).

Several groups have looked at the effect of these alkaloids *in vivo* (Harris et al., 2015; Marusich et al., 2017; Tan et al., 2022). Such studies have found that the minor tobacco alkaloids can partially substitute for nicotine in behavioural tests but have lower potency than nicotine itself. The different alkaloids are not equivalent in their behavioural effects. Differences in binding to different nAChR variants between the various nicotinic alkaloids would allow for differences in mode of action and potency even if all act through binding nAChRs. The combination of lower potency and lower concentration in tobacco make it unlikely that these alkaloids have significant effects on smoker behaviour.

Cotinine, a breakdown product of nicotine, has relatively long half-life in the blood [c.a. 16 h compared to c.a. 2 h for nicotine (Hukkanen et al., 2005)] and reaches low micromolar concentrations in a smoker's blood, several-fold higher than the concentration of nicotine. It may, therefore, be present in sufficient amounts to have a modulating effect on smoker behaviour, particularly for heavy smokers. Cotinine is a weak agonist of nAChRs, binding less strongly than nicotine (Tan et al., 2021) but has recently been shown to support self-administrative behaviour (Tan et al., 2022) although less robustly than nicotine. Cotinine upregulates the α7nAChRs and this activity is neuroprotective to glial cells (Iarkov et al., 2021). Cotinine also slows down the formation of α-synuclein fibrils, with a potency similar to that of nicotine, and may also have positive cognitive benefits.

Table 1 summarises the suggested beneficial effects of nicotine and cotinine.

MONOAMINE OXIDASE INHIBITORS

Monoamine oxidase inhibitors (MAOIs) in tobacco smoke have long been regarded as having potential significance in modulating the effects of smoking on the brain (Fowler et al., 2003; Dome et al., 2010; Hogg, 2016). Monoamine oxidase (MAO) enzymes in the brain are responsible for clearance of brain transmitters, notably dopamine, serotonin, adrenaline and noradrenaline (Lewis et al., 2007). Since inhibition of MAO activity will lead to reduced clearance of neurotransmitters such as dopamine it is suggested that MAOIs in the tobacco smoke might enhance the dopamine reward from nicotine and the addictiveness of smoking.

A variety of experimental evidence has been produced showing that MAO inhibition enhances behavioural responses to nicotine in rats (Guillem et al., 2005; Villegier et al., 2006, 2011; Smith et al., 2016b; Harris et al., 2020) but the extension to human smoking behaviour is less clear. MAO activity is well known to be reduced in smokers (Fowler et al., 1996a,b) with the inhibition being believed to be irreversible (Yu and Boulton, 1987). The time course of recovery of activity after smoking cessation extends

TABLE 1 | Nicotine, cotinine, and monoamine oxidase inhibitors in tobacco and tobacco smoke: potential positive effects.

Smoke component	Chemical structure	Mechanism	Disease state affected	References
Nicotine		nAChR activation	Cognitive improvement	Newhouse et al., 2004b; Majdi et al., 2021
Nicotine		↓ α-synuclein fibril formation: (cognition ↑)	Parkinson's disease	Kardani et al., 2017; Villafane et al., 2018
Nicotine		↓Amyloid ß-peptide aggregation: (cognition↑)	Alzheimer's disease	Zhang et al., 2006; Esteves et al., 2017
Nicotine		Cognition↑	Schizophrenia	Waterhouse et al., 2018
Nicotine		Cognition ↑	Depression	Gandelman et al., 2018; Conley et al., 2021
Cotinine		↓ α-synuclein fibril formation, neuroprotection	Parkinson's disease Alzheimer's disease	Terry et al., 2005; Riveles et al., 2008; Iarkov et al., 2021;
Naphthoquinones		MAO inhibition, neuroprotection (nitric oxide control)	Parkinson's disease	Venkatakrishnan et al., 2009
Harman/Norharman		MAO inhibition	Antidepressant	Farzin and Mansouri, 2006
Harman/Norharman		MAO inhibition	Antianxiolytic	Smith et al., 2013
2,3,6-Trimethyl-1,4-naphthoquinone		MAO inhibition	Neuroprotection, Parkinson's disease	Sari and Khalil, 2015

over several weeks after smoking cessation, consistent with the equivalent recovery time from inhibition by known irreversible MAO inhibitory drugs (Fowler et al., 2003). Although epigenetic (Launay et al., 2009) and microRNA (Higuchi et al., 2018) mechanisms for MAO activity reduction have been suggested, direct inhibition by components of tobacco smoke as the major cause of the observed reduction remains a likely mechanism by which MAO inhibition is accomplished in smokers.

The known MAOIs in tobacco smoke include the ß-carbolines harman and norharman, α-naphthylamine, farnesyl acetone and tetrahydroisoquinolines (TIQ's) (Lewis et al., 2007; Hogg, 2016). Harman and norharman have been proposed as the major contributors to the observed MAOI activity in tobacco smoke, causing the observed decrease in MAO activity (Rommelspacher et al., 2002; Herraiz and Chaparro, 2005), with the discrepancy between the amounts of ß-carboline measured in smokers, and the inhibition observed being ascribed to their accumulation in platelets (Rommelspacher et al., 2002) and in brain (Fekkes and Bode, 1993). However, harman and norharman make up less than 1/10th of the total direct MAO inhibitory activity in tobacco smoke (Truman et al., 2017) so the opportunity for other MAOIs in tobacco smoke to contribute substantially to the MAO activity reduction seen in smokers must exist. No irreversible MAO inhibitors have yet been reported from tobacco or tobacco smoke.

The question of whether MAO inhibitors in tobacco smoke can affect behaviour remains unresolved. As well as the potential for effects on addiction, MAO enzymes are drug targets for a variety of neurological disorders including depression, mood, anxiety, attention deficit hyperactivity, Tourette's syndrome, Parkinson's disease and Alzheimer's disease (Sharama, 2016; Borroni et al., 2017). Of the known MAO inhibitors in tobacco smoke, high concentrations of harman and norharman can affect responses to nicotine (Harris et al., 2020) and may act as antidepressants (Farzin and Mansouri, 2006; Smith et al., 2013) in animals. However, when used in amounts relevant to smokers, they were not seen to affect rat self-administration of nicotine (Smith et al., 2015) and no pharmacological effects have been reported from the known tobacco MAO inhibitors at physiologically relevant concentrations. In contrast, use of tobacco smoke extracts in self-administration or intracranial self-stimulation experiments has been found to affect responses to nicotine (Harris et al., 2010; Costello et al., 2014; Brennan et al., 2015). This discrepancy may be in part because the full range of tobacco smoke MAO inhibitors has not yet been identified.

It has also been suggested that further MAO inhibitory activity is formed from smoke components in the body. Acetaldehyde is formed during tobacco combustion, from the sugars in the plant material, and from sugars added as humectants and flavour additives during tobacco and cigarette manufacture. Acetaldehyde is typically present in cigarette smoke amounts ranging from 0.6 to over 2 milligrams per cigarette (Seeman et al., 2002). Acetaldehyde yields locomotor stimulation and reinforcing effects (Quertemont and Tambour, 2004) at concentrations higher than those seen in tobacco smoke and enhances self-administration of nicotine at concentrations similar to those in tobacco smoke (Belluzzi et al., 2005), although this finding was not confirmed by Smith and co-workers in similar trials (Smith et al., 2015).

It is suggested that acetaldehyde acts by reacting with chemicals naturally occurring in the brain to form MAO inhibitors (Talhout et al., 2007). A wide variety of TIQs are formed from acetaldehyde and catecholamines (dopamine, noradrenalin, adrenalin) (Naoi et al., 2004; Patsenka and Antkiewicz-Michaluk, 2004). Of particular note are the cyano derivatives, which inhibit MAO-A and -B with Ki values between 18 and 38 μM (Mendez-Alvarez et al., 1997). A group of tetrahydro-ß-carbolines (THBCs) are also formed from condensation of acetaldehyde and indoleamines (serotonin, tryptamine, tryptophan) (Talhout et al., 2007) and are more potent, inhibiting with Ki values under 10 μM. It seems likely that concentrations of these MAOIs, formed in the body after smoking, are sufficient to have some effect on smoking addiction in humans, as well as in rats, since a randomized double-blind trial of a method to reduce the amount of acetaldehyde entering a smoker's body had some success in encouraging cessation (Syrjanen et al., 2017). Thus far, together with the ß-carbolines, acetaldehyde is a leading candidate as a tobacco smoke component causing monoamine oxidase inhibition in smokers sufficient to modulate tobacco dependence, even though it does not directly cause MAO inhibition. The inhibitors formed from acetaldehyde appear to be reversible (Naoi et al., 2004).

The immediate effect on MAO activity of smoking a single cigarette could not be detected using PET methods (Fowler et al., 2003), whereas longer term effects of continued smoking results in an overall, apparently irreversible reduction of 30–40% in both MAO-A and -B activity, suggesting that long term exposure to tobacco smoke is required for this effect. Until we know which components of tobacco smoke cause the observed inhibition of MAO activity, and their mechanism of action, it will remain difficult to determine the extent, timing and overall effects of MAO inhibition in smokers.

Monoamine oxidase inhibitors in tobacco smoke may well have effects other than enhancing the addictive potential of nicotine. Smith and coworkers (Smith et al., 2016a) have examined the effects of MAO inhibitors and nicotine on brain function, measured using EEG techniques in non-smoking humans. They suggest that MAO inhibition alters brain function leading to lapses in cognition. Nicotine's effect in enhancing cognition is suggested to alleviate this.

Monoamine oxidase inhibitors are a major drug target, of interest for treatment of depression and anxiety, Parkinson's disease and Alzheimer's disease (Sharama, 2016). It has been suggested that the MAOI activity in tobacco smoke may enhance a smoker's mood (Pomerleau et al., 1984; Farzin and Mansouri, 2006; Smith et al., 2013; Choi et al., 2015) independently of their effects on dopamine reward from nicotine, and may contribute to smoker's dependence on tobacco by this indirect route (Arnold et al., 2014). However, rather than a positive effect on mood, it is possible that MAO inhibition serves to intensify withdrawal symptoms (Malin et al., 2013). Relief of withdrawal symptoms then serves to improve mood. This potential interplay of influences is not yet fully understood.

The potential role for MAO inhibition in tobacco dependence has led to suggestions that MAO inhibitors could be used for smoking cessation (Biberman et al., 2003; George and Weinberger, 2008). Current consensus is that, after some promising preliminary results, the known MAO inhibitory drugs are not particularly useful for smoking cessation (Howes et al., 2020).

The proposed effects of MAO inhibitors on smokers are listed in **Table 1**.

OTHER COMPONENTS OF TOBACCO SMOKE

Additional components of tobacco and tobacco smoke which have been associated with beneficial effects are listed in **Table 2**, below. They have been identified as a result of investigations of the active components supporting herbal use, but from plants other than tobacco. These compounds are all known components of tobacco (Rodgman and Perfetti, 2013), however, while their biological activity is of interest, their contribution to the overall effects of tobacco smoking is unknown.

CONCLUSION

It is accepted that nicotine has positive effects on cognition and attention in adults, even though having negative effects in infant and child development. For this reason, nicotine could be helpful in the management of a variety of mental health conditions and provide an explanation for high levels of smoking in disorders such as schizophrenia. Nicotine and its metabolite cotinine both stimulate cholinergic systems, which can be neuroprotective, and may also prevent ß-amyloid fibre aggregation in Alzheimer's disease, and α-synuclein fibre formation in Parkinson's disease. While the long-term overall effect of smoking is deleterious, the same may not be true for nicotine and cotinine. The therapeutic use of cotinine deserves further work, since it is expected to have a lower abuse potential than nicotine but may provide similar neuroprotective and cognitive effects.

The other most significant and potentially beneficial biological activity in tobacco smoke is monoamine oxidase inhibitory activity. This is likely to have an impact on the response to nicotine, and to increase the addictiveness of smoking, both by increasing the dopamine rewards from the nicotine, and by its effects (if any) on mood. The extent to which these effects on mood from smoking are due to relief of nicotine cravings, or directly caused by the modulation of monoamine oxidase enzyme activity in the brain remains uncertain. In this respect it is important to recall that the first generation of antidepressant drugs were selective MAO inhibitors, with some still currently used for treatment of major depressive disorders. This question will only be resolved when the causative agents of the MAO inhibition seen in smokers have been identified, and their effects can be studied independently of the effects of nicotine. It may prove possible to separate immediate MAO inhibitory activity from the long-term irreversible inhibitory factors and assess

TABLE 2 | Additional tobacco smoke components with beneficial biological activity.

Component	Chemical structure	Observed effect	Application	References
Catechol		↓ amyloid-β fibril formation	Alzheimer's disease	Huong et al., 2010
Hydroquinone		↓ a-synuclein fibrillation	Parkinson's disease	Hong et al., 2009
Solanesol		Neuroprotection	Stroke	Rajdev et al., 2020
Limonene		Neuroprotection	Alzheimer's disease	Shin et al., 2020
Cembranoids		Nicotinic activation, neuroprotection	Alzheimer's disease Parkinson's disease	Ferchmin et al., 2009
Quercetin		↓cognitive function ↓ amyloid-β fibril formation	Alzheimer's disease Parkinson's disease	Paula et al., 2019; Khan et al., 2019
Kaempferol		↑ striatal dopamine, SOD and GSH ↓ malondialdehyde	Parkinson's disease	Li and Pu, 2011
Eugenol		↓immobility in forced swim test	Depression	Irie et al., 2004
β-asarone		↑efficacy of memantine ↑cognitive deficit	Alzheimer's disease	Han et al., 2020; Liu et al., 2016; Chang and Teng, 2018
Vanillin		↓ oxidative stress response ↓behavioural impairment	Parkinson's disease	Dhanalakshmi et al., 2016
Ferulic acid		↑ serotonin and norepinephrine ↓immobility in forced swim test ↓brain capillary constriction	Depression Alzheimer's disease	Chen et al., 2015; Wang et al., 2021
Caffeic acid		↓ immobility in forced swim test	Depression	Takeda et al., 2002
Chlorogenic acid		↓mitochondrial disfunction ↓ oxidative stress	Parkinson's disease	Singh et al., 2020
Rutin		↓ immobility in tail suspension test ↓ effect of chronic induced stress	Depression, Anxiety	Parashar et al., 2017; Yusha'u et al., 2017
Naringenin		↓ amyloid-β toxicity ↓ immobility in tail suspension test ↑memory	Alzheimer's disease Depression	Yi et al., 2012; Yang et al., 2014; Md et al., 2018
Naringin		↓mitochondrial disfunction ↑memory	Alzheimer's disease	Sachdeva et al., 2014
Scopoletin		↓ anxiety-like behaviour	Anxiety	Luo et al., 2020
Esculetin		↓ immobility in forced swim test	Depression, Anxiety	Sulakhiya et al., 2016
Quinic acid		↓MAO-B, neuroprotection	Dementia	Liu et al., 2020

SOD, superoxide dismutase; GSH, glutathione peroxidase.

their effects separately once any irreversible inhibitors have been identified.

It is likely that MAO-B inhibitory activity in tobacco smoke, perhaps together with the combined neuroprotective effect of a variety of smoke components may be useful for symptom control in Parkinson's disease, while MAO-A inhibitory activity is more likely to contribute to the alleviation of mood disorders.

While the contribution of biologically active molecules other than nicotine remains to be established it is clear that tobacco smoke contains many components which might have beneficial effects. While this in no way compensates for the overall deleterious effects of smoking, these effects may help explain the strength of tobacco dependence many smokers experience and are worthy of further study, so that we can disentangle the deleterious and beneficial effects, to better help smokers to stop smoking.

AUTHOR CONTRIBUTIONS

PT, RP, and PT-S guided the initial literature search, performed by SH. SH and PT wrote the manuscript. BE added expert advice. All authors contributed to the final shape of the manuscript.

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Neurotoxicology 93 (2022) 163–172

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Review

A review of monoamine oxidase (MAO) inhibitors in tobacco or tobacco smoke

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ABSTRACT

Tobacco smoking is reputed to be the most difficult addiction of all to give up, and nicotine has been noted as the major addictive agent in tobacco smoke. However, research shows that nicotine addiction is due to more than nicotine alone. One hypothesis is that monoamine oxidase (MAO) inhibition from non-nicotinic components in, or derived from, tobacco smoke contributes to nicotine addiction. Harman and norharman, have been recognised as major and potent MAO inhibitors in tobacco smoke, but these two inhibitors together comprise perhaps less than 10% of the total MAO A inhibitory activity in cigarette smoke suggesting other unidentified components may make significant contributions to total inhibitory activity. Therefore, we reviewed an index of the chemical components of tobacco and tobacco smoke and identified those known to be MAO inhibitors. Amongst these inhibitors, phenols and phenolic acids with MAO inhibitory activity are commonly reversible and selective MAO A inhibitors, whereas trans,trans-farnesol, 2-methyl-1,4-naphthoquinone (menadione), 1,4-naphthoquinone, scopoletin, and diosmetin with MAO inhibitory activity are reversible and selective MAO B inhibitors. The compound, 1,4-benzoquinone is an irreversible MAO A inhibitor and to the best of our knowledge, this is the first irreversible MAO A inhibitor to be reported in tobacco smoke. MAO inhibitors have been used clinically to treat depression, anxiety, and Parkinson's disease. The MAO inhibitors identified from tobacco and tobacco smoke and summarized in this review, are potential pharmacological candidates to be investigated further. This review will enhance our knowledge of the way tobacco smoke affects MAO activity in smokers and will also be important in helping to understand nicotine addiction.

1. Introduction

Smoking is reputed to be one of the hardest addictions to give up, yet nicotine by itself has only moderate potential for addiction (Rose, 2006). One hypothesis is that monoamine oxidase (MAO) inhibitors in, or derived from, tobacco smoke decrease the breakdown of neurotransmitters associated with reward pathways, such as dopamine, and synergistically increase the addictive effects of nicotine in smokers (Hogg, 2016) by increasing the effective reward signal.

MAO enzymes are responsible for the oxidative deamination of monoamine substrates in the body and brain, including neurotransmitters such as serotonin, histamine, dopamine, norepinephrine and epinephrine. In humans, there are two isoforms, MAO A and MAO B, with different distributions in the body and the brain and with different substrate specificity (Youdim et al., 2006). Together they are involved in complex behaviour, including drug use as well as personality traits (Harro and Oreland, 2016). The MAO enzymes are also known drug targets: their inhibitors affect mood and have been used to treat depression, panic disorder, social phobia, bipolar, and anxiety (Menkes et al., 2016; Sub Laban and Saadabadi, 2021). MAO B inhibitors are also important in treatment of Parkinson's disease (PD) (Duarte et al., 2020).

Interestingly, positron emission tomography (PET) scans have shown that smokers have lowered levels of MAO A (28%; $P < 0.0003$) and MAO B (40%; $P < 0.0002$) activity in the brain relative to non-smokers (Fowler et al., 1996a, 1996b). Fowler et al. (2003) proposed that smoke-induced MAO inhibition requires prolonged exposure and is not reversed quickly. An earlier *in vitro* study had reported that unidentified components of cigarette smoke showed irreversible inhibition (Yu and Boulton, 1987). Hogg (2016) has suggested that several tobacco-derived substances may cause the MAO inhibition seen in smokers through additive or synergistic effects. Previous studies were conducted to identify and characterize MAO inhibitors in tobacco and tobacco smoke and

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Appendix 2.5 – Chapter 4 DRC 16 form

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GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.	
Student name:	Sa Weon Hong
Name and title of main supervisor:	Dr. Penelope Truman
In which chapter is the manuscript/published work?	Chapter 4
What percentage of the manuscript/published work was contributed by the student?	80%
Describe the contribution that the student has made to the manuscript/published work: The candidate was actively involved in the preliminary studies, design, performed experiments, analyzed the data, and wrote the manuscript.	
Please select one of the following three options:	
○	The manuscript/published work is published or in press Please provide the full reference of the research output:
○	The manuscript is currently under review for publication Please provide the name of the journal:
◉	It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Student's signature:	SWHong	Main supervisor's signature:	Penelope Truman

This form should be placed at the beginning of each relevant thesis chapter.

Appendix 2.6 – Chapter 5 DRC 16 form

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UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.	
Student name:	Sa Weon Hong
Name and title of main supervisor:	Dr. Penelope Truman
In which chapter is the manuscript/published work?	Chapter 5
What percentage of the manuscript/published work was contributed by the student?	80%
Describe the contribution that the student has made to the manuscript/published work: The candidate was actively involved in the preliminary studies, design, performed experiments, analyzed the data, and wrote the manuscript.	
Please select one of the following three options:	
○	The manuscript/published work is published or in press Please provide the full reference of the research output:
○	The manuscript is currently under review for publication Please provide the name of the journal:
◉	It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Student's signature:	SWHong	Main supervisor's signature:	Penelope Truman

This form should be placed at the beginning of each relevant thesis chapter.

Appendix 3 – Molecular docking studies

Appendix 3.1 – Molecular docking results of α-linolenic acid and linoleic acid with hMAO B.

Compound	*In vitro* IC_{50} (μM) ± SEM	ΔG (kcal mol^{-1})	*K*i (μM)	Amino acid	Distance (Å)	Type	Category
α-Linolenic acid (α-Linolenate)	31.9 ± 5.2	-8.1	1.147	Tyr435	2.47	Conventional	Hydrogen Bond
				Leu171	4.03	Alkyl	Hydrophobic
				Cys172	5.19	Alkyl	Hydrophobic
				Leu171	4.40	Alkyl	Hydrophobic
				Ile199	4.75	Alkyl	Hydrophobic
				Leu171	5.13	Alkyl	Hydrophobic
				Ile199	4.40	Alkyl	Hydrophobic
				Ile316	5.13	Alkyl	Hydrophobic
				Pro104	4.81	Alkyl	Hydrophobic
				Leu164	4.70	Alkyl	Hydrophobic
				Ile199	4.59	Alkyl	Hydrophobic
				Tyr60	5.22	Pi-Alkyl	Hydrophobic
				Phe103	4.96	Pi-Alkyl	Hydrophobic
				Trp119	4.73	Pi-Alkyl	Hydrophobic
				Tyr326	4.81	Pi-Alkyl	Hydrophobic
				Phe343	4.79	Pi-Alkyl	Hydrophobic
				Tyr398	5.13	Pi-Alkyl	Hydrophobic
Linoleic acid (Linoleate)	71.7 ± 11.0	-8.1	1.147	Tyr435	3.91	Pi-Anion	Hydrophobic
				FAD1502	3.91	Pi-Sigma	Hydrophobic
				Leu171	4.20	Alkyl	Hydrophobic
				Leu171	4.03	Alkyl	Hydrophobic
				Leu171	4.77	Alkyl	Hydrophobic
				Ile199	4.21	Alkyl	Hydrophobic
				Pro104	5.09	Alkyl	Hydrophobic
				Leu164	4.14	Alkyl	Hydrophobic
				Trp119	5.10	Pi-Alkyl	Hydrophobic
				Tyr326	4.33	Pi-Alkyl	Hydrophobic
				Tyr326	4.87	Pi-Alkyl	Hydrophobic
				Tyr398	5.37	Pi-Alkyl	Hydrophobic
				Tyr398	4.24	Pi-Alkyl	Hydrophobic

RMSD (Å): MAO B/Safinamide (1.288). The RMSD (Root Mean Square Deviation) cutoff of 2Å is a criterion used for the correct bound structure prediction.

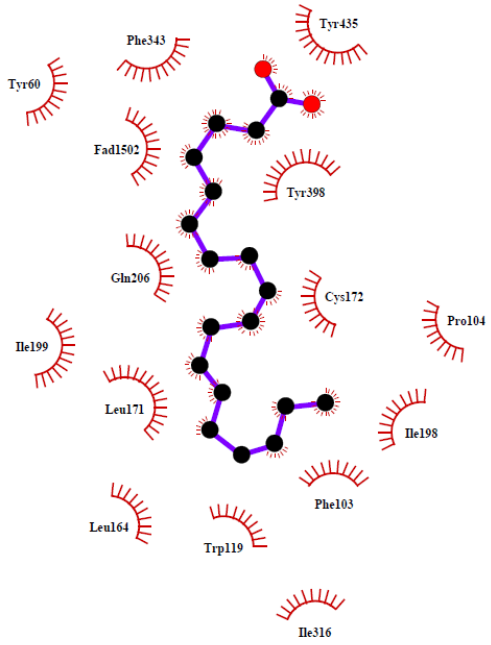
Appendix 3.2 – Molecular docking results of selected phenolic compounds and fatty acids in tobacco smoke.

	MAO A			MAO B			
Compound	*In vitro* IC_{50} (μM) ± SEM	Computational ΔG (kcal mol^{-1})	Computational K_i (μM)	*In vitro* IC_{50} (μM) ± SEM	Computational ΔG (kcal mol^{-1})	Computational K_i (μM)	SI[a]
Catechol	34.3 ± 6.5			46.4 ± 8.2	-5.5	92.5	1.35
4-Methylcatechol	14.1 ± 2.6			32.1 ± 5.5	-5.9	47.1	2.28
4-Ethylcatechol	12.6 ± 3.6			17.1 ± 5.1	-6.3	23.97	1.36
4-Vinylcatechol					-6.5	17.1	
Hydroquinone	15.2 ± 2.6			20.5 ± 4.2	-5.7	66.03	1.35
Linoleic acid (Linoleate)	23.8 ± 8.7			71.7 ± 11.0	-8.1	1.147	3.01
α-Linolenic acid (α-Linolenate)	15.7 ± 1.5			31.9 ± 5.2	-8.1	1.147	2.03
Harman*	0.34	-8.6	0.493				
Norharman	4.23 ± 1.16	-8.0	1.358	5.17 ± 0.35	-8.2	0.969	1.22
Harmine*	0.107	-8.9	0.297				
Safinamide					-10.3	0.279	
Selegiline*	67.25 ± 1.02						
Clorgyline*	0.0044 ± 0.462						

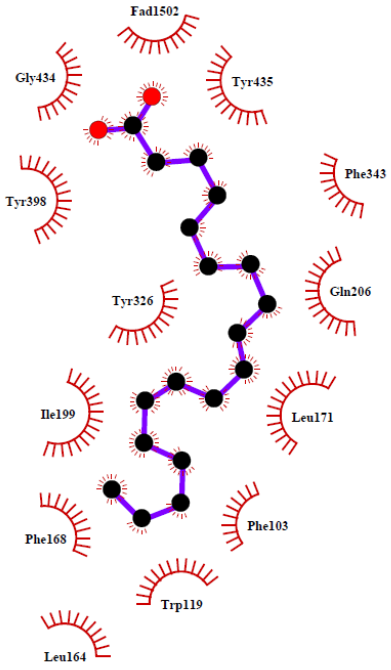
*IC_{50} data from References (Harman: K_i 55.54 ± 5.3 nM, Norharman: IC_{50} 6.47 ± 0.28, K_i 1.2 ± 0.18 μM, Safinamide: K_i MAO A 365.0 ± 18.7 μM, MAO B 0.45 ± 0.13 μM). The selectivity index ([a] SI) is defined as the ratio of [IC_{50} (MAO B)]/[IC_{50} (MAO A)]. RMSD (Å): MAO A/Harmine_$7H_2O$ (1.314), MAO B/Safinamide (1.288). The RMSD (Root Mean Square Deviation) cutoff of 2Å is a criterion used for the correct bound structure prediction.

Appendix 3.3 – Docking interactions of alpha-linolenate (A) and linoleate (B) with hMAO B.

A

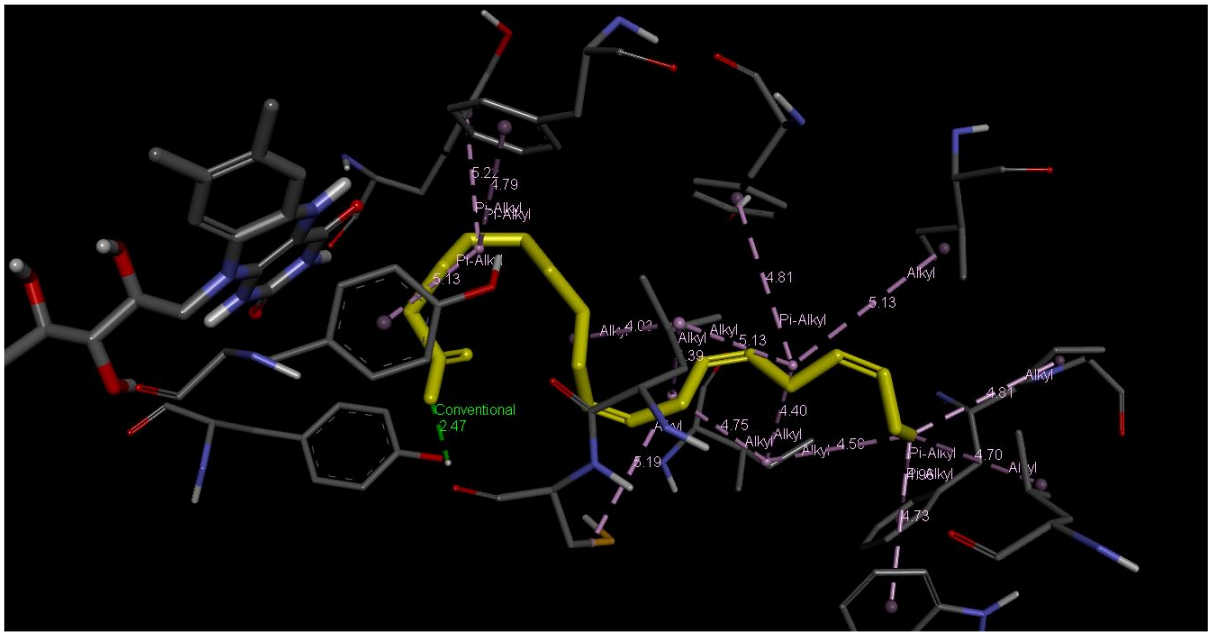


B

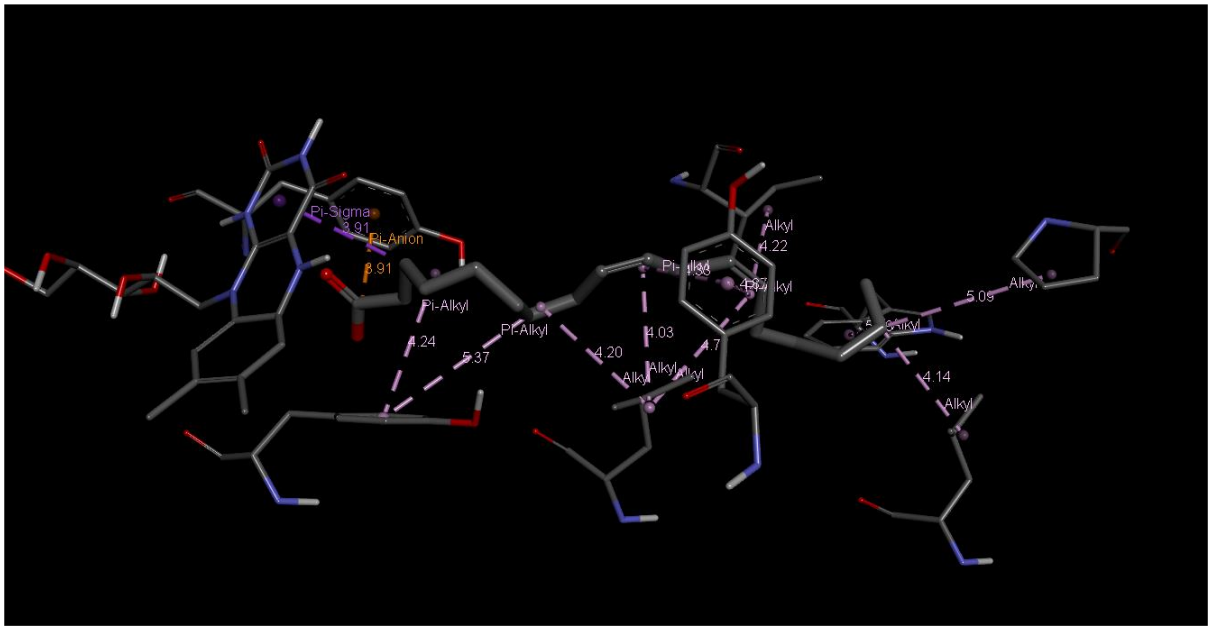


The results were visualized and analysed using Ligplot.

Appendix 3.4 – Docking interactions of alpha-linolenate with hMAO B.



Appendix 3.5 – Docking interactions of linoleate with hMAO B.



Appendix 4 – SwissADME

Appendix 4.1 – Computed parameter values of α-linolenic acid and linoleic acid.

Compound	MW	HBA	HBD	TPSA	iLogP	GI Absorption	BBB	Pgp substrate	Lipinski violations
Linoleic acid	280.45	2	1	37.3	4.14	High	Yes	No	1
α-Linolenic acid	278.43	2	1	37.3	3.36	High	Yes	No	1
Harman	182.22	1	1	28.68	1.75	High	Yes	Yes	0
Norharman	168.19	1	1	28.68	1.43	High	Yes	Yes	0
Selegiline	187.28	1	0	3.24	2.8	High	Yes	No	0
Clorgyline	272.17	2	0	12.47	3.43	High	Yes	No	0

MW: molecular weight; HBA: hydrogen bond acceptor; HBD: hydrogen bond donor; TPSA: topological polar surface area (TPSA needs to be between 20–130$Å^2$); iLogP: for implicit log P (the logarithm of the partition coefficient between n-octanol and water) (iLogP should be between -3.93–6.46); GI absorption: gastrointestinal absorption; BBB: blood-brain barrier; Pgp substrate: P-glycoprotein substrate (a drug efflux pump); Lipinski: number of Lipinski's rule of five violations (drug-likeness evaluation) (maximum value: 4).

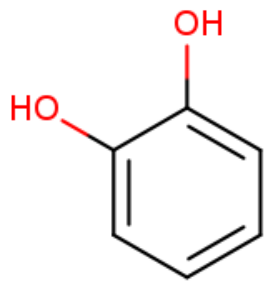
Appendix 4.2 – Computed parameter values of selected compounds in tobacco and tobacco smoke.

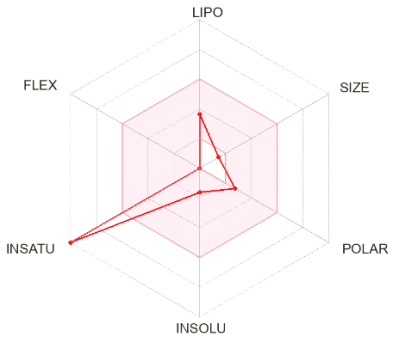
Compound	MW	HBA	HBD	TPSA	iLogP	GI Absorption	BBB	Pgp substrate	Lipinski violations
Harman	182.22	1	1	28.68	1.75	High	Yes	Yes	0
Norharman	168.19	1	1	28.68	1.43	High	Yes	Yes	0
Eugenol	164.2	2	1	29.46	2.37	High	Yes	No	0
Resveratrol	228.24	3	3	60.69	1.71	High	Yes	No	0
β-asarone	208.25	3	0	27.69	3.02	High	Yes	No	0
Ferulic acid	194.18	4	2	66.76	1.62	High	Yes	No	0
Caffeic acid	180.16	4	3	77.76	0.97	High	No	No	0
Gallic acid	170.12	5	4	97.99	0.21	High	No	No	0
Kaempferol	286.24	6	4	111.13	1.7	High	No	No	0
Naringenin	272.25	5	3	86.99	1.75	High	No	Yes	0
Naringin	580.53	14	8	225.06	2.07	Low	No	Yes	3
Rutin	610.52	16	10	269.43	0.46	Low	No	Yes	3
Quercetin	302.24	7	5	131.36	1.63	High	No	No	0
Esculetin	178.14	4	2	70.67	1.25	High	No	No	0
Scopoletin	192.17	4	1	59.67	1.86	High	Yes	No	0
Selegiline	187.28	1	0	3.24	2.8	High	Yes	No	0
Clorgyline	272.17	2	0	12.47	3.43	High	Yes	No	0

MW: molecular weight; HBA: hydrogen bond acceptor; HBD: hydrogen bond donor; TPSA: topological polar surface area (TPSA needs to be between 20–130Å^2); iLogP: for implicit log P (the logarithm of the partition coefficient between n-octanol and water) (iLogP should be between -3.93–6.46); GI absorption: gastrointestinal absorption; BBB: blood-brain barrier; Pgp substrate: P-glycoprotein substrate (a drug efflux pump); Lipinski: number of Lipinski's rule of five violations (drug-likeness evaluation) (maximum value: 4).

Appendix 4.3 – Prediction of ADME

Catechol

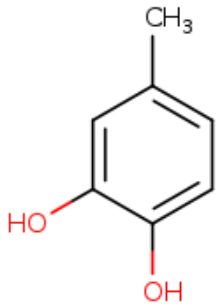


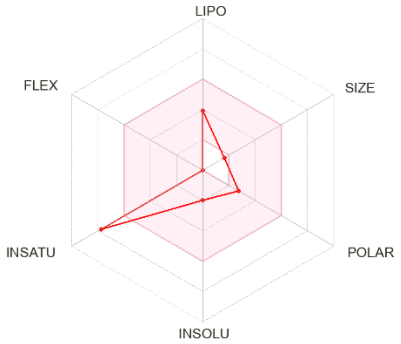


SMILES Oc1ccccc1O

Physicochemical Properties	
Formula	C6H6O2
Molecular weight	110.11 g/mol
Num. heavy atoms	8
Num. arom. heavy atoms	6
Fraction Csp3	0.00
Num. rotatable bonds	0
Num. H-bond acceptors	2
Num. H-bond donors	2
Molar Refractivity	30.49
TPSA	40.46 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	1.13
Log $P_{o/w}$ (XLOGP3)	0.88
Log $P_{o/w}$ (WLOGP)	1.10
Log $P_{o/w}$ (MLOGP)	0.79
Log $P_{o/w}$ (SILICOS-IT)	0.94
Consensus Log $P_{o/w}$	0.97
Water Solubility	
Log *S* (ESOL)	-1.63
Solubility	2.57e+00 mg/ml ; 2.33e-02 mol/l
Class	Very soluble
Log *S* (Ali)	-1.31
Solubility	5.34e+00 mg/ml ; 4.85e-02 mol/l
Class	Very soluble
Log *S* (SILICOS-IT)	-1.18
Solubility	7.21e+00 mg/ml ; 6.55e-02 mol/l
Class	Soluble
Pharmacokinetics	
GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-6.35 cm/s
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	No; 3 violations: MW<160, MR<40, #atoms<20
Veber	Yes
Egan	Yes
Muegge	No; 1 violation: MW<200
Bioavailability Score	0.55
Medicinal Chemistry	
PAINS	1 alert: catechol_A
Brenk	1 alert: catechol
Leadlikeness	No; 1 violation: MW<250
Synthetic accessibility	1.00

4-Methylcatechol





SMILES Cc1ccc(c(c1)O)O

Physicochemical Properties

Formula	C7H8O2
Molecular weight	124.14 g/mol
Num. heavy atoms	9
Num. arom. heavy atoms	6
Fraction Csp3	0.14
Num. rotatable bonds	0
Num. H-bond acceptors	2
Num. H-bond donors	2
Molar Refractivity	35.45
TPSA	40.46 Å²

Lipophilicity

Log $P_{o/w}$ (iLOGP)	1.39
Log $P_{o/w}$ (XLOGP3)	1.37
Log $P_{o/w}$ (WLOGP)	1.41
Log $P_{o/w}$ (MLOGP)	1.15
Log $P_{o/w}$ (SILICOS-IT)	1.35
Consensus Log $P_{o/w}$	1.33

Water Solubility

Log *S* (ESOL)	-1.97
Solubility	1.34e+00 mg/ml ; 1.08e-02 mol/l
Class	Very soluble
Log *S* (Ali)	-1.82
Solubility	1.87e+00 mg/ml ; 1.50e-02 mol/l
Class	Very soluble
Log *S* (SILICOS-IT)	-1.59
Solubility	3.19e+00 mg/ml ; 2.57e-02 mol/l
Class	Soluble

Pharmacokinetics

GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-6.08 cm/s

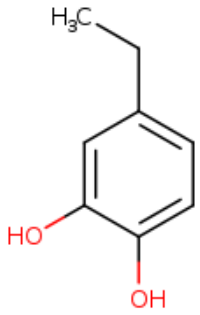
Druglikeness

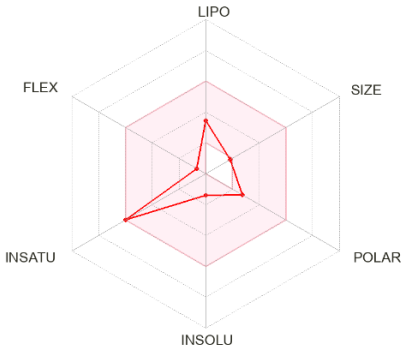
Lipinski	Yes; 0 violation
Ghose	No; 3 violations: MW<160, MR<40, #atoms<20
Veber	Yes
Egan	Yes
Muegge	No; 1 violation: MW<200
Bioavailability Score	0.55

Medicinal Chemistry

PAINS	1 alert: catechol_A
Brenk	1 alert: catechol
Leadlikeness	No; 1 violation: MW<250
Synthetic accessibility	1.00

4-Ethylcatechol





SMILES CCc1ccc(c(c1)O)O

Physicochemical Properties

Formula	C8H10O2
Molecular weight	138.16 g/mol
Num. heavy atoms	10
Num. arom. heavy atoms	6
Fraction Csp3	0.25
Num. rotatable bonds	1
Num. H-bond acceptors	2
Num. H-bond donors	2
Molar Refractivity	40.26
TPSA	40.46 Å²

Lipophilicity

Log $P_{o/w}$ (iLOGP)	1.64
Log $P_{o/w}$ (XLOGP3)	0.50
Log $P_{o/w}$ (WLOGP)	1.66
Log $P_{o/w}$ (MLOGP)	1.48
Log $P_{o/w}$ (SILICOS-IT)	1.65
Consensus Log $P_{o/w}$	1.39

Water Solubility

Log *S* (ESOL)	-1.39
Solubility	5.63e+00 mg/ml ; 4.08e-02 mol/l
Class	Very soluble
Log *S* (Ali)	-0.92
Solubility	1.66e+01 mg/ml ; 1.20e-01 mol/l
Class	Very soluble
Log *S* (SILICOS-IT)	-2.01
Solubility	1.35e+00 mg/ml ; 9.77e-03 mol/l
Class	Soluble

Pharmacokinetics

GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-6.79 cm/s

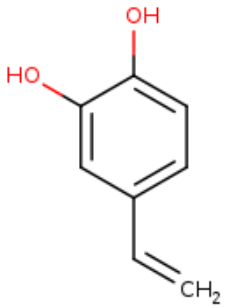
Druglikeness

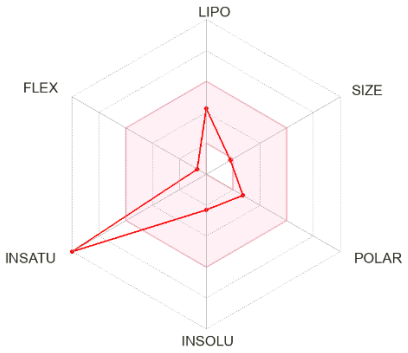
Lipinski	Yes; 0 violation
Ghose	No; 1 violation: MW<160
Veber	Yes
Egan	Yes
Muegge	No; 1 violation: MW<200
Bioavailability Score	0.55

Medicinal Chemistry

PAINS	1 alert: catechol_A
Brenk	1 alert: catechol
Leadlikeness	No; 1 violation: MW<250
Synthetic accessibility	1.00

4-Vinylcatechol





SMILES C=Cc1ccc(c(c1)O)O

Physicochemical Properties

Formula	C8H8O2
Molecular weight	136.15 g/mol
Num. heavy atoms	10
Num. arom. heavy atoms	6
Fraction Csp3	0.00
Num. rotatable bonds	1
Num. H-bond acceptors	2
Num. H-bond donors	2
Molar Refractivity	40.58
TPSA	40.46 Å²

Lipophilicity

Log $P_{o/w}$ (iLOGP)	1.49
Log $P_{o/w}$ (XLOGP3)	1.97
Log $P_{o/w}$ (WLOGP)	1.63
Log $P_{o/w}$ (MLOGP)	1.39
Log $P_{o/w}$ (SILICOS-IT)	1.65
Consensus Log $P_{o/w}$	1.63

Water Solubility

Log *S* (ESOL)	-2.30
Solubility	6.77e-01 mg/ml ; 4.97e-03 mol/l
Class	Soluble
Log *S* (Ali)	-2.45
Solubility	4.89e-01 mg/ml ; 3.59e-03 mol/l
Class	Soluble
Log *S* (SILICOS-IT)	-1.66
Solubility	2.96e+00 mg/ml ; 2.18e-02 mol/l
Class	Soluble

Pharmacokinetics

GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-5.73 cm/s

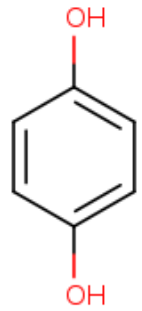
Druglikeness

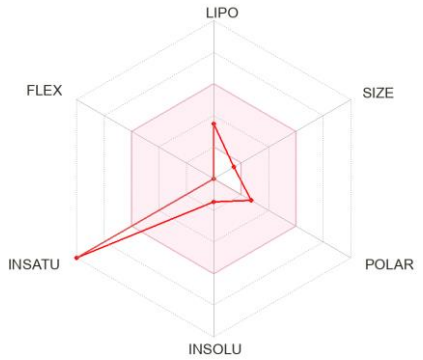
Lipinski	Yes; 0 violation
Ghose	No; 2 violations: MW<160, #atoms<20
Veber	Yes
Egan	Yes
Muegge	No; 1 violation: MW<200
Bioavailability Score	0.55

Medicinal Chemistry

PAINS	1 alert: catechol_A
Brenk	1 alert: catechol
Leadlikeness	No; 1 violation: MW<250
Synthetic accessibility	1.23

Hydroquinone





SMILES Oc1ccc(cc1)O

Physicochemical Properties

Formula	C6H6O2
Molecular weight	110.11 g/mol
Num. heavy atoms	8
Num. arom. heavy atoms	6
Fraction Csp3	0.00
Num. rotatable bonds	0
Num. H-bond acceptors	2
Num. H-bond donors	2
Molar Refractivity	30.49
TPSA	40.46 Å²

Lipophilicity

Log $P_{o/w}$ (iLOGP)	0.92
Log $P_{o/w}$ (XLOGP3)	0.59
Log $P_{o/w}$ (WLOGP)	1.10
Log $P_{o/w}$ (MLOGP)	0.79
Log $P_{o/w}$ (SILICOS-IT)	0.94
Consensus Log $P_{o/w}$	0.87

Water Solubility

Log *S* (ESOL)	-1.45
Solubility	3.91e+00 mg/ml ; 3.55e-02 mol/l
Class	Very soluble
Log *S* (Ali)	-1.01
Solubility	1.07e+01 mg/ml ; 9.70e-02 mol/l
Class	Very soluble
Log *S* (SILICOS-IT)	-1.18
Solubility	7.21e+00 mg/ml ; 6.55e-02 mol/l
Class	Soluble

Pharmacokinetics

GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-6.55 cm/s

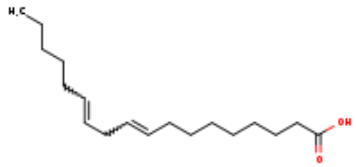
Druglikeness

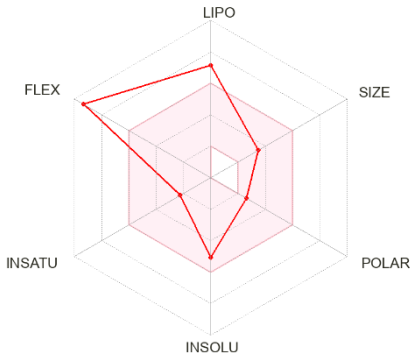
Lipinski	Yes; 0 violation
Ghose	No; 3 violations: MW<160, MR<40, #atoms<20
Veber	Yes
Egan	Yes
Muegge	No; 1 violation: MW<200
Bioavailability Score	0.55

Medicinal Chemistry

PAINS	0 alert
Brenk	1 alert: hydroquinone
Leadlikeness	No; 1 violation: MW<250
Synthetic accessibility	1.00

Linoleic acid

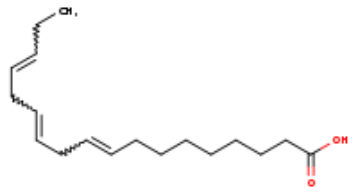


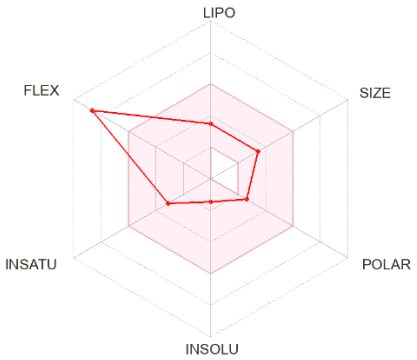


SMILES CCCCCC=CCC=CCCCCCCCC(=O)O

Physicochemical Properties	
Formula	C18H32O2
Molecular weight	280.45 g/mol
Num. heavy atoms	20
Num. arom. heavy atoms	0
Fraction Csp3	0.72
Num. rotatable bonds	14
Num. H-bond acceptors	2
Num. H-bond donors	1
Molar Refractivity	89.46
TPSA	37.30 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	4.14
Log $P_{o/w}$ (XLOGP3)	6.98
Log $P_{o/w}$ (WLOGP)	5.88
Log $P_{o/w}$ (MLOGP)	4.47
Log $P_{o/w}$ (SILICOS-IT)	5.77
Consensus Log $P_{o/w}$	5.45
Water Solubility	
Log *S* (ESOL)	-5.05
Solubility	2.49e-03 mg/ml ; 8.87e-06 mol/l
Class	Moderately soluble
Log *S* (Ali)	-7.58
Solubility	7.42e-06 mg/ml ; 2.64e-08 mol/l
Class	Poorly soluble
Log *S* (SILICOS-IT)	-4.67
Solubility	5.93e-03 mg/ml ; 2.11e-05 mol/l
Class	Moderately soluble
Pharmacokinetics	
GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-3.05 cm/s
Druglikeness	
Lipinski	Yes; 1 violation: MLOGP>4.15
Ghose	No; 1 violation: WLOGP>5.6
Veber	No; 1 violation: Rotors>10
Egan	No; 1 violation: WLOGP>5.88
Muegge	No; 1 violation: XLOGP3>5
Bioavailability Score	0.85
Medicinal Chemistry	
PAINS	0 alert
Brenk	1 alert: isolated_alkene
Leadlikeness	No; 2 violations: Rotors>7, XLOGP3>3.5
Synthetic accessibility	3.10

Linolenic acid





SMILES CCC=CCC=CCC=CCCCCCCCC(=O)O

Physicochemical Properties

Formula	C18H30O2
Molecular weight	278.43 g/mol
Num. heavy atoms	20
Num. arom. heavy atoms	0
Fraction Csp3	0.61
Num. rotatable bonds	13
Num. H-bond acceptors	2
Num. H-bond donors	1
Molar Refractivity	88.99
TPSA	37.30 Å²

Lipophilicity

Log $P_{o/w}$ (iLOGP)	3.36
Log $P_{o/w}$ (XLOGP3)	6.46
Log $P_{o/w}$ (WLOGP)	5.66
Log $P_{o/w}$ (MLOGP)	4.38
Log $P_{o/w}$ (SILICOS-IT)	5.59
Consensus Log $P_{o/w}$	5.09

Water Solubility

Log *S* (ESOL)	4.78
Solubility	4.64e-03 mg/ml ; 1.67e-05 mol/l
Class	Moderately soluble
Log *S* (Ali)	7.04
Solubility	2.55e-05 mg/ml ; 9.16e-08 mol/l
Class	Poorly soluble
Log *S* (SILICOS-IT)	-3.96
Solubility	3.08e-02 mg/ml ; 1.11e-04 mol/l
Class	Soluble

Pharmacokinetics

GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-3.41 cm/s

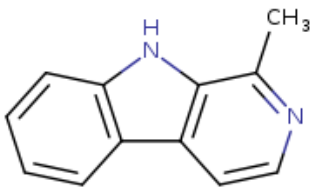
Druglikeness

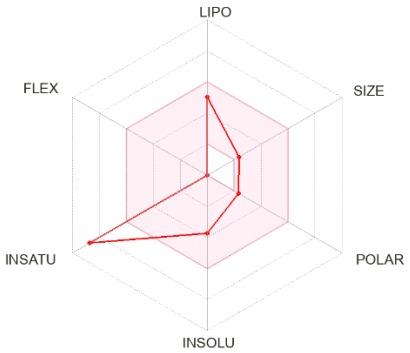
Lipinski	Yes; 1 violation: MLOGP>4.15
Ghose	No; 1 violation: WLOGP>5.6
Veber	No; 1 violation: Rotors>10
Egan	Yes
Muegge	No; 1 violation: XLOGP3>5
Bioavailability Score	0.85

Medicinal Chemistry

PAINS	0 alert
Brenk	1 alert: isolated_alkene
Leadlikeness	No; 2 violations: Rotors>7, XLOGP3>3.5
Synthetic accessibility	3.03

Harman

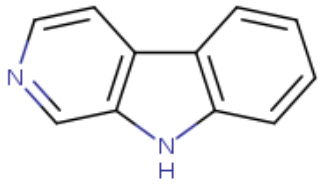


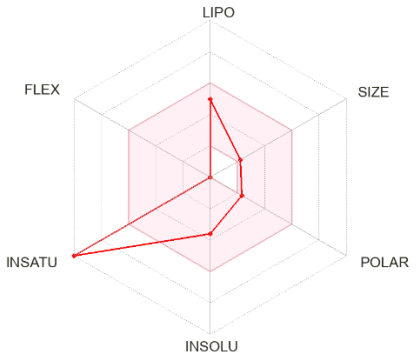


SMILES Cc1nccc2c1[nH]c1c2cccc1

Physicochemical Properties	
Formula	C12H10N2
Molecular weight	182.22 g/mol
Num. heavy atoms	14
Num. arom. heavy atoms	13
Fraction Csp3	0.08
Num. rotatable bonds	0
Num. H-bond acceptors	1
Num. H-bond donors	1
Molar Refractivity	58.57
TPSA	28.68 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	1.75
Log $P_{o/w}$ (XLOGP3)	3.28
Log $P_{o/w}$ (WLOGP)	3.02
Log $P_{o/w}$ (MLOGP)	1.90
Log $P_{o/w}$ (SILICOS-IT)	3.53
Consensus Log $P_{o/w}$	2.70
Water Solubility	
Log *S* (ESOL)	-3.72
Solubility	3.45e-02 mg/ml ; 1.89e-04 mol/l
Class	Soluble
Log *S* (Ali)	-3.56
Solubility	5.05e-02 mg/ml ; 2.77e-04 mol/l
Class	Soluble
Log *S* (SILICOS-IT)	-4.97
Solubility	1.94e-03 mg/ml ; 1.06e-05 mol/l
Class	Moderately soluble
Pharmacokinetics	
GI absorption	High
BBB permeant	Yes
P-gp substrate	Yes
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-5.08 cm/s
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	No; 1 violation: MW<200
Bioavailability Score	0.55
Medicinal Chemistry	
PAINS	0 alert
Brenk	0 alert
Leadlikeness	No; 1 violation: MW<250
Synthetic accessibility	1.50

Norharman





SMILES c1ccc2c(c1)[nH]c1c2ccnc1

Physicochemical Properties

Formula	C11H8N2
Molecular weight	168.19 g/mol
Num. heavy atoms	13
Num. arom. heavy atoms	13
Fraction Csp3	0.00
Num. rotatable bonds	0
Num. H-bond acceptors	1
Num. H-bond donors	1
Molar Refractivity	53.60
TPSA	28.68 Å²

Lipophilicity

Log $P_{o/w}$ (iLOGP)	1.43
Log $P_{o/w}$ (XLOGP3)	3.17
Log $P_{o/w}$ (WLOGP)	2.72
Log $P_{o/w}$ (MLOGP)	1.62
Log $P_{o/w}$ (SILICOS-IT)	3.09
Consensus Log $P_{o/w}$	2.41

Water Solubility

Log *S* (ESOL)	-3.62
Solubility	4.04e-02 mg/ml ; 2.40e-04 mol/l
Class	Soluble
Log *S* (Ali)	-3.44
Solubility	6.06e-02 mg/ml ; 3.61e-04 mol/l
Class	Soluble
Log *S* (SILICOS-IT)	-4.58
Solubility	4.43e-03 mg/ml ; 2.64e-05 mol/l
Class	Moderately soluble

Pharmacokinetics

GI absorption	High
BBB permeant	Yes
P-gp substrate	Yes
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-5.08 cm/s

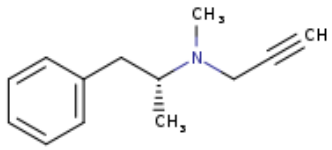
Druglikeness

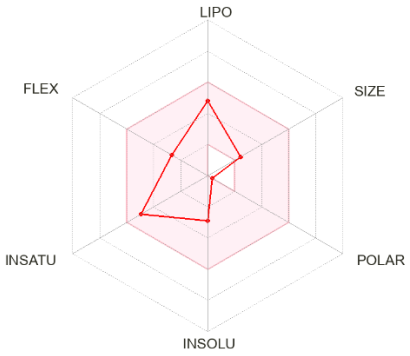
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	No; 1 violation: MW<200
Bioavailability Score	0.55

Medicinal Chemistry

PAINS	0 alert
Brenk	0 alert
Leadlikeness	No; 1 violation: MW<250
Synthetic accessibility	1.46

Selegiline





SMILES C[C@@H](N(CC#C)C)Cc1ccccc1

Physicochemical Properties

Formula	C13H17N
Molecular weight	187.28 g/mol
Num. heavy atoms	14
Num. arom. heavy atoms	6
Fraction Csp3	0.38
Num. rotatable bonds	4
Num. H-bond acceptors	1
Num. H-bond donors	0
Molar Refractivity	61.31
TPSA	3.24 Å²

Lipophilicity

Log $P_{o/w}$ (iLOGP)	2.80
Log $P_{o/w}$ (XLOGP3)	2.90
Log $P_{o/w}$ (WLOGP)	2.26
Log $P_{o/w}$ (MLOGP)	3.25
Log $P_{o/w}$ (SILICOS-IT)	2.85
Consensus Log $P_{o/w}$	2.81

Water Solubility

Log *S* (ESOL)	-2.88
Solubility	2.46e-01 mg/ml ; 1.31e-03 mol/l
Class	Soluble
Log *S* (Ali)	-2.63
Solubility	4.40e-01 mg/ml ; 2.35e-03 mol/l
Class	Soluble
Log *S* (SILICOS-IT)	-3.45
Solubility	6.64e-02 mg/ml ; 3.55e-04 mol/l
Class	Soluble

Pharmacokinetics

GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-5.38 cm/s

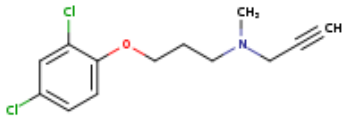
Druglikeness

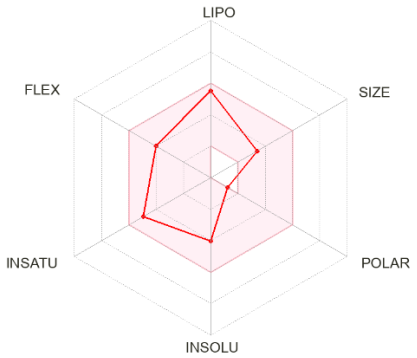
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	No; 2 violations: MW<200, Heteroatoms<2
Bioavailability Score	0.55

Medicinal Chemistry

PAINS	0 alert
Brenk	1 alert: triple_bond
Leadlikeness	No; 1 violation: MW<250
Synthetic accessibility	2.11

Clorgyline





SMILES C#CCN(CCCOc1ccc(cc1Cl)Cl)C

Physicochemical Properties	
Formula	C13H15Cl2NO
Molecular weight	272.17 g/mol
Num. heavy atoms	17
Num. arom. heavy atoms	6
Fraction Csp3	0.38
Num. rotatable bonds	6
Num. H-bond acceptors	2
Num. H-bond donors	0
Molar Refractivity	72.85
TPSA	12.47 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	3.43
Log $P_{o/w}$ (XLOGP3)	4.18
Log $P_{o/w}$ (WLOGP)	3.41
Log $P_{o/w}$ (MLOGP)	3.64
Log $P_{o/w}$ (SILICOS-IT)	3.89
Consensus Log $P_{o/w}$	3.71
Water Solubility	
Log *S* (ESOL)	-4.03
Solubility	2.56e-02 mg/ml ; 9.42e-05 mol/l
Class	Moderately soluble
Log *S* (Ali)	-4.15
Solubility	1.92e-02 mg/ml ; 7.07e-05 mol/l
Class	Moderately soluble
Log *S* (SILICOS-IT)	-4.79
Solubility	4.36e-03 mg/ml ; 1.60e-05 mol/l
Class	Moderately soluble
Pharmacokinetics	
GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-4.99 cm/s
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability Score	0.55
Medicinal Chemistry	
PAINS	0 alert
Brenk	1 alert: triple_bond
Leadlikeness	No; 1 violation: XLOGP3>3.5
Synthetic accessibility	2.24

Appendix 5 – PreADMET

Appendix 5.1 – ADME prediction of selected phenolic compounds and fatty acids in tobacco smoke.

Compound	PPB[a]	HIA[b]	Caco2[c]	CYP2D6 substrate	BBB[d]	Pgp inhibition	Pure water Solubility (mg/L)
Catechol	97.32	87.41	16.55	Non	1.56	Non	11470.3
4-Methylcatechol	91.59	87.82	18.71	Non	2.34	Non	3848.03
4-Ethylcatechol	100.00	88.27	18.47	Non	3.25	Non	1570.4
4-Vinylcatechol	17.77	89.00	18.47	Non	2.46	Non	919.703
Hydroquinone	96.28	87.42	16.55	Non	1.56	Non	45961.6
α-Linoleic acid	100.00	98.37	28.08	Non	7.32	Inhibitor	2.65109
Linolenic acid	100.00	98.27	27.97	Non	6.17	Inhibitor	7.66782
Harman	100.00	94.10	24.00	Weakly	5.59	Non	213.977
Norharman	100.00	94.05	30.99	Weakly	4.76	Non	466.949
Selegiline	53.20	100.00	31.95	Substrate	4.44	Inhibitor	4487.36
Clorgyline	81.07	100.00	52.49	Substrate	6.74	Inhibitor	109.758

[a] PPB (plasma protein binding): weak binding (< 90%) and strong binding (> 90%). [b] HIA (percent human intestinal absorption): poor absorption (0%–20%), moderate absorption (20–70%), and well absorption (70–100%). [c] Caco2 (human colon carcinoma cell line) permeability: low permeability (<4), middle permeability (4–70), and high permeability (>70). CYP2D6 (cytochrome P450 2D6). [d] BBB (blood–brain barrier) penetration: low absorption (< 0.1), middle absorption (0.1–2.0), and high absorption (> 2.0). BBB is denoted by BB = [Brain]/[Blood], where [Brain] and [Blood] are the steady-state concentrations of radioactively labeled chemicals (Ma, X. L., Chen, C., & Yang, J. (2005). Predictive model of blood-brain barrier penetration of organic compounds. Acta Pharmacologica Sinica, 26(4), 500-512). Pgp (P-glycoprotein: a drug efflux pump) inhibition.

Appendix 5.2 – Prediction of ADME/Tox

Catechol	
ID	**Value**
ADME	
BBB	1.5648
Buffer_solubility_mg_L	33215.1
Caco2	16.5456
CYP_2C19_inhibition	Inhibitor
CYP_2C9_inhibition	Inhibitor
CYP_2D6_inhibition	Non
CYP_2D6_substrate	Non
CYP_3A4_inhibition	Inhibitor
CYP_3A4_substrate	Non
HIA	87.412808
MDCK	157.375
Pgp_inhibition	Non
Plasma_Protein_Binding	97.323518
Pure_water_solubility_mg_L	11470.3
Skin_Permeability	-3.18885
SKlogD_value	1.439320
SKlogP_value	1.439320
SKlogS_buffer	-0.520500
SKlogS_pure	-0.982260
Druglikeness	
CMC_like_Rule	Not qualified
CMC_like_Rule_Violation_Fields	Molecular_weight, AMolRef, No_Total_atoms
CMC_like_Rule_Violations	3
Lead-like_Rule_Violation_Fields	
Lead_like_Rule	Suitable if its binding affinity is greater than 0.1 microM
Lead_like_Rule_Violations	0
MDDR_like_Rule	Nondrug-like
MDDR_like_Rule_Violation_Fields	No_Rings, No_Rigid_bonds, No_Rotatable_bonds
MDDR_like_Rule_Violations	3
Rule_of_Five	Suitable
Rule_of_Five_Violation_Fields	
Rule_of_Five_Violations	0
WDI_like_Rule	Out of 90% cutoff
WDI_like_Rule_Violation_Fields	Balaban_index_JX
WDI_like_Rule_Violations	1
Toxicity	
algae_at	0.105721
Ames_test	mutagen
Carcino_Mouse	negative
Carcino_Rat	positive
daphnia_at	0.726936
hERG_inhibition	low_risk
medaka_at	0.573193
minnow_at	0.182615
TA100_10RLI	positive
TA100_NA	positive
TA1535_10RLI	positive
TA1535_NA	negative

4-Methylcatechol	
ID	**Value**
ADME	
BBB	2.34069
Buffer_solubility_mg_L	15275.6
Caco2	18.7096
CYP_2C19_inhibition	Inhibitor
CYP_2C9_inhibition	Inhibitor
CYP_2D6_inhibition	Non
CYP_2D6_substrate	Non
CYP_3A4_inhibition	Inhibitor
CYP_3A4_substrate	Non
HIA	87.823486
MDCK	396.118
Pgp_inhibition	Non
Plasma_Protein_Binding	91.594240
Pure_water_solubility_mg_L	3848.03
Skin_Permeability	-2.72995
SKlogD_value	1.964530
SKlogP_value	1.964530
SKlogS_buffer	-0.909910
SKlogS_pure	-1.508670
Druglikeness	
CMC_like_Rule	Not qualified
CMC_like_Rule_Violation_Fields	Molecular_weight, AMolRef, No_Total_atoms
CMC_like_Rule_Violations	3
Lead-like_Rule_Violation_Fields	
Lead_like_Rule	Suitable if its binding affinity is greater than 0.1 microM
Lead_like_Rule_Violations	0
MDDR_like_Rule	Nondrug-like
MDDR_like_Rule_Violation_Fields	No_Rings, No_Rigid_bonds, No_Rotatable_bonds
MDDR_like_Rule_Violations	3
Rule_of_Five	Suitable
Rule_of_Five_Violation_Fields	
Rule_of_Five_Violations	0
WDI_like_Rule	Out of 90% cutoff
WDI_like_Rule_Violation_Fields	Balaban_index_JX
WDI_like_Rule_Violations	1
Toxicity	
algae_at	0.0611539
Ames_test	mutagen
Carcino_Mouse	negative
Carcino_Rat	positive
daphnia_at	0.473268
hERG_inhibition	low_risk
medaka_at	0.251156
minnow_at	0.125259
TA100_10RLI	positive
TA100_NA	positive
TA1535_10RLI	positive
TA1535_NA	negative

4-Ethylcatechol	
ID	**Value**
ADME	
BBB	3.25364
Buffer_solubility_mg_L	9223.81
Caco2	18.4748
CYP_2C19_inhibition	Inhibitor
CYP_2C9_inhibition	Inhibitor
CYP_2D6_inhibition	Non
CYP_2D6_substrate	Non
CYP_3A4_inhibition	Inhibitor
CYP_3A4_substrate	Non
HIA	88.274006
MDCK	407.209
Pgp_inhibition	Non
Plasma_Protein_Binding	100.000000
Pure_water_solubility_mg_L	1570.4
Skin_Permeability	-2.25889
SKlogD_value	2.355950
SKlogP_value	2.355950
SKlogS_buffer	-1.175490
SKlogS_pure	-1.944390
Druglikeness	
CMC_like_Rule	Not qualified
CMC_like_Rule_Violation_Fields	Molecular_weight, AMolRef, No_Total_atoms
CMC_like_Rule_Violations	2
Lead-like_Rule_Violation_Fields	
Lead_like_Rule	Suitable if its binding affinity is greater than 0.1 microM
Lead_like_Rule_Violations	0
MDDR_like_Rule	Nondrug-like
MDDR_like_Rule_Violation_Fields	No_Rings, No_Rigid_bonds, No_Rotatable_bonds
MDDR_like_Rule_Violations	2
Rule_of_Five	Suitable
Rule_of_Five_Violation_Fields	
Rule_of_Five_Violations	0
WDI_like_Rule	Out of 90% cutoff
WDI_like_Rule_Violation_Fields	Balaban_index_JX
WDI_like_Rule_Violations	1
Toxicity	
algae_at	0.0439998
Ames_test	mutagen
Carcino_Mouse	negative
Carcino_Rat	positive
daphnia_at	0.301489
hERG_inhibition	low_risk
medaka_at	0.106015
minnow_at	0.0686735
TA100_10RLI	positive
TA100_NA	positive
TA1535_10RLI	positive
TA1535_NA	positive

4-Vinylcatechol	
ID	**Value**
ADME	
BBB	2.46163
Buffer_solubility_mg_L	1022.53
Caco2	18.4731
CYP_2C19_inhibition	Inhibitor
CYP_2C9_inhibition	Inhibitor
CYP_2D6_inhibition	Non
CYP_2D6_substrate	Non
CYP_3A4_inhibition	Inhibitor
CYP_3A4_substrate	Non
HIA	89.000297
MDCK	412.534
Pgp_inhibition	Non
Plasma_Protein_Binding	17.755473
Pure_water_solubility_mg_L	919.703
Skin_Permeability	-2.16299
SKlogD_value	2.237130
SKlogP_value	2.237130
SKlogS_buffer	-2.124340
SKlogS_pure	-2.170370
Druglikeness	
CMC_like_Rule	Not qualified
CMC_like_Rule_Violation_Fields	Molecular_weight, AMolRef, No_Total_atoms
CMC_like_Rule_Violations	3
Lead-like_Rule_Violation_Fields	
Lead_like_Rule	Suitable if its binding affinity is greater than 0.1 microM
Lead_like_Rule_Violations	0
MDDR_like_Rule	Nondrug-like
MDDR_like_Rule_Violation_Fields	No_Rings, No_Rigid_bonds, No_Rotatable_bonds
MDDR_like_Rule_Violations	3
Rule_of_Five	Suitable
Rule_of_Five_Violation_Fields	
Rule_of_Five_Violations	0
WDI_like_Rule	Out of 90% cutoff
WDI_like_Rule_Violation_Fields	Balaban_index_JX
WDI_like_Rule_Violations	1
Toxicity	
algae_at	0.0798119
Ames_test	mutagen
Carcino_Mouse	positive
Carcino_Rat	positive
daphnia_at	0.189667
hERG_inhibition	medium_risk
medaka_at	0.0468426
minnow_at	0.0185028
TA100_10RLI	positive
TA100_NA	positive
TA1535_10RLI	positive
TA1535_NA	positive

Hydroquinone	
ID	**Value**
ADME	
BBB	1.56261
Buffer_solubility_mg_L	8216.66
Caco2	16.5499
CYP_2C19_inhibition	Inhibitor
CYP_2C9_inhibition	Inhibitor
CYP_2D6_inhibition	Non
CYP_2D6_substrate	Non
CYP_3A4_inhibition	Inhibitor
CYP_3A4_substrate	Non
HIA	87.422832
MDCK	78.3462
Pgp_inhibition	Non
Plasma_Protein_Binding	96.283474
Pure_water_solubility_mg_L	45961.6
Skin_Permeability	-3.23085
SKlogD_value	0.956480
SKlogP_value	0.956480
SKlogS_buffer	-1.127140
SKlogS_pure	-0.379440
Druglikeness	
CMC_like_Rule	Not qualified
CMC_like_Rule_Violation_Fields	Molecular_weight, AMolRef, No_Total_atoms
CMC_like_Rule_Violations	3
Lead-like_Rule_Violation_Fields	
Lead_like_Rule	Suitable if its binding affinity is greater than 0.1 microM
Lead_like_Rule_Violations	0
MDDR_like_Rule	Nondrug-like
MDDR_like_Rule_Violation_Fields	No_Rings, No_Rigid_bonds, No_Rotatable_bonds
MDDR_like_Rule_Violations	3
Rule_of_Five	Suitable
Rule_of_Five_Violation_Fields	
Rule_of_Five_Violations	0
WDI_like_Rule	Out of 90% cutoff
WDI_like_Rule_Violation_Fields	Balaban_index_JX
WDI_like_Rule_Violations	1
Toxicity	
algae_at	0.107092
Ames_test	mutagen
Carcino_Mouse	negative
Carcino_Rat	positive
daphnia_at	0.726936
hERG_inhibition	low_risk
medaka_at	0.573193
minnow_at	0.174334
TA100_10RLI	positive
TA100_NA	positive
TA1535_10RLI	positive
TA1535_NA	negative

Linoleic acid	
ID	**Value**
ADME	
BBB	7.31647
Buffer_solubility_mg_L	645.765
Caco2	28.0819
CYP_2C19_inhibition	Inhibitor
CYP_2C9_inhibition	Inhibitor
CYP_2D6_inhibition	Non
CYP_2D6_substrate	Non
CYP_3A4_inhibition	Inhibitor
CYP_3A4_substrate	Non
HIA	98.370635
MDCK	72.0045*
Pgp_inhibition	Inhibitor
Plasma_Protein_Binding	100.000000
Pure_water_solubility_mg_L	2.65109
Skin_Permeability	-0.538849
SKlogD_value	5.535650
SKlogP_value	6.783650
SKlogS_buffer	-2.637780
SKlogS_pure	-5.024430
Druglikeness	
CMC_like_Rule	Not qualified
CMC_like_Rule_Violation_Fields	AlopP98_value
CMC_like_Rule_Violations	1
Lead-like_Rule_Violation_Fields	AlopP98_value
Lead_like_Rule	Violated
Lead_like_Rule_Violations	1
MDDR_like_Rule	Mid-structure
MDDR_like_Rule_Violation_Fields	No_Rings
MDDR_like_Rule_Violations	1
Rule_of_Five	Suitable
Rule_of_Five_Violation_Fields	AlopP98_value
Rule_of_Five_Violations	1
WDI_like_Rule	Out of 90% cutoff
WDI_like_Rule_Violation_Fields	AlopP98_value, Balaban_index_JX, Kier_flexibility, Kier_alpha_02, Kier_alpha_03
WDI_like_Rule_Violations	5
Toxicity	
algae_at	0.0034348
Ames_test	mutagen
Carcino_Mouse	positive
Carcino_Rat	positive
daphnia_at	0.00809795
hERG_inhibition	low_risk
medaka_at	0.000106144
minnow_at	9.55755e-005
TA100_10RLI	negative
TA100_NA	negative
TA1535_10RLI	negative
TA1535_NA	positive

Linolenic acid	
ID	**Value**
ADME	
BBB	6.16921
Buffer_solubility_mg_L	2711.95
Caco2	27.9738
CYP_2C19_inhibition	Inhibitor
CYP_2C9_inhibition	Inhibitor
CYP_2D6_inhibition	Non
CYP_2D6_substrate	Non
CYP_3A4_inhibition	Inhibitor
CYP_3A4_substrate	Non
HIA	98.273607
MDCK	74.7897
Pgp_inhibition	Inhibitor
Plasma_Protein_Binding	100.000000
Pure_water_solubility_mg_L	7.66782
Skin_Permeability	-0.538273
SKlogD_value	5.214910
SKlogP_value	6.462910
SKlogS_buffer	-2.011440
SKlogS_pure	-4.560050
Druglikeness	
CMC_like_Rule	Not qualified
CMC_like_Rule_Violation_Fields	AlopP98_value
CMC_like_Rule_Violations	1
Lead-like_Rule_Violation_Fields	AlopP98_value
Lead_like_Rule	Violated
Lead_like_Rule_Violations	1
MDDR_like_Rule	Mid-structure
MDDR_like_Rule_Violation_Fields	No_Rings
MDDR_like_Rule_Violations	1
Rule_of_Five	Suitable
Rule_of_Five_Violation_Fields	AlopP98_value
Rule_of_Five_Violations	1
WDI_like_Rule	Out of 90% cutoff
WDI_like_Rule_Violation_Fields	AlopP98_value, Balaban_index_JX, Kier_flexibility, Kier_alpha_02, Kier_alpha_03
WDI_like_Rule_Violations	5
Toxicity	
algae_at	0.00527731
Ames_test	mutagen
Carcino_Mouse	positive
Carcino_Rat	positive
daphnia_at	0.0113075
hERG_inhibition	medium_risk
medaka_at	0.000208334
minnow_at	0.000238603
TA100_10RLI	negative
TA100_NA	negative
TA1535_10RLI	negative
TA1535_NA	positive

Harman	
ID	**Value**
ADME	
BBB	5.58863
Buffer_solubility_mg_L	4173.17
Caco2	23.9955
CYP_2C19_inhibition	Inhibitor
CYP_2C9_inhibition	Inhibitor
CYP_2D6_inhibition	Non
CYP_2D6_substrate	Weakly
CYP_3A4_inhibition	Inhibitor
CYP_3A4_substrate	Non
HIA	94.096543
MDCK	371.074
Pgp_inhibition	Non
Plasma_Protein_Binding	100.000000
Pure_water_solubility_mg_L	213.977
Skin_Permeability	-4.06106
SKlogD_value	2.443770
SKlogP_value	2.443770
SKlogS_buffer	-1.640140
SKlogS_pure	-2.930240
Druglikeness	
CMC_like_Rule	Qualified
CMC_like_Rule_Violation_Fields	0
CMC_like_Rule_Violations	
Lead-like_Rule_Violation_Fields	Suitable if its binding affinity is greater than 0.1 microM
Lead_like_Rule	0
Lead_like_Rule_Violations	Mid-structure
MDDR_like_Rule	No_Rotatable_bonds
MDDR_like_Rule_Violation_Fields	1
MDDR_like_Rule_Violations	Suitable
Rule_of_Five	
Rule_of_Five_Violation_Fields	0
Rule_of_Five_Violations	In 90% cutoff
WDI_like_Rule	
WDI_like_Rule_Violation_Fields	0
WDI_like_Rule_Violations	0
Toxicity	
algae_at	0.080218
Ames_test	mutagen
Carcino_Mouse	positive
Carcino_Rat	negative
daphnia_at	0.151356
hERG_inhibition	medium_risk
medaka_at	0.0361193
minnow_at	0.0360958
TA100_10RLI	positive
TA100_NA	negative
TA1535_10RLI	positive
TA1535_NA	positive

Norharman	
ID	**Value**
ADME	
BBB	4.75908
Buffer_solubility_mg_L	838.783
Caco2	30.9898
CYP_2C19_inhibition	Inhibitor
CYP_2C9_inhibition	Inhibitor
CYP_2D6_inhibition	Non
CYP_2D6_substrate	Weakly
CYP_3A4_inhibition	Inhibitor
CYP_3A4_substrate	Non
HIA	94.047668
MDCK	365.666
Pgp_inhibition	Non
Plasma_Protein_Binding	100.000000
Pure_water_solubility_mg_L	466.949
Skin_Permeability	-4.13876
SKlogD_value	2.213880
SKlogP_value	2.213880
SKlogS_buffer	-2.302170
SKlogS_pure	-2.556550
Druglikeness	
CMC_like_Rule	Qualified
CMC_like_Rule_Violation_Fields	0
CMC_like_Rule_Violations	
Lead-like_Rule_Violation_Fields	Suitable if its binding affinity is greater than 0.1 microM
Lead_like_Rule	0
Lead_like_Rule_Violations	Mid-structure
MDDR_like_Rule	No_Rotatable_bonds
MDDR_like_Rule_Violation_Fields	1
MDDR_like_Rule_Violations	Suitable
Rule_of_Five	
Rule_of_Five_Violation_Fields	0
Rule_of_Five_Violations	In 90% cutoff
WDI_like_Rule	
WDI_like_Rule_Violation_Fields	0
WDI_like_Rule_Violations	0
Toxicity	
algae_at	0.117563
Ames_test	mutagen
Carcino_Mouse	positive
Carcino_Rat	negative
daphnia_at	0.217036
hERG_inhibition	medium_risk
medaka_at	0.0708295
minnow_at	0.0481736
TA100_10RLI	positive
TA100_NA	negative
TA1535_10RLI	positive
TA1535_NA	positive

Selegiline	
ID	**Value**
ADME	
BBB	4.43519
Buffer_solubility_mg_L	1594.05
Caco2	31.9458
CYP_2C19_inhibition	Inhibitor
CYP_2C9_inhibition	Non
CYP_2D6_inhibition	Inhibitor
CYP_2D6_substrate	Substrate
CYP_3A4_inhibition	Non
CYP_3A4_substrate	Substrate
HIA	100.000000
MDCK	164.082
Pgp_inhibition	Inhibitor
Plasma_Protein_Binding	53.201657
Pure_water_solubility_mg_L	4487.36
Skin_Permeability	-0.793164
SKlogD_value	1.195920
SKlogP_value	2.760380
SKlogS_buffer	-2.070000
SKlogS_pure	-1.620510
Druglikeness	
CMC_like_Rule	Qualified
CMC_like_Rule_Violation_Fields	
CMC_like_Rule_Violations	0
Lead-like_Rule_Violation_Fields	AlopP98_value
Lead_like_Rule	Violated
Lead_like_Rule_Violations	1
MDDR_like_Rule	Mid-structure
MDDR_like_Rule_Violation_Fields	No_Rings, No_Rotatable_bonds
MDDR_like_Rule_Violations	2
Rule_of_Five	Suitable
Rule_of_Five_Violation_Fields	
Rule_of_Five_Violations	0
WDI_like_Rule	In 90% cutoff
WDI_like_Rule_Violation_Fields	
WDI_like_Rule_Violations	0
Toxicity	
algae_at	0.039242
Ames_test	mutagen
Carcino_Mouse	negative
Carcino_Rat	negative
daphnia_at	0.100513
hERG_inhibition	medium_risk
medaka_at	0.0131139
minnow_at	0.0165755
TA100_10RLI	negative
TA100_NA	negative
TA1535_10RLI	negative
TA1535_NA	positive

Clogyline	
ID	**Value**
ADME	
BBB	6.73708
Buffer_solubility_mg_L	1180.77
Caco2	52.4947
CYP_2C19_inhibition	Non
CYP_2C9_inhibition	Non
CYP_2D6_inhibition	Non
CYP_2D6_substrate	Substrate
CYP_3A4_inhibition	Non
CYP_3A4_substrate	Non
HIA	100.000000
MDCK	2.18479
Pgp_inhibition	Inhibitor
Plasma_Protein_Binding	81.071829
Pure_water_solubility_mg_L	109.758
Skin_Permeability	-1.30887
SKlogD_value	2.217700
SKlogP_value	3.782160
SKlogS_buffer	-2.362680
SKlogS_pure	-3.394410
Druglikeness	
CMC_like_Rule	Qualified
CMC_like_Rule_Violation_Fields	
CMC_like_Rule_Violations	0
Lead-like_Rule_Violation_Fields	AlopP98_value
Lead_like_Rule	Violated
Lead_like_Rule_Violations	1
MDDR_like_Rule	Mid-structure
MDDR_like_Rule_Violation_Fields	No_Rings
MDDR_like_Rule_Violations	1
Rule_of_Five	Suitable
Rule_of_Five_Violation_Fields	
Rule_of_Five_Violations	0
WDI_like_Rule	In 90% cutoff
WDI_like_Rule_Violation_Fields	
WDI_like_Rule_Violations	0
Toxicity	
algae_at	0.0117493
Ames_test	mutagen
Carcino_Mouse	positive
Carcino_Rat	negative
daphnia_at	0.0239034
hERG_inhibition	medium_risk
medaka_at	0.00100535
minnow_at	0.00250202
TA100_10RLI	negative
TA100_NA	positive
TA1535_10RLI	negative
TA1535_NA	positive

Appendix 6 – Covid-19 impacts form

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

Note for Examiners
Explanation of COVID-19 Impacts

Thank you for taking the time to examine this thesis, which has been undertaken during the Covid-19 pandemic. The New Zealand Government's response to Covid-19 includes a system of Alert Levels which have impacted upon researchers. Our University's pandemic plan applied the Government's expectations to our research environment to ensure the health and safety of our researchers, however, research was impacted by restrictions and disruptions, as outlined below.

For a six-week period from March 26 to April 27 2020, New Zealand was placed under very strict lockdown conditions (Level 4 – Lockdown), with students and staff unable to physically access University facilities, unless they were involved in essential research related to Covid-19. All field work ceased and data collection with humans was restricted to online methods, if appropriate. The restrictions were partially lifted on April 27, but students and staff were not generally allowed back into University facilities until May 13.

Ongoing disruptions have also been encountered for some students due to uncertainties over the potential for future Covid-19-related restrictions on activities, and a Covid-19 cluster outbreak based in Auckland in New Zealand on 12 August 2020 led to the imposition of rolling Level 2 (Reduce) and Level 3 (Restrict) conditions until 23 September 2020. Auckland campus based students remained on Level 2 until 7 October 2020.

This Alert Level system continues to be utilised throughout 2021, and in particular from 17 August 2021 when the whole of New Zealand again moved to Level 4 lockdown for an extended period. The Auckland region remained in alert level 3 or 4 for a number of months. Please see the NZ Government website for more information on lockdown dates.

These changing Alert Levels have meant that some research students had experimental, clinical, laboratory, field work, and/or data collection or analysis interrupted, and consequently may have had to adjust their research plans. For some students, the impacts of Covid-19 have been substantial as they may have had to significantly revise their research plans.

Overseas travel is not permitted by the University and restrictions have been placed on the New Zealand borders which are closed to non-New Zealand citizens and permanent residents. This meant that international students who were based offshore at the time of lockdown, were unable to return to New Zealand. A small number of offshore students were provided permission to return to New Zealand in early 2021. Many students have also suffered from anxiety and stress-related issues, and have had financial impacts, meaning their research progress has been significantly delayed.

This form, as completed by the supervisor and student, outlines the extent that the research has been affected by Covid-19 conditions.

Approved by DRC 10/Feb/2021
DRC 21/02/03
Updated September 2021

Please consider the factors listed below in your assessment of the work.

This statement has been prepared by the candidate's supervisor in consultation with the student and has been endorsed by the relevant Head of Academic Unit.

Student Name:	Sa Weon Hong	ID Number:	[redacted]
Supervisor Name:	Penelope Truman	Date:	30-Mar-23
Thesis title:	Biochemical characterisation of six novel monoamine oxidase inhibitors identified in tobacco smoke		

Considerations to be taken into account. Note: This statement will remain in the final copy of the thesis which will be available from the Massey University Library following the examination process.
[*Enter key considerations here for the examiners. This can include but is not limited to change of scope, scale, topic, focus; limitations in relation to data collection, access to necessary literature or archival materials, laboratories, field sites; disruptions as a result of lockdown and various alert levels, medical or health considerations etc*]

The impact of Covid-19 on Sa Weon's thesis work has been significant, in my view.

Sa Weon was able to work through lockdowns, by concentrating on reading papers and writing up. I have no doubt that this was difficult, with a small child at home. It must have been emotionally very hard, also, being so completely separated from his family in Korea. However, Sa Weon has never complained, nor asked for special consideration, and is submitting his thesis on schedule.

Covid-19 also provided disruption to supply chains. Specifically, he was unable to obtain kynuramine dihydrobromide. Sigma-Aldridge, our usual supplier, kept promising delivery in two months - then in another two months - for over a year, and is still unable to supply. The only other reputable supplier with this product in stock that we could find was charging an unaffordable price. Sa Weon managed to scrape together enough reagent to fill the biggest holes in his data, but this was an inefficient use of his time and must have caused significant stress.

A further significant difficulty was compounded by Covid-19. Victoria University of Wellington (VUW) had a backlog of people using its mass spectrometry facilities at the same time time that Sa Weon also wanted access. We wanted to use the VUW facilities in part because they are local, and in part because this made best use of Professor Teesdale-Spittle's input as a co-supervisor. However, an equipment breakdown at VUW took several months to resolve, as the service agent was unable to travel from Australia. As a result, even once the repairs were effected, several students all needed urgent access. Thus Sa Weon could not undertake the mass spectrometric work to look for protein adducts as planned. He will now do this work, originally planned for September-November last year, in April this year, after his thesis has been submitted.

Approved by DRC 10/Feb/2021
DRC 21/02/03
Updated September 2021

Confidential for Examiners Only: [*Please enter any other considerations which are confidential for examiners only and should not be placed in the final thesis version submitted to Library following the examination process]*

Signed, confirming this is a fair reflection of the impact of Covid-19 on this research.

Student SWHong

Supervisor Penelope Truman

Head of Academic Unit (or nominee)

Approved by DRC 10/Feb/2021
DRC 21/02/03
Updated September 2021