The synthesis and evaluation of phthalimide analogues as inhibitors of monoamine oxidase B

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Thesis submitted in fulfilment of the requirements
for the degree Philosophiae Doctor in Pharmaceutical Chemistry,
at the North-West University, Potchefstroom Campus, South Africa

Promoter: Prof. J.P. Petzer
Co-promoter: Prof. J.J. Bergh

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DEDICATED TO THE ALMIGHTY GOD FOR THE STRENGTH AND COURAGE THROUGHOUT THE STUDY

"If you Behold, you will Become"

Thank you Lord for leading me. You Deserve All the Glory.

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PREFACE

The experimental work described in this thesis was carried out in the School of Pharmacy of the North-West University, Potchefstroom Campus, South Africa. André Joubert and Johan Jordaan of the SASOL Centre for Chemistry, North-West University, Potchefstroom Campus, South Africa recorded the NMR spectra, while the MS spectra were recorded by Marelize Ferreira of the Mass Spectrometry Service, School of Chemistry, University of the Witwatersrand. Support with the HPLC analysis was provided by Jan du Preez from the Analytical Technology Laboratory, North-West University.

The thesis is presented in an article format and each paper is an individual entity. The research conducted represents original work undertaken by the author, and has not been previously submitted for degree purposes to any other University. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of this thesis. Permission of the co-authors of the papers used in the study as well as guide to authors for each journal have also been included.

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DECLARATION

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

I, Clarina Ilara Manley-King, hereby declare that the dissertation with the title:

The synthesis and evaluation of phthalimde analogues as inhibitors of monoamine oxidase B

is my own work and has not been submitted at any other University either in whole or in part.

Signed at Potchefstroom on the 14th day of November, 2011.

Clarina Ilara Manley-king
November, 2011

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ACRONYMS AND ABBREVIATIONS

AD	Alzheimers' disease
ALS	Amylotrophic lateral sclerosis
CNS	Central nervous system
COMT	Catechol-O-methyltransferase
CSC	(E)-8-(3-Chlorostyryl)caffeine
E	Enzyme
ES	Enzyme-substrate complex
FAD	Flavin adenine dinucleotide
GABA	Gamma-aminobutyric acid
GSH	Glutathione
HRP	Horseradish peroxidise
HPLC	High Performance Liquid Chromatography
K_{cat}	The turnover number
K_i	Enzyme-inhibitor dissociation constant
K_m	The Michaelis constant
L-AAAD	L-Aromatic amino acid decarboxylase
LBs	Lewy bodies
L-DOPA	Levodopa
MAO	Monoamine oxidase
MAO-A	Monoamine oxidase A
MAO-B	Monoamine oxidase B
MPP^+	1-Methyl-4-phenylpyridinium
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NMDA	*N*-Methyl-D-aspartate
ODT	Orally disintegrating tablet
PCP	Phencyclidine
PD	Parkinson's disease
PEA	Phenylethylamine
ROS	Reactive oxygen species
S	Substrate
SET	Single electron transfer
SOD	Superoxide dismutase
UPS	Ubiquitin proteasome system
V	Reaction rate
V_{max}	Maximum velocity

ABSTRACT

Parkinson's disease (PD) is a multifactorial neurodegenerative disease believed to be caused by a number of factors. This has made the successful treatment of the disease very difficult, as the underlying cause of degeneration is still unknown. Monoamine oxidase (MAO-B) inhibitors have been used in the treatment of PD. MAO-B is known to be involved in the catalytic oxidation of biogenic amines, a reaction which produces aldehydes and hydrogen peroxide as by-products. Both these by-products can be toxic if not rapidly cleared. Inhibitors of MAO-B conserve the depleted supply of dopamine and also stoichiometrically decreases the amount of toxic by-products formed. Thus, MAO-B inhibitors may offer both symptomatic and neuroprotective effects that can aid in the treatment of PD.

This study is part of the ongoing investigation into the development of new selective reversible inhibitors of MAO-B. Literature reports that isatin, a small, reversible, endogenous MAO inhibitor, found in the brain, can inhibit both MAO-A and MAO-B enzymes. Previous studies have shown that *(E)*-5-styrylisatin and *(E)*-6-styrylisatin are reversible inhibitors of human MAO-A and -B. Both homologues are reported to exhibit selective binding to the MAO-B isoform with *(E)*-5-styrylisatin being the most potent inhibitor. To further investigate these structure–activity relationships (SAR), in the present study, additional C5- and C6-substituted isatin analogues were synthesized and evaluated as inhibitors of recombinant human MAO-A and MAO-B. A series of structurally related corresponding anilines, which are synthetic precursors in the synthesis of isatin derivatives, were also evaluated as MAO inhibitors. This study is part of an attempt to identify new inhibitors with enhanced potencies and specificities for both MAO-A or MAO-B.

In general, C5- and C6-substitution of isatin leads to enhanced binding affinity to both MAO isozymes, compared to isatin, and in most instances result in selective binding to the MAO-B isoform. The most potent MAO-B inhibitor 5-(4-phenylbutyl)isatin, exhibited an IC_{50} value of 0.66 nM and the most potent MAO-A inhibitor was found to be 5-phenylisatin with an IC_{50} value of 562 nM. Crystallographic and modelling studies suggest that the isatin ring binds to the substrate cavities of MAO-A and -B and is stabilized by hydrogen bond interactions between the NH and the C2 carbonyl oxygen of the dioxoindolyl moiety and water molecules present in the substrate cavities of MAO-A and -B.

Based on these observations and the close structural resemblance between isatin and its phthalimide isomer, a series of phthalimide analogues were synthesized and evaluated as MAO inhibitors. The results showed that the C5 substituted phthalimides were very potent competitive inhibitors with IC_{50} values ranging 0.007 to 2.5 μM for MAO-B and IC_{50} values ranging 0.22 to 9.0 μM for MAO-A. The 5-(4-benzyloxy)phthalimide was the most potent MAO-B inhibitor in the phthalimide series, with an IC_{50} value of 0.007 μM. The results of modelling studies showed that hydrogen-bond interactions between the phthalimide carbonyl oxygen and the enzyme amino acid residues and the integral water molecules are important for the binding of phthalimide to the active site of MAO-B.

The potent competitive inhibition and activities of the C5 substituted phthalimide analogues towards MAO-B has led us to investigate a structurally similar series of C4-substituted phthalonitriles. A series of C4-substituted phthalonitriles were prepared and evaluated as inhibitors of MAO-B. In general, the phthalonitriles were very potent competitive inhibitors of MAO-B with IC_{50} values ranging from 0.005–6.02 μM. 5-(4-benzyloxy)phthalonitrile was found to be the most potent inhibitor for human MAO-B with an IC_{50} value of 0.005 μM.

To further investigate the effect of the nitrile group in this class of compounds, C3 and C4 substituted benzonitriles were prepared and evaluated for MAO inhibition. The results showed that similar to the phthalonitriles, the benzonitriles were also potent inhibitors of human MAO-B, with IC_{50} values ranging from 0.785-1.39 μM. The benzonitriles, however, were not as potent as the corresponding phthalonitriles. These findings suggest that, although two nitrile groups are more optimal for inhibition, the presence of a second nitrile group is not a necessity for potent MAO-B inhibition. Placement of the nitrile group at C3 resulted in more potent MAO-B inhibition compared to placement of the nitrile at C4.

OPSOMMING

Parkinson se siekte (PD) is 'n multifaktoriële, neurodegeneratiewe siekte. Dit word aanvaar dat PD deur 'n aantal faktore veroorsaak word. Suksesvolle behandeling van die siekte is egter problematies omdat die onderliggende oorsake daarvan nog onbekend is. Monoamienoksidase-B (MAO-B) -remmers is reeds gebruik vir die behandeling van PD. MAO-B is betrokke by die katalitiese oksidasie van biogeniese amiene, waartydens aldehiede en waterstofperoksied as neweprodukte gevorm word. Beide hierdie neweprodukte mag toksies wees indien dit nie vinnig opgeruim word nie. MAO-B-remmers veroorsaak dat die verminderde voorraad dopamien bewaar word en verlaag die hoeveelheid toksiese neweprodukte wat stoïgiometries vorm. MAO-B-remmers mag dus simptomatiese verligting sowel as neurobeskerming bied, wat bruikbaar kan wees vir die behandeling van PD.

Hierdie studie vorm deel van 'n deurlopende ondersoek na die ontwikkeling van nuwe, selektiewe, omkeerbare MAO-B-remmers. Volgens die literatuur is isatien 'n klein, omkeerbare, endogene MAO-remmer wat in die brein aangetref word en wat daartoe in staat is om beide MAO-A- en MAO-B-ensieme te inhibeer. Vorige ondersoeke het getoon dat *(E)*-5-stirielisatien en *(E)*-6-stirielisatien menslike MAO-A en -B omkeerbaar inhibeer. Beide homoloë bind selektief aan die MAO-B isoform, met *(E)*-5-stirielisatien wat die mees potente remming vertoon. Ten einde hierdie struktuuraktiwiteitsverwantskappe (SAV), verder te ondersoek in die huidige studie, is addisionele C5- en C6-gesubstitueerde isatienanaloë gesintetiseer en geëvalueer as remmers van rekombinante, menslike MAO-A en -B. 'n Reeks struktureelverwante, ooreenstemmende aniliene, wat sintetiese voorlopers in die sintese van isatienderivate is, is ook as MAO-remmers geëvalueer. Hierdie studie vorm deel van 'n poging om nuwe remmers met verbeterde potensie en spesifisiteit vir beide MAO-A en MAO-B bekend te stel.

In die algemeen lei C5- en C6-substitusie van isatien tot verhoogde bindingsaffiniteit vir beide MAO-isosieme, vergeleke met isatien, en in die meeste gevalle ook tot selektiewe binding aan die MAO-B-isoform. Die mees potente MAO-B-remmer, 5-(4-fenielbutiel)isatien, se IC_{50}-waarde was 0.66 nM en die mees potente MAO-B-remmer, 5-fenielisatien, het 'n IC_{50}-waarde van 562 nM getoon. Kristallografiese en modelleringstudies dui daarop dat die isatienring in die substraatholtes van MAO-A en -B bind en gestabiliseer word deur waterstofbindingsinteraksies tussen die NH en die C2-karbonielsuurstof van die dioksoïndoliel-eenheid en watermolekules wat in die substraatholtes van MAO-A en -B teenwoordig is.

Na aanleiding van hierdie waarnemings en die noue strukturele verwantskap tussen isatien en sy ftaalimiedisomeer, is 'n reeks ftaalimiedanaloë gesintetiseer en as MAO-remmers geëvalueer. Die resultate toon dat die C5-gesubstitueerde ftaalimiede besonder sterk kompetitiewe remmers is met IC_{50}-waardes van 0.007 tot 2.5 µM vir MAO-B en IC_{50}-waardes van 0.22 tot 9.0 µM vir MAO-A. 5-(4-Bensieloksie)ftaalimied was die sterkste MAO-B-remmer in die ftaalimiedreeks met 'n IC_{50}-waarde van 0.007 µM. Modelleringstudies het getoon dat die waterstofbindingsinteraksies tussen die karbonielsuurstof van die ftaalimied en die aminosuurresidue van die ensiem en die integrale watermolekules belangrik is vir die binding van ftaalimied aan die aktiewe setel van MAO-B.

Die kragtige kompetitiewe inhibisie en aktiwiteit van die C5-gesubstitueerde ftaalimiedanaloë vir MAO-B het gelei tot die ondersoek van 'n struktureel soortgelyke reeks van C4-gesubstitueerde ftalonitriele. 'n Reeks C4-gesubstitueerde ftalonitriele is gesintetiseer en as MAO-B-remmers geëvalueer. Oor die algemeen was die ftalonitriele hoogs potente MAO-B-remmers met IC_{50}-waardes wat van 0.005 tot 6.02 µM gestrek het. Dit is bevind dat 5-(4-bensieloksie)ftalonitriel die kragtigste remmer vir menslike MAO-B was met 'n IC_{50}-waarde van 0.005 µM.

Ten einde die invloed van die nitrielgroep op hierdie klas verbindings verder te ondersoek, is die C3- en C4-gesubstitueerde bensonitriele berei en geëvalueer as MAO-remmers. Die resultate het getoon dat die bensonitriele, soos die ftalonitriele, ook kragtige remmers van MAO-B is, met IC_{50}-waardes van 0.785-1.39 µM. Die bensonitriele was egter minder potent as die ftalonitriele. Hierdie bevindings dui daarop dat, alhoewel twee nitrielgroepe optimaal vir remming is, die teenwoordigheid van 'n tweede nitrielgroep nie noodsaaklik is vir potente MOA-B-remming nie. Verbindings met die nitrielgroep in die C3-posisie was kragtiger MAO-B-remmers as dié met die nitrielgroep in die C4-posisie.

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LETTER OF PERMISSION

TO WHOM IT MAY CONCERN

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14th November, 2011

Dear Sir / Madam,

CO-AUTHORSHIP ON RESEARCH PAPERS

The undersigned, as co-authors of the research articles listed below, hereby give permission to Miss Clarina Ilara Manley-King to submit the papers as part of the degree PhD in Pharmaceutical Chemistry at the North-West University, Potchefstroom Campus:

I. **INHIBITION OF MONOAMINE OXIDASE BY SELECTED C5- AND C6-SUBSTITUTED ISATIN ANALOGUES**

II. **INHIBITION OF MONOAMINE OXIDASE BY C5-SUBSTITUTED PHTHALIMDE ANALOGUES**

III. **MONOAMINE OXIDASE INHIBITION BY C4-SUBSTITUTED PHTHALONITRILES**

Yours sincerely,

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J. P. Petzer J. J. Bergh