Syntheses of sulfanylphthalimide and xanthine analogues and their evaluation as inhibitors of monoamine oxidase and as antagonists of adenosine receptors

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I dedicate this thesis to the one true and faithful God.

Preface

This doctoral thesis is submitted in the article format. As required from the A-rules of the North-West University (NWU), the stated purpose for what each co-author's relevant share was in each of the presented research articles, as well as the written declaration for consent of each co-author, are provided in Chapters 6, 7 and 8. All three research articles presented in this thesis were compiled for submission to Bioorganic & Medicinal Chemistry Letters. The statement of copy right and relevant author instructions for this journal is offered in section 6.4 and 6.5, respectively.

All scientific research for this thesis was conducted by Mrs. M. M. van der Walt at the NWU, Potchefstroom campus.

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Abstract

Currently L-DOPA is the drug most commonly used for the treatment of Parkinson's disease (PD). However, the long-term use of L-DOPA is associated with the development of motor fluctuations and dyskinesias. Treatment mainly addresses the dopaminergic features of the disease and leaves its progressive course unaffected. An optimal treatment would be a combination of both motor and non-motor symptom relief with neuroprotective properties. Two drug targets have attracted the attention for PD treatment, namely monoamine oxidase B (MAO-B) and adenosine A_{2A} receptors. MAO-B inhibitors enhance the elevation of dopamine levels after L-DOPA treatment, improve motor functions and may also possess neuroprotective properties. The antagonistic interaction between A_{2A} and dopamine receptors in the striatopallidal pathway, which modulates motor behaviour, has also become a potential strategy for PD treatment. Blockade of the A_{2A} receptor exerts both anti-symptomatic and neuroprotective activities and offer benefit for motor symptoms and motor complications. This thesis seeks to synthesize novel drug treatments for PD by exploring both MAO-B inhibitors and adenosine A_{2A} receptor antagonists and to assess the prospects for drug modification to increase activity.

MAO-B inhibitors

Based on a recent report that the phthalimide moiety may be a useful scaffold for the design of potent MAO-B inhibitors, the present study examines a series of 5-sulfanylphthalimide analogues as potential inhibitors of both human MAO isoforms. The results document that 5-sulfanylphthalimides are highly potent and selective MAO-B inhibitors with all of the examined compounds possessing IC_{50} values in the nanomolar range. The most potent inhibitor, 5-(benzylsulfanyl)phthalimide, exhibits an IC_{50} value of 0.0045 µM for the inhibition of MAO-B with a 427–fold selectivity for MAO-B compared to MAO-A. We conclude that 5-sulfanylphthalimides represent an interesting class of MAO-B inhibitors and may serve as lead compounds for the design of antiparkinsonian therapy.

It has recently been reported that nitrile containing compounds frequently act as potent MAO-B inhibitors. In an attempt to identify additional potent and selective inhibitors of MAO-B and to contribute to the known structure-activity relationships of MAO inhibition by nitrile containing compounds, the present study examined the MAO inhibitory properties of series of novel sulfanylphthalonitriles and sulfanylbenzonitriles. The results document that the evaluated compounds are potent and selective MAO-B inhibitors with most homologues possessing IC_{50} values in the nanomolar range. In general, the sulfanylphthalonitriles exhibited higher binding affinities for MAO-B than the corresponding sulfanylbenzonitrile homologues. Among the compounds evaluated, 4-[(4-bromobenzyl)sulfanyl]phthalonitrile is a particularly promising

inhibitor since it displayed a high degree of selectivity (8720-fold) for MAO-B over MAO-A, and potent MAO-B inhibition (IC_{50} = 0.025 µM). Based on these observations, this structure may serve as a lead for the development of therapies for neurodegenerative disorders such as Parkinson's disease.

Adenosine A_{2A} receptor antagonism

Most adenosine A_{2A} receptor antagonists belong to two different chemical classes, the xanthine derivatives and the amino-substituted heterocyclic compounds. In an attempt to discover high affinity A_{2A} receptor antagonists for PD and to further explore the structure-activity relationships of A_{2A} antagonism by the xanthine class of compounds, this study examines the A_{2A} antagonistic properties of series of (*E*)-8-styrylxanthine, 8-(phenoxymethyl)xanthine and 8-(3-phenylpropyl)xanthine derivatives. The results document that among these series, the (*E*)-8-styrylxanthines are the most potent antagonists with the most potent homologue, (*E*)-1,3-dietyl-7-methyl-8-[(3-trifluoromethyl)styryl]xanthine, exhibiting a K_i value of 11.9 nM. This compound was also effective in reversing haloperidol-induced catalepsy in rats. The importance of substitution at C8 with the styryl moiety was demonstrated by the finding that none of the 8-(phenoxymethyl)xanthines and 8-(3-phenylpropyl)xanthines exhibited high binding affinities for the A_{2A} receptor. It was also concluded that (*E*)-8-styrylxanthines are potent A_{2A} antagonists with particularly the 1,3-dietyl-7-methylxanthine substitution pattern being most appropriate for high affinity binding.

Conclusion

The results of these studies have established that all of the sulfanylphthalimides, sulfanylphthalonitriles and sulfanylbenzonitriles examined display significant MAO-B inhibitory properties *in vitro* with IC_{50} values in the low µM to nM range. Good A_{2A} receptor affinity was demonstrated by the xanthines containing a styryl moiety, while the phenoxymethyl and phenylpropyl xanthines exhibited poor activity.

Keywords: Parkinson's disease, monoamine oxidase, phthalimide, phthalonitrile, benzonitrile, inhibition, adenosine A_{2A} receptors, antagonist, xanthine

Uittreksel

L-DOPA is tans die voorkeurgeneesmiddel vir die behandeling van Parkinson se siekte (PD). Ongelukkig word langtermyn gebruik met L-DOPA geassosieer met die ontwikkeling van motoriese fluktuasies en diskineses. PD-behandeling is hoofsaaklik gerig op die dopamienergiese eienskappe van die siekte, met die gevolg dat die progressie van die siekte nie gestuit word nie. ‘n Geneesmiddel wat beide die motoriese en nie-motoriese simptome verlig en ook neurobeskermende eienskappe toon sou die ideale behandeling bied.

Monoamienoksidase-B (MAO-B) en adenosien A_{2A}-reseptore is twee belowende geneesmiddelteikens wat na vore getree het vir die behandeling van PD. MAO-B-remmers verhoog dopamien, wanneer dit toegedien word tydens of na behandeling met L-DOPA. Dit verbeter motoriese fluktuasies en het ook moontlike neurobeskermende eienskappe. Die antagonistiese interaksies tussen A_{2A}- en dopamienreseptore in die striatopallidale weg, wat motoriese beweging reguleer, is ook ‘n potensiële teiken vir die behandeling van PD. Deur A_{2A}-reseptore te blokkeer, word simptome verlig en neurobeskermende effekte uitgeoefen, wat voordelig is vir die verligting van motoriese simptome en motoriese komplikasies. In hierdie studie word gepoog om nuwe geneesmiddels te sintetiseer wat eerstens as MAO-B-remmers en tweedens as A_{2A}-reseptor antagoniste optree vir die behandeling van PD en om geneesmiddelmolekules te modifiseer vir verhoogde aktiwiteit.

MAO-B-remmers

Na aanleiding van onlangse bevindinge, dat ftaalimiede gebruik kan word om aktiewe MAO-B-remmers te ontwerp, het die huidige studie 5-sulfanielftaalimied-analoë ondersoek as potensiële remmers van beide menslike MAO-subtipes. Die resultate het getoon dat 5-sulfanielftaalimiede potente selektiewe MAO-B-remmers is en dat al die verbindings in die reeks se IC_{50}-waardes in die nM-gebied was. Die beste inhibeerder, 5-(bensielsulfaniel)ftaalimied, met ‘n IC_{50}-waarde van 0.0045 µM vir MAO-B-remming, was 427-keer meer selektief vir MAO-B as vir MAO-A. Hierdie bevinding toon dat die 5-sulfanielftaalimiede ‘n interessante klas MAO-B-remmers is en as uitgangsverbindings gebruik kan word om meer potente MAO-remmers te ontwerp vir die behandeling van PD.

Daar is onlangs gerapporteer dat nitrielbevattende verbindings as kragtige MAO-B-remmers funksioneer. Die huidige studie poog om ook addisionele potente en selektiewe remmers van MAO-B te identifiseer, deur sintese van sulfanielftalonitriel- en sulfanielbensonitriel-analoë en om sodoende ‘n bydrae te maak tot die huidige kennis van struktuur-aktiwiteitsverwantskappe van MAO-inhibisie deur nitrielbevattende verbindings. Die resultate van hierdie studie toon dat die verbindings potente en selektiewe MAO-B-remmers is en dat die meerderheid verbindings in dié reeks se IC_{50}-waardes in die nM-gebied was. In die algemeen het die sulfanielftalonitriele ‘n

hoër bindingsaffiniteit vir MAO-B getoon as hul sulfanielbensonitriel eweknieë. Verbinding 4-[(4-bromobensiel)sulfaniel]ftalonitriel beskik oor 'n IC_{50}-waarde van 0.025 µM vir MAO-B-inhibisie en 'n 8720 maal hoër selektiwiteit vir MAO-B as vir MAO-A. Uit die resultate kan die gevolgtrekking gemaak word dat die sulfanielnitriele as uitgangsverbindings vir ontwikkeling van geneesmiddelbehandelings vir neurodegeneratiewe siektes soos vir PD gebruik kan word.

Adenosien A_{2A}-antagonisme

Die meerderheid adenosien A_{2A}-reseptorantagoniste maak deel uit van twee verskillende chemiese klasse, naamlik die xantien-derivate en amino-gesubstitueerde heterosikliese verbindings. In 'n poging om hoë affiniteit A_{2A}-reseptorantagoniste vir PD daar te stel en om die struktuur-aktiwitieitsverwantskappe vir A_{2A}-antagonisme deur die xantiene verder te ondersoek, evalueer hierdie studie die A_{2A}-antagonistiese eienskappe van 'n reeks van (*E*)-8-stirielxantiene, 8-(fenoksiemetiel)xantien- en 8-(3-fenielpropiel)xantien-derivate. Die resulate toon dat die (*E*)-8-stirielxantiene die mees potente antagoniste is, met (*E*)-1,3-dietiel-7-metiel-8-[(3-trifluorometiel)stiriel]xantien wat 'n K_i-waarde van 11.9 nM toon. Hierdie verbinding was ook in staat om haloperidol-geïnduseerde katalepsie in rotte om te keer. Die belang van substitusie op C8 met 'n stirielketting is beklemtoon deur die bevinding dat die 8-(fenoksiemetiel)xantiene en 8-(3-fenielpropiel)xantiene geen affiniteit vir die A_{2A}-reseptor getoon het nie. Daar is ook gevind dat 1,3-dietiel-7-metielxantien-substitusie, in die (*E*)-8-stirielxantiene, tot 'n verhoogde bindingsaffiniteit vir die A_{2A}-reseptore gelei het.

Gevolgtrekking

Die resultate van die bogenoemde studies het bevestig dat al die geëvalueerde sulfanielftaalimiede, sulfanielftalonitriele en sulfanielbensonitriele goeie *in vitro* MAO-B-remmende eienskappe toon, met IC_{50}-waardes in die lae µM- tot nM-gebied. Die stirielxantiene was goeie A_{2A}-antagoniste, maar die fenoksiemetiel- en fenielpropiel-xantiene het swak A_{2A}-reseptoraffiniteit getoon.

Sleutelwoorde: Parkinson se siekte, monoamienoksidase, ftaalimied, ftalonitriel, bensonitriel, inhibisie, adenosien A_{2A}-reseptore, antagonis, xantien

Abbreviations

A

AAD	Aromatic L-amino acid decarboxylase
AMP	Adenosine monophosphate
AR-JP	Autosomal recessive juvenile Parkinson's disease
ATP	Adenosine triphosphate

C

cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
COMT	Catechol-O-methyltransferase
CSC	(*E*)-8-(3-Chlorostyryl)caffeine
C-terminus	Carboxy-terminus

D

DMPX	3,7-Dimethyl-1-propargylxanthine
DOPAC	3,4-Dihydroxyphenylacetic acid
DPCPX	8-Cyclopentyl-1,3-dipropylxanthine

F

FAD	Flavine adenine dinucleotide

G

GABA	Gamma-aminobutyric acid
G-protein	Guanine nucleotide-binding protein
GPCRs	G-protein-coupled receptors
GPi	Globus pallidus internal
GPe	Globus pallidus external

L

L-DOPA	3,4-Dihydroxy-L-phenylalanine

M

MAO	Monoamine oxidase
MAO-A	Monoamine oxidase, type A
MAO-B	Monoamine oxidase, type B

mp	Melting point
$MPDP^+$	1-Methyl-4-phenyl-2,3-dihydropyridinium
MPP^+	1-Methyl-4-phenylpyridinium
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
3-O-MD	3-O-Methyldopa
3-MT	3-methoxytyramine

N

N-terminus	Amino-terminus

O

6-OHDA	6-hydroxydopamine

P

Pael-R	Parkin-associated endothelin receptor-like receptor
PD	Parkinson’s disease

R

r.t.	Room temperature

S

SAR	Structure-activity relationship
SNpc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
STN	Subthalamic nucleus

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Preparation of the key starting material for article 3

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"Trust in the LORD with all your heart
and lean not on your own understanding..."

Proverbs 3:5