

Inhibition of monoamine oxidase by caffeine- and phthalide analogues

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Preface

This thesis is submitted in article format consisting of three original research articles. Two of the stated articles were submitted to the academic journals, *European Journal of Medicinal Chemistry* and *Arzneimittelforschung*. The author guidelines for each submitted article are also included. All scientific research for the purpose of this thesis was conducted by Ms B. Strydom at the North-West University, Potchefstroom campus.

Letters of agreement from the co-authors of the research articles and the publishing agreements from the editors of the stated journals are included.

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, Belinda Strydom hereby declare that the dissertation with the title: INHIBITION OF MONOAMINE OXIDASE BY CAFFEINE- AND PHTHALIDE ANALOGUES is my own work and has not been submitted at any other university either whole or in part.

Strydom, Belinda
25 October 2012

Letter of agreement



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To whom it may concern,

Dear Sir/Madam

CO-AUTHORSHIP ON RESEACH PAPERS

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

- 8-Aryl- and alkyloxycaffeine analogues as inhibitors of monoamine oxidase, *European Journal of Medicinal Chemistry*
- Structure-activity relationships for the inhibition of monoamine oxidase by 8-(2-phenoxyethoxy)caffeine analogues, submitted as: The inhibition of monoamine oxidase by 8-(2-phenoxyethoxy)caffeine analogues, *Arzneimittelforschung/ Drug Research*
- Inhibition of monoamine oxidase by phthalide analogues

Yours sincerely,

Prof. J.P Petzer

Prof. J.J. Bergh

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“Every one who is seriously involved in the pursuit of science becomes convinced that a spirit is manifest in the laws of the Universe—a spirit vastly superior to that of man, and one in the face of which we with our modest powers must feel humble.”

-Albert Einstein-

Abstract

Monoamine oxidase (MAO) consists of two isoforms, MAO-A and MAO-B. MAO is responsible for the oxidation of neurotransmitter and dietary amines. The MAO-B isoform, in particular, is considered to be a drug target for the treatment of neurodegenerative disorders such as Parkinson's disease (PD). Inhibition of MAO-B may conserve dopamine in the brain, resulting in symptomatic relief of PD. Furthermore, inhibition of MAO-B may prevent the formation of neurotoxic metabolic products and thus MAO-B inhibitors may exert a neuroprotective effect. For these reasons, MAO-B inhibitors have been used as antiparkinsonian agents. The MAO-B inhibitors that are currently being used in the treatment of PD are irreversible inhibitors. In addition to the potential adverse effects associated with irreversible inhibition, the recovery of MAO activity after inactivation by an irreversible inhibitor may require several weeks. Reversible inhibitors may have less adverse effects and enzyme recovery after withdrawal is almost immediate.

In this study three series of novel reversible MAO inhibitors were synthesized and their MAO inhibitory properties were examined. For the first two series, caffeine was employed as a scaffold with alkyloxy substitution on C8 of the caffeine moiety. Caffeine is a weak inhibitor of MAO-B, but substitution on C8 increases its inhibitory activity towards MAO-B as illustrated with the potent MAO-B inhibitor, (*E*)-8-(3-chlorostyryl)caffeine (CSC). In a previous study it was demonstrated with 8-benzyloxycaffeine that an alkyloxy side chain may result in potent inhibitors of both MAO-A and -B. Based on these reports the first two series of caffeine analogues contained alkyloxy side chains on C8 of the caffeine. The target caffeine analogues were synthesized by condensing an appropriately substituted alcohol with 8-chlorocaffeine.

For the third series, 6-benzyloxyphthalide was used as lead compound and a series of phthalide analogues were synthesized with various alkyloxy side chains on the C6 position. In previous studies it has been shown that benzyloxy substitution on the C5 positions of isatin and phthalimide resulted in potent MAO inhibitors. Isatin and phthalimide are structurally related to phthalide and the C5 position on isatin and phthalimide are homologues to the C6 position on phthalide. Therefore, the third series explored the MAO inhibitory properties of phthalide analogues with alkyloxy

side chains on C6. To investigate the importance of the oxy moiety for MAO inhibitory activity, the effects of benzylamino substitution on the C6 position of the phthalide ring was also examined. The alkyloxyphthalide analogues were synthesized by reacting 6-hydroxyphthalide with an appropriately substituted alkyl bromide. The benzylaminophthalide was synthesized, in turn, according to the same procedure from 6-aminophthalide and benzyl bromide.

The synthesized compounds were evaluated as inhibitors of recombinant human MAO-A and -B. Kynuramine, a MAO-A/B mixed substrate served as enzyme substrate. Kynuramine is oxidized by the MAOs to yield 6-hydroxyquinoline, a compound which fluoresces in alkaline media. Concentration measurements of 6-hydroxyquinoline can conveniently be made via fluorescence spectrophotometry since both kynuramine and the inhibitors are non-fluorescent under these assay conditions. The inhibition potencies of the test compounds were expressed as the corresponding IC_{50} values. Representative inhibitors in each series were selected to evaluate the reversibility of inhibition of the compounds.

In the first series, the 8-aryl and alkyloxycaffeine analogues were found to be reversible inhibitors of MAO-A and -B with greater selectivity towards MAO-B. A particularly potent MAO inhibitor among the first series was 8-[2-(4-bromophenoxy)ethoxy]caffeine with IC_{50} values of 1.65 μ M and 0.166 μ M towards MAO-A and -B, respectively. Based on the promising inhibitory activities of the first series, in the second series, the MAO inhibitory properties of the 8-(2-phenoxyethoxy)caffeine analogues were further explored. For this purpose, 8-(2-phenoxyethoxy)caffeine analogues with different substituents on C4 of the phenyl ring were synthesized. Structure-activity relationship (SAR) studies were carried out in order to correlate selected physicochemical properties of the C4 substituents with the inhibitory activities towards MAO. It was found that substituents on C4 of the phenyl ring which are more lipophilic, electron withdrawing and has greater bulkiness may result in more potent inhibition towards MAO-A. The most potent MAO-A inhibitor of this series was 8-[2-(4-iodophenoxy)ethoxy]caffeine with an IC_{50} value of 0.924 μ M. The results also documented that MAO-B inhibition potency correlated with the electronic parameters of the substituent on C4 of the phenyl ring, with highly electron withdrawing substituents yielding potent MAO-B inhibitors. This behaviour is exemplified by 8-[2-(4-trifluoromethylphenoxy)ethoxy]caffeine, which inhibited MAO-B with an IC_{50} value of 0.061 μ M. In the third series the phthalide analogues also

proved to be potent reversible inhibitors of both MAO-A and –B. As observed with the caffeine derived compounds of series 1 and 2, these compounds were also more selective towards MAO-B. The most potent MAO-B inhibitor was 6-[4-(trifluoromethyl)benzyloxy]phthalide with an IC_{50} value of 0.0014 μ M while the most potent MAO-A inhibitor was 6-(3-phenylpropoxy)phthalide with an IC_{50} value of 0.1 μ M. The least potent inhibitor of MAO-A and –B was 6-(benzylamino)phthalide which inhibited MAO-A with an IC_{50} value of 59.9 μ M and displayed no inhibition towards MAO-B.

To examine the reversibility of the target compounds, two methods were employed. For the first two series, the caffeine derived inhibitors, 8-[2-(4-bromophenoxy)ethoxy]caffeine was selected as representative inhibitor. MAO-A and –B were pre-incubated with the representative inhibitor for time periods of 0, 15, 30 and 60 minutes and the residual catalytic rates were measured. The results showed that there was no significant time-dependent decrease in catalytic rate, which is an indication that the representative inhibitor is a reversible inhibitor of MAO-A and –B. For the third series, reversibility of MAO-A and –B inhibition was determined by using 6-(3-phenylpropoxy)phthalide and 6-[4-(trifluoromethyl)benzyloxy]phthalide as representative inhibitors, respectively. MAO was pre-incubated with the respective inhibitors at concentrations of 10-fold IC_{50} and 100-fold IC_{50} . After dilution of the incubations to 0.1-fold IC_{50} and 1-fold IC_{50} , the residual enzyme activities were measured. After the dilution to 1-fold IC_{50} , the activities of MAO-A and –B were recovered to 86% and 68%, respectively. This behavior is consistent with reversible inhibition. To further establish the reversibility of inhibition by the representative caffeine and phthalide inhibitors, Lineweaver-Burk plots were constructed. The results showed that the Lineweaver-Burk plots intersected on the y-axis, which is an indication that the representative inhibitors are competitive and therefore reversible inhibitors of MAO-A and –B. Reversible inhibition is in general a desirable trait since irreversible MAO inhibitors are frequently associated with unfavourable and potentially fatal side effects.

Based on the findings that the caffeine and phthalide analogues examined in this study are potent and reversible inhibitors of MAO-A and –B, these compounds represent suitable candidates for the development of novel MAO inhibitors for the treatment of PD.

Uittreksel

Die ensiem, monoamienoksidase (MAO) bestaan uit twee isovorme, naamlik, MAO-A en MAO-B. MAO is verantwoordelik vir die oksidasie van neurotransmitter en diëetverwante amiene. MAO-B word beskou as 'n belangrike geneesmiddelteiken vir die behandeling van neurodegeneratiewe siektes soos Parkinson se siekte. Inhibisie van MAO-B verhoed die metaboliese afbraak van dopamien in die brein, en veroorsaak sodoende verligting van die simptome van Parkinson se siekte. Die inhibisie van MAO-B kan moontlik ook die vorming van neurotoksiese metaboliese produkte voorkom en sodoende 'n neurobeskermende effek tot gevolg hê. MAO-B-remmers word tans gebruik vir die behandeling van Parkinson se siekte omdat dit 'n simptomatiese sowel as 'n neurobeskermende effek het. Huidige MAO-B-remmers wat vir die behandeling van Parkinson se siekte aangewend word, bind onomkeerbaar aan die ensiem en lei dikwels tot ernstige newe-effekte. Met staking van behandeling met onomkeerbare remmers, kan dit etlike weke neem vir ensiemaktiwiteit om terug te keer na normale vlakke. In teenstelling hiermee lei omkeerbare remmers tot minder newe-effekte en, na staking van behandeling, keer ensiemaktiwiteit terug na normale vlakke sodra die remmer uit die weefsel opgeruim is.

Die huidige studie beskryf die sintese van drie reekse nuwe, omkeerbare MAO-remmers en ondersoek vervolgens die MAO-inhiberende effekte van die verbindings. Kafeïen is in die eerste twee reekse as leidraadverbinding gebruik en die effek van alkieloksiesubstitusie op C8 van die kafeïengroep op die MAO-inhiberende aktiwiteit is ondersoek. Kafeïen is 'n swak remmer van MAO-B, maar soos getoon met (*E*)-8-(3-chlorostiriël)kafeïen (CSC), kan C8-substitusie op kafeïen die inhibisie van MAO-B aansienlik verbeter. 'n Vorige studie het getoon dat alkieloksiesykettings op C8, soos in die geval van 8-bensieloksiekafeïen, lei tot potente inhibeerders van beide MAO-A en -B. Die eerste twee reekse kafeïenanaloeë wat in dié studie ondersoek is, is dus gesubstitueer met 'n alkieloksiesyketting op C8 van die kafeïenring. Die verlangde kafeïenanaloeë is gesintetiseer deur die kondensering van 8-chlorokafeïen met 'n gepaste gesubstitueerde alkohol.

In die derde reeks is 6-bensieloksieftalied as leidraadverbinding gebruik en 'n reeks ftaliedanaloeë, met verskeie alkieloksiesykettings op die C6-posisie, is gesintetiseer.

Vorige studies het getoon dat isatien en ftaalimied, met bensieloksiesykettings op die C5-posisies, as potente MAO-remmers optree. Isatien en ftaalimied is struktureel verwant en die C5 posisie op isatien en ftaalimied is homolog aan die C6-posisie op ftalied. Om hierdie rede is ftaliedanaloe, met alkieloksiesykettings op C6, in die derde reeks as potensiële MAO-remmers ondersoek. Om die rol van die suurstofgedeelte in die inhibisie van MAO te bestudeer, is die ftaliedanaloo wat die bensielamino-substituent op die C6-posisie bevat, ook gesintetiseer. Die alkieloksieftaliedanaloe is gesintetiseer deur die reaksie van 6-hidroksieftalied met 'n toepaslike gesubstitueerde alkielbromied. Bensielaminoftalied is gesintetiseer deur die reaksie van 6-aminoftalied met bensielbromied.

Die gesintetiseerde verbindings is as remmers van rekombinante menslike MAO-A en -B geëvalueer. Kinuramien, wat 'n gemengde MAO-A/B substraat is, is as substraat gebruik in hierdie studie. Kinuramien word geoksideer deur MAO om 6-hidroksiekinolien te vorm. Hierdie metaboliet fluoresseer in alkaliese oplossing. Die konsentrasie van 6-hidroksiekinolien is deur fluoressensiespektrometrie gemeet aangesien kinuramien en die remmers nie by hierdie eksperimentele kondisies fluoresseer nie. Die aktiwiteite van die remmers is as hul IC_{50} -waardes uitgedruk. Verteenwoordigende remmers in elke reeks is vir die bepaling van die omkeerbaarheid van inhibisie gekies.

Met die eerste reeks is daar gevind dat 8-ariel- en alkieloksiekafeïenanaloe omkeerbare remmers van MAO-A en -B is, met selektiewe inhibisie van MAO-B. Daar is gevind dat 8-[2-(4-bromofenoksie)etoksie]kafeïen 'n besondere potente remmer van MAO-A en -B is, met IC_{50} -waardes van 1.65 μM en 0.166 μM , respektiewelik. Op grond van die belowende aktiwiteite van die eerste reeks, is daar besluit om in die tweede reeks op die inhibisie-eienskappe van 8-(2-fenoksie-etoksie)kafeïenanaloe te fokus. 'n Reeks 8-(2-fenoksie-etoksie)kafeïenanaloe is dus gesintetiseer met verskillende substituentte op C4 van die fenielring. Struktuuraktiwiteitsverwantskap-studies is gedoen in 'n poging om die fisies-chemiese eienskappe van die C4-substituentte te korreleer met die MAO-inhiberende aktiwiteite. Hierdie studie het onthul dat substituentte op C4 van die fenielring, wat 'n hoë mate van lipofiliteit besit, wat elektrononttrekkend is en wat steries groot is, beter remming van MAO-A tot gevolg het. 'n Voorbeeld hiervan is 8-[2-(4-iodofenoksie)etoksie]kafeïen wat die beste MAO-A-remmer was, met 'n IC_{50} -waarde van 0.924 μM . Vir potente MAO-B-inhibisie is gevind dat elektrononttrekkende substituentte op C4 van die fenielring optimaal is. 'n

Voorbeeld hiervan is 8-[2-(4-trifluorometielfenoksie)etoksie]kafeïen wat 'n IC_{50} -waarde van 0.061 μM vir die remming van MAO-B gehad het. Die derde reeks, die ftaliedanalooë, het ook potente, omkeerbare MAO inhibeerders opgelewer. Daar is ook gevind dat die ftaliedanalooë selektiewe inhibeerders van MAO-B is. 6-[4-(Trifluorometiel)bensieloksie]ftalied was die mees potente MAO-B-remmer, met 'n IC_{50} -waarde van 0.0014 μM . Die mees potente MAO-A-remmer, daarenteen, was 6-(3-fenielpropoksie)ftalied, met 'n IC_{50} -waarde van 0.1 μM . 6-(Bensielamino)ftalied was die swakste MAO-inhibeerder in die derde reeks met 'n IC_{50} -waarde van 59.9 μM vir die inhibisie van MAO-A. Hierdie verbinding het geen remming van MAO-B getoon nie.

Die omkeerbaarheid van remming is bestudeer deur gebruik te maak van twee metodes. 8-[2-(4-Bromofenoksie)etoksie]kafeïen is as verteenwoordigende inhibeerder van die eerste twee reekse gekies. MAO-A en -B is in die teenwoordigheid van hierdie verbinding vir 0, 15, 30 en 60 minute geïnkubeer. Die residuele tempo van katalise is vervolgens gemeet. Geen tydsafhanklike afname in die tempo van katalise is opgemerk nie. Hierdie gedrag is 'n aanduiding dat die verteenwoordigende inhibeerder 'n omkeerbare remmer van MAO-A en -B is. In die derde reeks is die omkeerbaarheid van die remming van MAO-A en -B bepaal deur die gebruik van 6-(3-fenielpropoksie)ftalied en 6-[4-(trifluorometiel)bensieloksie]ftalied, respektiewelik, as verteenwoordigende inhibeerders. Die MAO-ensieme is met die onderskeie inhibeerders geïnkubeer by konsentrasies van tienvoudig die IC_{50} -waarde en honderdvoudig die IC_{50} -waarde. Die residuele ensiemaktiwiteit is gemeet nadat die inkubasies verdun is om konsentrasies van die remmer, wat gelyk is aan 'n tiende van die IC_{50} waarde en gelyk is aan die IC_{50} waarde, onderskeidelik, te lewer. Die resultate het getoon dat die aktiwiteite van MAO-A en -B met verdunning na 'n konsentrasie van die inhibeerder wat gelyk is aan die IC_{50} waarde, terugkeer na 86% en 68% van die kontrole waarde respektiewelik. Hierdie bevinding is 'n aanduiding dat die verteenwoordigende inhibeerders omkeerbare MAO-remmers is. Om verder te bevestig of die verteenwoordigende remmers in reeks 1 en 3 omkeerbaar is, is daar gebruik gemaak van Lineweaver-Burk grafieke. Vir die remming van beide MAO-A en -B is daar gevind dat die Lineweaver-Burk grafieke op die y-as sny, wat 'n aanduiding is dat die verbindings kompeterende remmers is, en dus omkeerbare inhibisie veroorsaak. Omkeerbare remmers van MAO-A en -B is van waarde omdat onomkeerbare remmers ernstige newe-effekte tot gevolg kan hê.

Uit hierdie studie kan daar dus tot die gevolgtrekking gekom word dat kafeïen- en ftaliedanalooë potente, omkeerbare remmers van MAO-A en -B is. Hierdie verbindings kan dus as goeie kandidate vir die ontwikkeling van nuwe MAO-remmers vir die behandeling van Parkinson se siekte geag word.

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Abbreviations

6-OHDA	-	6-Hydroxydopamine
AADC	-	Aromatic amino acid decarboxylase
BBB	-	Blood-brain barrier
COMT	-	Catechol-O-methyltransferase
CSC	-	(<i>E</i>)-8-(3-Chlorostyryl)caffeine
CYP	-	Cytochrome P450
DA	-	Dopamine
DAT	-	Dopamine transporter
Dyn	-	Dynorphin
Enk	-	Enkephalin
FAD	-	Flavin adenine dinucleotide
HRMS	-	High resolution mass spectra
L-dopa	-	Levodopa
MAO	-	Monoamine oxidase
mp	-	Melting point
MPDP ⁺	-	1-Methyl-4-phenyl-2,3-dihydropyridinium
MPPP	-	1-Methyl-4-phenyl-4-propionoxypiperidine
MPTP	-	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NA	-	Noradrenaline
PD	-	Parkinson's disease
ROS	-	Reactive oxygen species
SAR	-	Structure activity relationship
SI	-	Selectivity Index
SNpc	-	Substantia nigra pars compacta
UCH-L1	-	Ubiquitin C-terminal hydrolase-L1