Identification of monoamine oxidase inhibitors using a molecular modelling approach

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Preface

This dissertation is submitted in article format and consists of two original research articles. Those results not included in the articles are presented in Chapters 4 and 5 of the dissertation. The articles were submitted for publication to the academic journals, *Arzneimittelforschung/Drug Research* and *Life Sciences*, respectively. The author guidelines for each journal are also included in the annexure. The research described in this dissertation was conducted by Ms. A Pienaar at the North-West University, Potchefstroom campus.

Letters of agreement from the co-authors of the research articles and the journal instructions of the stated journals are included in the annexure.

Abstract

Monoamine oxidase (MAO) is an enzyme located on the outer mitochondrial membrane and is considered to be a target for the treatment of diseases such as Parkinson's disease and depression. MAO may be classified into two isoforms, MAO-A and MAO-B. Since MAO-A and MAO-B catalyzes the metabolism of serotonin and dopamine, respectively, MAO-A inhibitors are used in the therapy of depression while MAO-B inhibitors are useful in the treatment of Parkinson's disease.

The older nonselective and irreversible MAO inhibitors, however, are not frequently used because they may ellicit potentially dangerous side effects such as the "cheese reaction". The cheese reaction occurs when irreversible MAO-A inhibitors block the metabolism of tyramine in the gastrointestinal tract. Excessive amounts of tyramine subsequently enter the systemic circulation and cause a hypertensive reaction.

This problem may be overcome by the development of selective MAO-B inhibitors and reversible MAO-A inhibitors. Selective MAO-B inhibitors do not cause the cheese reaction, because tyramine is metabolized, in the intestines, by MAO-A. Tyramine also has the ability to displace reversible MAO-A inhibitors and can subsequently be normally metabolized, thus not causing the cheese reaction. Several reseach groups are therefore involved in the discovery of reversible MAO-A and MAO-B inhibitors. As mentioned above, such drugs may be used in the treatment of depression and Parkinson's disease. One approach is the *de novo* design of novel molecules with affinities for MAO-A and MAO-B active sites. In a second approach, existing drugs may be reappropriated as MAO inhibitors. With this approach, approved drugs are screened for the possibility that they, in addition to their action at the indicated target, also act as inhibitors of MAO-A and/or MAO-B. Such drugs may then be applied as MAO inhibitors in the treatment of depression and Parkinson's disease. From a toxicological point of view, it is also of importance to identify MAO-A inhibitory activities among existing drugs as this will alert to the occurance of potential side effects such as the cheese reaction. In this study the second approach will be followed. This study will screen a virtual library of approved drugs for inhibitory activity towards MAO-A and MAO-B.

Molecular modeling may be used to screen virtual libraries of drugs as potential inhibitors of the MAO enzymes. This may conveniently be achieved by employing structure-based or ligand-based pharmacophore models.

In this study a virtual library of approved drugs was screened for secondary inhibitory activities towards the MAO isoforms with the use of structure-based pharmacophore models. There are several advantages to this approach. Molecular modeling aims at reducing the overall cost associated with the discovery and development of a new drug by identifying the most promising candidates to focus the experimental efforts on. It aids in understanding how a ligand binds to the active site of an enzyme. It is relatively easier to re-register a drug for a second pharmacological activity. This approach may also lead to drugs with a multi-target mode of action.

The structure-based pharmacophores were constructed using the known crystallographic structures of MAO-A and MAO-B with the inhibitors, harmine and safinamide, complexed in the active sites, respectively. Employing the MAO-A and MAO-B structure-based pharmacophore model in the virtual screening of a library of approved drugs, 45 compounds were found to map to the MAO-A and MAO-B pharmacophore models.

Among the hits, 29 compounds were selected for *in vitro* evaluation as MAO-A and MAO-B inhibitors. The IC_{50} values for these compounds were determined. After *in vitro* evaluation, 13 compounds showed inhibitory activity towards MAO. Of the 13 compounds 3 showed interesting inhibitory activities. These compounds included caffeine (IC_{50} = 0.761 µM for MAO-A and 5.08 µM for MAO-B), esomeprazole (IC_{50} = 23.2 µM for MAO-A and 48.3 µM for MAO-B) and leflunomide (IC_{50} = 19.1µM for MAO-A and 13.7 µM for MAO-B). The MAO inhibitory properties of caffeine and esomeprazole were further investigated.

The reversibility of MAO inhibition by caffeine and esomeprazole were determined by dialysis and dilution studies. Sets of Lineweaver-Burk plots were constructed to determine the modes of binding of these inhibitors to the MAO enzymes. Both caffeine and esomeprazole were found to be reversible and competitive inhibitors of MAO.

Dialysis of mixtures of caffeine with MAO-A and MAO-B resulted in the recovery of enzyme activity to levels of 97% and 96%, respectively. Dialysis of mixtures of esomeprazole with MAO-A and MAO-B resulted in the recovery of enzyme activity to levels of 93% and 88%, respectively. Similarly, dilution of mixtures containing esomeprazole and MAO-A/MAO-B resulted in the recovery of enzyme activity to levels of 94% and 87%, respectively.For the inhibition of MAO-A and MAO-B by caffeine and esomeprazole, the Lineweaver-Burk plots were indicative of a competitive mode of inhibition.

In an attempt to gain further insignt, caffeine, esomeprazole and leflunomide were docked into models of the active sites of MAO-A and MAO-B. An analysis of the interactions between the enzyme models and the ligands were carried out and the results are discussed in the dissertation

The results of the present study show that screening of a virtual database of molecules with a pharmacophore model may be useful in identifying existing drugs with potential MAO inhibitory activities. The search for new reversible MAO inhibitors for the treatment of diseases, including Parkinson's disease and depression, may be facilitated by employing a virtual screening approach. Such an approach also may be more cost-effective than *de novo* inhibitor design. In addition, the virtual screening approach may alert to potential side effects of existing drugs that may arise as a consequence of a secondary inhibition of MAO.

Keywords: Monoamine oxidase, Esomeprazole, Inhibition, Competitive, Reversible, Caffeine

Uittreksel

Monoamienoksidase (MAO) is ’n ensiem wat op die buitenste mitochondriale membraan voorkom en word as ’n teiken vir die behandeling van Parkinson se siekte en depressie beskou. MAO word as twee isovorme, MAO-A en MAO-B, geklassifiseer. Aangesien MAO-A en MAO-B onderskeidelik verantwoordelik is vir die metabolisme van serotonien en dopamien, word MAO-A-remmers vir die terapie vir depressie gebruik, terwyl MAO-B-remmers vir die behandeling van Parkinson se siekte gebruik word.

Die ouer, nie-selektiewe en onomkeerbare MAO-remmers word egter nie dikwels gebruik nie omdat hulle potensieel gevaarlike newe-effekte soos die kaasreaksie mag ontlok. Die kaasreaksie kom voor wanneer onomkeerbare MAO-A-remmers die metabolisme van tiramien in die spysverteringskanaal blokkeer. Tiramien kry gevolglik toegang tot die sistemiese sirkulasie en veroorsaak ’n hipertensiewe reaksie.

Hierdie probleem kan oorkom word deur die ontwikkeling van selektiewe MAO-B-remmers en omkeerbare MAO-A-remmers. Selektiewe MAO-B-remmers veroorsaak nie die kaasreaksie nie, aangesien tiramien in die ingewande deur MAO-A gemetaboliseer word. Tiramien besit ook die vermoë om omkeerbare MAO-A-remmers te verplaas, om sodoende normaal gemetaboliseer te word. Omkeerbare MAO-A-remmers veroorsaak dus ook nie die kaasreaksie nie. Verskeie navorsingsgroepe fokus tans op die ontdekking van omkeerbare MAO-A en MAO-B remmers. Soos hierbo genoem, kan hierdie geneesmiddels vir die behandeling van depressie en Parkinson se siekte gebruik word. Een benadering wat gevolg kan word, is die *de novo* ontwerp van nuwe molekules met affiniteite vir die MAO-A en MAO-B aktiewe setels. In ’n tweede benadering kan bestaande geneesmiddels heraangewend word as MAO-remmers. Met hierdie benadering word bestaande geneesmiddels getoets vir die moontlikheid dat hulle, bykomend tot hul bekende aktiwiteite, ook as MAO-A- en/of MAO-B-remmers optree. Hierdie geneesmiddels kan gevolglik aangewend word as MAO-remmers vir die behandeling van depressie en Parkinson se siekte. Uit ’n toksikologiese oogpunt is dit ook belangrik om te bepaal of bestaande geneesmiddels as MAO-A-remmers optree omdat MAO-A-inhibisie tot newe-effekte soos die kaasreaksie kan lei. In hierdie ondersoek sal die tweede benadering gevolg word. Hierdie studie gaan ’n virtuele

biblioteek van bestaande geneesmiddels ondersoek vir verbindings wat MAO-A en MAO-B rem.

Molekulêre modellering kan gebruik word om ’n virtuele biblioteek van geneesmiddels te ondersoek vir verbindings wat MAO-A en MAO-B rem. Vir hierdie doel kan struktuur-gebaseerde of ligand-gebaseerde farmakofoormodelle aangewend word.

In hierdie studie is ’n virtuele biblioteek van bestaande geneesmiddels ondersoek vir verbindings wat die MAO-isovorme rem, deur gebruik te maak van struktuur-gebaseerde farmakofoormodelle. Hierdie benadering het verskeie voordele. Molekulêre modellering kan die koste van die ontdekking en ontwikkeling van ’n nuwe geneesmiddel verlaag deur identifisering van die mees belowende middels waarop die eksperimentele pogings gefokus word. Dit is ook meer koste-effektief om ’n geneesmiddel te herregistreer vir ’n sekondêre farmakologiese aktiwiteit as om ’n nuwe geneesmiddel te registreer. Hierdie benadering mag ook lei tot identifisering van geneesmiddels met meervoudige werkingsmeganismes.

Die struktuur-gebaseerde farmakofoormodelle is ontwerp deur gebruik te maak van die kristallografiese strukture van MAO-A en MAO-B met die remmers, harmien en safienamied, gekomplekseer in die aktiewe setels van die onderskeie ensieme. Deur die MAO-A en MAO-B struktuur-gebaseerde farmakofoormodelle te gebruik, is ’n virtuele biblioteek van goedgekeurde geneesmiddels ondersoek vir verbindings wat as remmers kan optree. Daar is gevind dat 45 verbindings die MAO-A en MAO-B farmakofoormodelle pas.

Van hierdie 45 verbindings is 29 geneesmiddels vir *in vitro* evaluasie, as MAO-A- en MAO-B–remmers geselekteer. Vir hierdie doel is die IC_{50}-waardes van hierdie verbindings bepaal. Die resultate het aangedui dat 13 verbindings MAO remming teweegbring. Van hierdie 13 verbindings het 3 geneesmiddels noemenswaardige resultate getoon, naamlik, kafeïen, (IC_{50} =0.761 µM vir MAO-A en 5.08 µM vir MAO-B), esomeprasool, (IC_{50} = 23.2 µM vir MAO-A en 48.3 µM vir MAO-B) en leflunomied, (IC_{50} = 19.1 µM vir MAO-A en 13.7 µM vir MAO-B). Die MAO-remmende vermoë van kafeïen en esomeprasool is verder ondersoek.

Die omkeerbaarheid van MAO-remming deur kafeïen en esomeprasool is met verdunningstudies bepaal. Lineweaver-Burk-grafieke is opgestel om die meganisme van MAO-remming te ondersoek. Daar is gevind dat beide kafeïen en esomeprasool omkeerbare en kompeterende remmers van MAO-A en MAO-B is.

Na dialise van reaksies wat kafeïen en die MAO-ensieme bevat, is ensiemaktiwiteit tot vlakke van 97% en 96% vir MAO-A en MAO-B, onderskeidelik, herwin. Na dialise van reaksies wat esomeprasool en die MAO-ensieme bevat, is ensiemaktiwiteit tot vlakke van 93% en 88% vir MAO-A en MAO-B, onderskeidelik, herwin. Verdunning van reaksies wat esomeprasool en die MAO-ensieme bevat, het ook gelei tot die herwinning van ensiemaktiwiteit tot vlakke van 94% en 87% vir MAO-A en MAO-B, onderskeidelik. Vir die inhibisie van MAO-A en MAO-B deur kafeïen en esomeprasool, was die Lineweaver-Burk grafieke aanduidend van ’n kompeterende remmingsmeganisme.

In ’n poging om verdere insig te kry, is die strukture van kafeïen, esomeprasool en leflunomied in aktiewe setel-modelle van MAO-A en MAO-B gepas. ’n Analise van die interaksies tussen die ensiemmodelle en die ligande is uitgevoer en die resultate word in die verhandeling bespreek.

Die resultate van die huidige studie wys dat farmakofoormodelle gebruik kan word om ’n virtuele databasis te ondersoek vir bestaande geneesmiddels wat MAO-A en MAO-B kan rem. Hierdie benadering kan dus aangewend word vir die identifisering van nuwe omkeerbare MAO-remmers vir die behandeling van siektes soos Parkinson se siekte en depressie. Hierdie benadering is ook meer koste-effektief as die *de novo* ontwerp van nuwe geneesmiddels en kan ook ’n waarskuwing rig dat bestaande geneesmiddels newe-effekte kan hê as gevolg van hul sekondêre werking, naamlik remming van MAO.

Sleutelwoorde: Monoamienoksidase, Esomeprasool, Inhibisie, Kompeterend, Omkeerbaar, Kaffeïen

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List of abbreviations

A

Asn	aspargine

C

Cys	Cysteine
COMT	Catechol-O-methyltransferase

D

3D	Three-dimensional

E

Eso	Esomeprazole

F

FAD	Flavin adenine dinucleotide
FDA	Food and Drug Administration

G

Gln	Glutamine
Glu	Glutamate

H

HPLC	High performance liquid chromatography
HRP	Horseradish peroxidase

I

IC_{50}	Inhibitor concentration at 50% inhibition

Ile	Isoleucine

K

K_m	Michaelis constant

L

Lys	Lysine
Leu	Leucine
Laz	Lazabemide

M

MAO	Monoamine oxidase
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MPP^+	1-Methyl-4-phenylpyridinium

P

Phe	Phenyl
PDB	Protein Data Bank
PPAR-γ	Peroxisome proliferator-activated receptor gamma

R

ROS	Reactive oxygen species
RIMA's	Reversible inhibitors of MAO-A

S

SSRIs	Selective serotonin reuptake inhibitors
SET	Single electron transfer

T

Tyr	Tyrosine
Tol	toloxatone

V

V_{max}	Maximum velocity

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used, was derived from the structure of harmine using the *structure-based* approach

Table 3.4. A virtual library of 20 test compounds (11 MAO-A inhibitors and 9 non-inhibitors) was screened with the pharmacophore model. Below is given a list of compounds that were not four feature hits. The shaded entries are known MAO-A inhibitors (IC_{50} < 5 μM). The structures, not shaded, are known to not bind to MAO-A. The pharmacophore model used, was derived from the structure of harmine using the *structure-based* approach

Table 3.5. The interaction energies of safinamide with the active site residues and waters of MAO-B. Selected interactions among those that are most productive are shaded.

Table 3.6. A list of the compounds in the DrugBank which mapped to the pharmacophore model derived from the structure of safinamide using the *structure-based* approach. These compounds represent drugs which are used systemically by humans. The shaded entries were found to be four feature hits

Table 3.7. A virtual library of 30 test compounds (20 MAO-B inhibitors and 10 non-inhibitors) was screened with the pharmacophore model. Below is given a list of compounds that were found to be four feature hits. The shaded entries are known MAO-B inhibitors (IC_{50} < 5 μM). The structures, not shaded, are known to not bind to MAO-B. The pharmacophore model used, was derived from the structure of safinamide using the *structure-based* approach

Table 3.8 A virtual library of 30 test compounds (20 MAO-B inhibitors and 10 non-inhibitors) was screened with the pharmacophore model. Below is given a list of compounds that were not four feature hits. The shaded entries are known MAO-B inhibitors (IC_{50} < 5 μM). The structures, not shaded, are known to not bind to MAO-B. The pharmacophore model used, was

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