Inhibition of monoamine oxidase B by selective 7-substituted coumarin derivatives

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Introduction
Alzheimer’s disease (AD) and Parkinson’s disease (PD) are the most frequent neurodegenerative disorders and cause of disability among western societies. In the last years, due to their therapeutic potential in this age-related neurodegeneration, currently, acetyl cholinesterase (AChE) inhibitors are the only drugs approved for treatment of cognitive dysfunction in AD and dopamine (DA) replacement therapy with levodopa (L-Dopa) in conjunction with irreversible MAO-B inhibitors (e.g. Deprenyl) is the present first-line treatment regime in PD.

Neurodegeneration
Catecholamines (particular dopamine) are either metabolised by endogenous enzymes such as monoamine oxidases (MAO) or spontaneously degraded by auto-oxidation to yield hydrogen peroxide (H$_2$O$_2$). The H$_2$O$_2$ produced are fed into the reactive oxygen species (ROS) cycle and leads to exacerbation of inflammation and tissue damage. Neuronal tissue MAO-B levels increase ~4 fold with age, resulting in higher dopamine metabolism, rising hydrogen peroxide formation and the development of Aβ plaques.

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mimics the same sequel of neurodegeneration that occurs in man. Its MAO-B-catalysed oxidation leads to the dihydropyridinium intermediate 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP$^+$), which undergoes further oxidation to yield 1-methyl-4-phenyl-pyridinium (MPP$^+$), which is transported via VMAT2 (vesicular monoamine transporter) into the dopaminergic neurons where it inhibits mitochondrial electron transport complex I, resulting in decreased ATP production and cell death.

Monoamine oxidase (MAO)
Monoamine oxidase (MAO) is a ~60 kDa mitochondrial outer membrane FAD (Flavin adenine dinucleotide cofactor) containing enzyme. According to substrate and inhibitor specificities, MAO consists of two isoforms, namely MAO-A and MAO-B. MAO-B catalyses the deamination of β-phenylethylamine and benzylamine and inhibition (e.g. by selegiline) of this enzyme results in anti-Parkinson’s and anti-Alzheimer’s effects.

MAO-B consists of 520 amino acids and is located in the outer mitochondrial membranes of neuronal, glial and other cells. It has 2 cavities (Figure 1) namely the polar entrance cavity (290 Å$^3$) and the hydrophobic substrate cavity (420 Å$^3$) that forms the active site of the enzyme.

Purpose
(See Scheme 1)
In this study the stereoelectronic properties of novel ester and methoxy ester coumarin compounds were investigated to optimise a pharmacophore for MAO-B inhibition. This structure was subsequently conjugated to the neuroprotective xanthinyl moieties of caffeine and the polycyclic structure as these drugs may act via a dual mechanism for the treatment of neurodegenerative disorders and act as an accurate model in the manipulation and development of new, potent, selective reversible MAO-B inhibitors.

Rationale of compounds
Coumarins exhibit a broad spectrum pharmacological activity ranging from HIV-1 reverse transcriptase inhibitory properties to competitive, reversible and selective MAO-B inhibitors and AChE inhibitors. SAR studies revealed that substitution on position 7 (Figure 2A) showed high activity toward both enzymes, while A/B selectivity is modulated through substitution at position 3 and/or 4.

A$^+$$B^-$ antagonists are currently being investigated as possible therapeutic agents for the symptomatic treatment of motor deficits in PD. (E)-8-(3-chlorostyryl) caf-
feine (Figure 2B) was recently reported to be a potent and selective competitive MAO-B inhibitor\textsuperscript{15} and an antagonist of adenosine \textsuperscript{A\textsubscript{2A}}.\textsuperscript{16}

Polycyclic cage compounds (Figure 2C) have been reported to have important pharmaceutical applications in the symptomatic and proposed curative treatment of neurodegenerative diseases by antagonising the NMDA receptor thus preventing cytoplasmic Ca\textsuperscript{2+} influx and eventually neuronal cell death. These compounds might modify and improve the pharmacokinetic and pharmacodynamic properties (e.g. blood-brain barrier permeability) of current drugs.\textsuperscript{17}

**Experimental**

Coumarin analogues were synthesised via etherification, esterification and hydrolysis, and conjugated to the neuroprotective xanthinyl moieties by means of activation chemistry with EDC and DCC, esterification and amination (Figure 3).

**Biological evaluation**

The synthesised compounds were evaluated in vitro as competitive inhibitors of MAO-B, using a spectrophotometric assay that utilised MMTP, an analogue of the neurotoxin MPTP as substrate. MMTP serves as a good substrate for both forms of the enzyme, the dihydropyridinium metabolite undergoes no further oxidation and has a relatively large molar extinction coefficient (\(\varepsilon = 525,000 \text{ M}^{-1}\)). Its maximal absorbance at 420 nm, which is far removed from mitochondrial background MMDP\textsuperscript{+} production, can spectrophotometrically be measured at 420 nm, a wavelength at which neither the substrate nor the test inhibitors absorb light.\textsuperscript{6}

As enzyme source, we employed the mitochondrial fraction obtained from baboon liver tissue since it is reported to be devoid of MAO-A activity while exhibiting a high degree of MAO-B catalytic activity. Therefore, even though MMTP is a MAO-A/B mixed substrate, its oxidation by baboon liver mitochondria can be exclusively attributed to the action of the MAO-B isoform.\textsuperscript{6} The potency of MAO-B inhibition were expressed as the enzyme-inhibitor dissociation constant (\(K_i\) value).

The test inhibitors slowed the rate of the MAO-B catalysed oxidation of 1-methyl-4-(1-methylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine (MMTP) to the corresponding dihydropyridinium (MMDP\textsuperscript{+}) metabolite.

**Molecular modelling**

The recent publication of the human MAO-B crystal structure\textsuperscript{11} permits modeling and docking of substrates and inhibitors into the active site of the enzyme. Docking is a useful tool to determine the possibility of a theoretical model to bind to a receptor and is extensively utilised to determine the properties necessary for high potency. Good correlations between the calculated and experimental inhibition constants (\(K_i\) values) were obtained in previous MAO-B studies.\textsuperscript{18}

Molecular modelling studies were carried out with the published MAO-B crystal structure (PDB: 1V5Y1) and proposed inhibitors were docked in the Ligandfit module of Cerius\textsuperscript{2} to identify the size and functional groups for optimum binding to the catalytic site of MAO-B.

**Results**

The final products were obtained as oils or amorphous solids from chromatography and were crystallised from organic solvents. MS confirmed the molecular masses and NMR and IR were used to identify the characteristic signals of the compounds.
REFERENCES: