Discovery of MAO-B Inhibitors - Present Status and Future Directions Part I: Oxygen Heterocycles and Analogs

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Abstract: Parkinson's disease (PD) is one of the most common neurodegenerative disorders. The role of monoamine oxidase (MAO) inhibitors has expanded in the PD treatment. The present review will summarize the current structure-activity relationship information available on MAOs inhibitors of unrelated families of compounds of oxygen heterocyclic type based on coumarin, chromone and chalcone scaffolds.

As the current hitting-one-target therapeutic strategy has been proved to be quite inefficient in PD, this review will also discuss about the development of multi-target drugs, in which MAO inhibition plays a counter-part, as a novel and promising treatment approach for PD.

Keywords: Parkinson's disease, MAO inhibitors, SAR, multi-target, coumarins, chromones, chalcones.

1. INTRODUCTION

Monoamine Oxidases (EC 1.4.3.4; MAOs) are outer mitochondrial membrane-bound flavoproteins present practically in all human tissues. Monoamine Oxidases exist in two isoforms named MAO-A and MAO- B (MAO-A and MAO-B) that belong to the protein family of flavin-containing amine oxidoreductases. The structure, function, and mechanism of mitochondrial monoamine oxidases have been the focus of extensive investigations: since the 1950s, approximately 30,000 published papers are listed in databases such as PubMed [1].

Monoamine Oxidases catalyze the oxidative deamination of monoamines, namely xenobiotic and dietary amines, and metabolize amine neurotransmitters in the brain and other tissues [2]. The MAOs metabolic reaction involves the oxidation of the amine function via oxidative cleavage of the α-CH bond of the substrate with the ensuing generation of an imine intermediate. This pathway is accomplished by the reduction of the flavin cofactor that is reoxidized by molecular oxygen, with simultaneous hydrogen peroxide release. Subsequently, the imine intermediate is hydrolyzed by a non-enzymatic pathway yielding ammonia and the corresponding aldehyde (Fig. **1**) [1, 3].

All mammals contain two MAO isoforms, MAO-A and MAO-B, that share 70% of sequence identity and are encoded by separate genes. These isoforms can be distinguished by their respective substrate preferences, their tissue distribution and by their substrate specificities. Substrates of both MAO types include dopamine (DA), tyramine, tryptamine, kynuramine and 3-methoxytyramine [4]. However, serotonin is preferentially metabolized by MAO-A and dopamine by MAO-B [2, 5, 6].

MAO-B is the predominant isoform in the human brain, where it acts in the breakdown of DA as well as in the deamination of β-phenylethylamine an endogenous amine that stimulates the release of DA and inhibits its neuronal reuptake [7]. Expression levels of MAO-B in neuronal tissue enhance 4-fold with aging, especially in glial cells, resulting in an increased level of DA metabolism and in the production of higher levels of dopanal and hydrogen peroxide (H_2O_2). Dopanal has been implicated in R-synuclein aggregation [8], and the increased levels of cellular H_2O_2 promote apoptotic signaling events resulting in a decreased level of DA-producing cells [9]. These events are thought to play a major role in the etiology of neurodegen-erative diseases such as Parkinson and Alzheimer [9, 10].

The intensive research effort in this area has its origins by the observation that MAO inhibitors were originally found to function as antidepressants. However a renewed interest in the inhibition of MAO-B has resulted from the observed age-related increase of MAO-B levels in humans and its possible connection to neurodegenerative diseases of the elderly such as Parkinson's Disease (PD) [11].

2. ROLE OF MAO-B INHIBITORS IN PARKINSON'S DISEASE THERAPY

During the last 20 years, an enormous research effort and hundreds of millions of dollars have been spent attempting to

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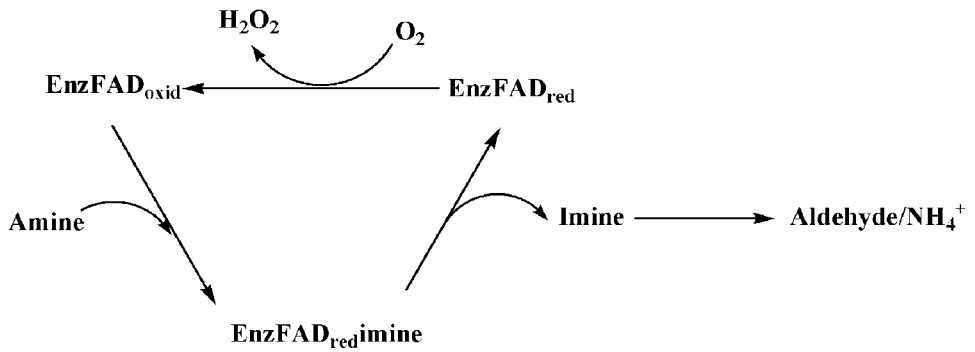


Fig. (1). Monoamine Oxidases general catalytic reaction pathways.

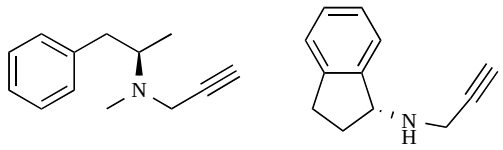
develop and prove drugs that may slow the rate of progression of PD. However, up to date no drug has yet satisfied the rigorous criteria set by clinicians and licensing authorities for a neuroprotective agent [12].

Despite this apparent failure, numerous important lessons have been learned, and some enthusiastic areas have emerged. The loss of dopaminergic neurons in the *substantia nigra* [13] and the role of MAO in DA metabolism are the best known aspects of the PD that have also been connected with the movement and balance problems. Currently, any treatment used for Parkinson's disease aims to end the relief of symptoms, reduce disability, stopping or slowing the neurodegenerative process and prevent long-term complications through early in the therapy. Consequently, the current therapies approved so far for the treatment of PD are only palliative. The mainstay of PD treatment focuses on the replacement of lost DA with DA agonists, including L-dopa monotherapy, or with a combined administration with for instance a MAO-B or a catechol-O-methyltransferase (COMT) inhibitor [13, 14]. The use of MAO-B inhibitors as initial therapy is also believed to postpone the need for levodopa initiation and, thus, delays the onset of levodopa-related motor fluctuations.

The use of MAO inhibitors as DA-sparing agents or as adjuncts to L-DOPA was considered as a treatment for early-stage Parkinson's disease, but such approach with non-selective inhibitors was withdrawn due to side-effects. Nevertheless, it was found that selective irreversible MAO-B inhibitors have no such effects [15, 16].

L-deprenyl (selegiline) (Fig. **2**) was the first selective MAO-B inhibitor investigated and approved by the Food and Drug Administration (FDA) as an adjuvant to levodopa (l-DOPA). When applied transdermally, the drug does not enter the gastrointestinal system, thereby decreasing the dangers of dietary interactions associated with oral administration of monoamino oxidase inhibitors (MAOIs). This drug provides a safer alternative and is now used as first-line therapy [2, 17-20].

Rasagiline (Fig. **2**) is a second-generation irreversible MAO-B inhibitor indicated for the treatment of PD. It was approved in 2006 by the FDA for initial monotherapy as well as adjunctive therapy in patients taking levodopa [21]. Rasagiline compared with selegiline, was shown to be more potent as MAO-B inhibitor both *in vitro* and *in vivo* models. The discrepancy among the neuroprotective effects of these inhibitors may be related to differences in their biotransformation. Selegiline undergoes extensive hepatic first-pass metabolism by cytochrome P450 enzyme, which reduces its bioavailability and forms high levels of neurotoxic metabolites: L-methamphetamine and metabolic analogs [5]. The amphetamine metabolites might be associated with a decrease in the activity and with the production of cardiac and psychiatric adverse events in patients with PD [2]. On the other hand the principal metabolite of rasagiline, (R)-aminoindan, is not an amphetamine-like compound, which offers potential advantages over selegiline. Novel findings demonstrated that this metabolite has antioxidant and neuroprotective capabilities and thus, may contribute to increase the activity performance of its parent compound [22, 23]. Like deprenyl, rasagiline is an acetylenic compound that forms an irreversible flavin N(5) flavocyanine covalent adduct.



Deprenyl **Rasagiline**

Fig. (2). MAO-B inhibitors approved by FDA.

The MAO-A inhibitors are effective in the treatment of depression. MAO-A is more sensitive to inhibition by clorgyline and moclobemide and MAO-B is selectively inhibited by low concentrations of selegiline (R-(-)-deprenyl) and rasagiline [24, 25]. MAO-B selective inhibitors, such as selegiline, co-administrated with levo-dopa represent an effective treatment for relieving symptoms resulting from the loss of dopaminergic neurons [2].

3. DEVELOPMENT OF MAO-B INHIBITORS BASED ON OXYGEN HETEROCYCLES AND ANALOGS

Although the development of monoamine oxidase (MAO) inhibitors has been the focus of extensive investigations since the 1950s [1] considerable efforts have been performed at the present time into the rational design and development of reversible MAO inhibitors [4, 14, 26-28]. Given the role of the isoform B of monoamine oxidase (MAO-B) in the metabolism of dopamine (DA), selective

and reversible MAO-B inhibitors are still a therapeutic need for patients with Parkinson's disease (PD) [2].

One of the most important stages of the drug discovery process is the generation of lead compounds. Structure-activity-relationships (SARs) are well-integrated in modern drug discovery and have been largely used for the process of finding new leads and optimization of their receptor or enzyme affinity as well as of pharmacokinetic and physicochemical properties [53]. In spite of the considerable progresses in the understanding of the interactions of MAO isoforms with their specific substrates or inhibitors [29], there are not any available rules for the rational design of new potent and selective MAO inhibitors. Privileged structures, such as indoles, arylpiperazines, biphenyls and benzopyranes are currently ascribed as supportive approaches in drug discovery. Even though different families of oxygen heterocycles, such as xanthones, chromones, coumarins and their precursors (chalcones), have been extensively used as scaffolds in medicinal chemistry programs for searching novel potent and reversible MAO inhibitors [30-38].

Thus, the present review intends to gather the data obtained so far encompassing the oxygen heterocycle scaffold enabling, in a near future, the development of suitable predictive QSAR models.

Coumarins

During the two last decades diverse types of MAO inhibitors have been reported among which coumarins exhibited a recognized outline. In fact, some of the members of this family have shown to be potent and selective MAO-B inhibitors. Their synthetic accessibilities have allowed to conduct a number of chemical modifications and diverse structure-activity studies have been carried out [30, 32, 33, 39-42].

Coumarins (1-benzopyran-2(2H)-ones) are benzopyrones well-known for displaying a variety of pharmacology properties that are dependent on the type of substitution. Over the last decade, coumarin derivatives have been identified as inhibitors of therapeutically important enzymes such as aromatase [43, 44], acetylcholinesterase (AChE) [45] and MAO [30]. Several examples were found in the literature, but one of the most relevant was the work of Gnerre *et al.* [30] that have tested 34 coumarin derivatives (**1**), 5 chromones (**2**), 9 compounds with bicyclic structure containing a benzo moiety fused with various five (or six) membered heterocycles (**3**) and 23 congeners of 3,4-dimethylcoumarin with a 7-benzyloxyl substitutent (**4**) towards rat MAO (*r*MAO) (Fig. **3**).

According to the biological data the majority of the compounds acted as *r*MAO-B inhibitors with IC_{50} values in the micromolar to low nanomolar range. From a SAR point of view several structural features can be remarked in the coumarin nucleus (**1**). The introduction of substitutents at position 3 and/or 4 of the coumarin nucleus (**1**) modulate *r*MAO-B inhibitory activity and *r*MAO-A/*r*MAO-B selectivity: monosubstitution in position 4 with a methyl group iconduct to more active compounds whereas phenyl, trifluoromethyl and hydroxyl substituents at the same position cause a decrease in the activity. It is noteworthy that the simultaneous substitution at position 3 and 4 with methyl groups was more favorable for the *r*MAO-B inhibition activity. A loss of *r*MAO activity occurs when the substituents at position 3 and 4 were phenyl and methyl groups, respectively. However, the 3,4-annelation with 5- and 6-membered rings, only for cycloalkyl and cyclopentenyl groups, have a positive effect on the activity. The authors have also shown that the introduction of substituents in the 7-position of the coumarin nucleus, namely a benzyloxyl substitution, elicits positive interactions within the *r*MAO-B binding pocket. The increasing of the length of the ether bridge, with a further methylene group, did not significantly change the activity. Nevertheless, a decrease of both *r*MAO-A and *r*MAO-B inhibition was observed when a CH_2NH bridge was introduced at the 7-position.

Based on the overall data Gnerre *et al.* was selected as [30] 7-(benzyloxy)-3,4-dimethylcoumarin (5) as a hit for *r*MAO-B inhibition. Consequently hit refinement generates a new series ((4), Fig. (**4**)) that was mainly based on the modification of the aromatic pattern of 7-benzyloxy group. From the SAR study it was concluded that the *ortho*-substitution appeared to be unfavorable while the presence of *para*-substituents has a positive influence. Furthermore derivatives bearing halogen substituents conducted the highest *r*MAO-B inhibitory activity (Fig. **4**). The most active MAO-B inhibitor was 7-(3,4-difluorobenzyloxy)-3,4-dimethylcoumarin (**6**) (Fig. **4**) with a pIC_{50} value of 8.94.

To attain a better understanding about the importance of coumarin as a privileged scaffold a 3D-QSAR model, using comparative molecular field analysis (CoMFA), was developed by the same authors [30]. The 3D QSAR technique is particularly useful when the 3D structure of the receptor is unknown. The main advantage of the 3D-QSAR models (based in CoMFA analysis) is that they can allow to predict the activities of nearby analogs (close because we cannot extrapolate outside the scope of the model), e.g. coumarins, and they can suggest the sites in the molecules where one can built favorable modifications. Nevertheless, a critical step in CoMFA is the positioning and subsequent alignment of the molecules, a requirement that limits its applications to homogeneous series of compounds.

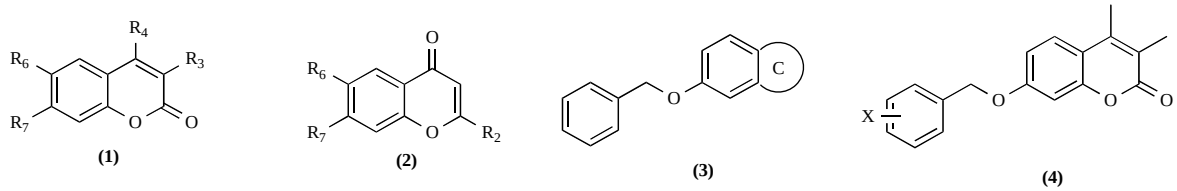


Fig. (3). General chemical structures of the compounds tested by Gnerre *et al.* [30].

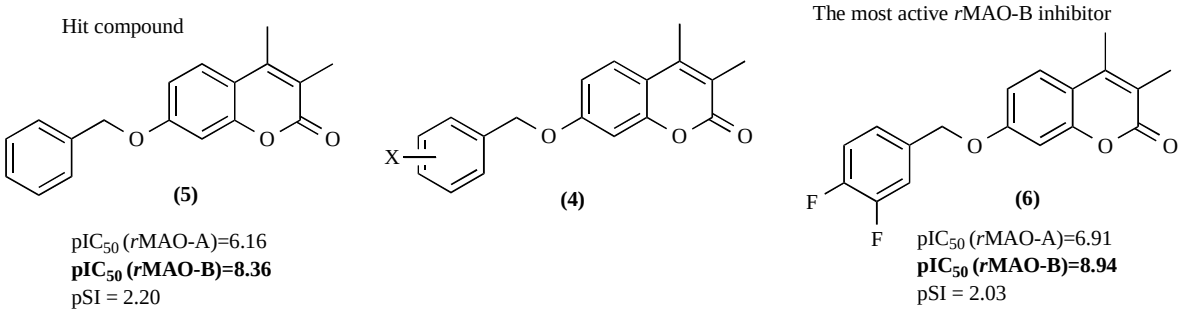


Fig. (4). SAR strategy followed by Gnerre et al. to generate potent and selective rMAO-B inhibitors [30].
$pSI = pI_{50}(MAO\text{-}B) - pIC_{50}(MAO\text{-}A)$.

Out of 71 chemicals, 41 coumarins acting as *r*MAO-A and 44 as *r*MAO-B inhibitors were selected by the authors to conform the training set and then the CoMFA analysis was performed. The criterion followed to choose the training set was the selection of inhibitors containing the coumarin ring and substitutions on the 3-, 4- or 7-positions. The model used to explain the inhibition on *r*MAO-A only includes the molecular electrostatic potential field while the QSAR model used to predict the *r*MAO-B activity was developed combining three fields; steric, electrostatic and lipophilic. The following conclusions were achieved: a) substitutions at positions 3 and 4 of the coumarin ring have a favorable steric contribution; b) substitutions on the phenyl ring of the benzyloxy substituent in *meta* and *para* positions are sterically favorable but in a less important manner; c) the lipophilic character of the CH_2 group in the oxymethylene bridge is favorable for the activity. In addition, it was concluded that the presence of electron withdrawing substituents at the 3- and 4-positions of the benzyloxy group produces favorable zones for the activity.

Until now, the coumarin nucleus has been substituted at positions 3 with phenyl and methyl groups or with hydrogen and following the initial data [30] it looks that small substituents at position 6 of the same nucleus are well tolerated. On the basis of this structural information, Chimenti *et al*. [39] synthesized 14 coumarin derivatives substituted at position 3 with carboxylic acid, ethyl ester or acyl chloride groups, at position 6 with halogen, nitro and methyl groups, and unsubstituted at position 7 (**7**, Fig. (**5**)) with the aim of verifying their MAO (bovine MAO-*b*MAO) inhibitory potencies and selectivities. The experimental results allow to conclude that esters are the compounds with the lowest inhibitory activity against both *b*MAO isoforms, while acids have high potency and selectivity towards *b*MAO-B, with the exception of 6-nitrocoumarin. Coumarin-3-carboxylic acid (**8**) Fig. (**5**) was found to be highly selective against the *b*MAO-B isoform. Moreover, coumarin-3-acyl chlorides presented strong *b*MAO inhibitory activity against both isoforms, particularly the compound (**9**) disclose the highest activity against *b*MAO-B. Unfortunately this compound showed a very low selectivity index.

Here it remains a question: Why the influence of an acyl group at the position 3 when the coumarin nucleus is substituted at the position 7 was not reported?

The same authors performed the synthesis and the evaluation of the *b*MAO inhibitory activity of the *N,N′*-bis[2-oxo-2H-benzopyran]-3-carboxamides (dicoumarinoyl diamides) and concluded that most of them display *b*MAO-A selective inhibitory activity [46].

Carotti *et al.* [47, 48] has explored the influence of new type of substituents at position 7 of the coumarin nucleus. The substituents incorporate at the 7-positions were, ether, ketone, ester, carbamate, amide functions of different size and lipophilicity [47] or a phenyl ring linked to a coumarin core by bridges of different size, length, lipophilic and electronic nature [48]. In all cases, the coumarin nucleus was

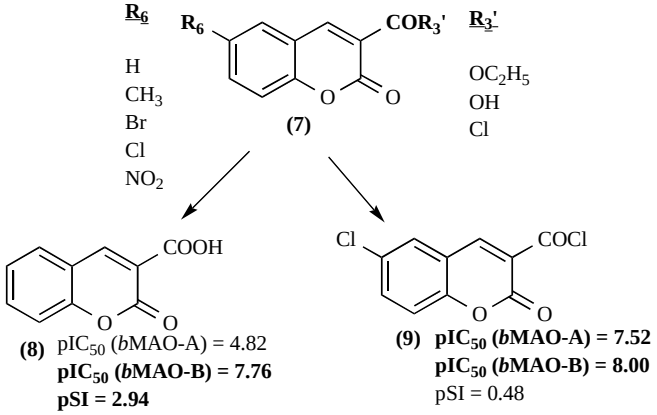


Fig. (5). SAR strategy followed by Chimenti *et al.* [39] to generate selective bMAO-B inhibitors.

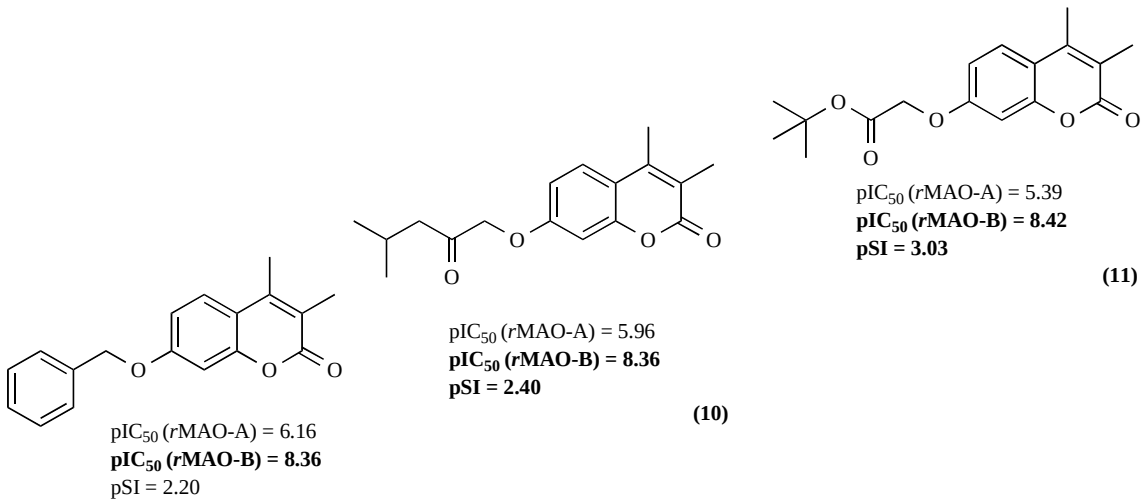


Fig. (6). The influence of some substituents at position 7 of coumarin nucleus on the selectivity.

substituted at position 3 and 4 with methyl groups or without substituents. From the acquired biological data it was confirmed that the majority of 7-substituted coumarin derivatives act as *r*MAO-B inhibitors, with none or little effect on MAO-A. Different substituents, such as 4-methyl-2-oxopentyl or *terc*-butyl acetate, positioned at position 7 of coumarin nucleus (see compounds (**10**) and (**11**) respectively, Fig. (**6**)) cause similar MAO-B inhibition data [47], but are more selective than the previously reported 7-(benzyloxy)-3,4-dimethylcoumarin (**5**) [30].

The Fig. (**7**) summarizes the main SAR features found for 7-(benzyloxy)-3,4-dimethylcoumarin derivatives as *r*MAO-B inhibitors [48]. The substructure marked with *i* highlights the presence of one or two halogens improve the *r*MAO-B inhibitory activity; the part marked *ii* elucidate that the length of the bridge between phenyl ring and the oxygen atom (from ether group) is very important for the activity: the spacer must be no longer than two carbon atoms (without ramifications, F_8) and must not contain an oxygen atom (F_3, F_5, F_7 and F_{10}); finally, the part *iii* illustrate that the introduction of methyl groups in positions 3 and 4 efficiently modulates the *r*MAO-B inhibition.

With the recent access to purified recombinant human MAO-B [49] as well as conditions for co-crystallization of inhibitor-enzyme [50] the scientific community began to screen and define the molecular and structural scaffold features towards human MAOs (*h*MAO). From the X-ray structural data on *h*MAO-B it was concluded that this isoenzyme has a longer and narrower active site cavity *h*MAOA a fact that is consistent with the data of SAR studies performed on *r*MAO-B and *b*MAO-B. Recent studies have shown that the specificities of different MAO-B inhibitors are up to 2 orders of magnitude different between human and rat [51, 52]. Similar differences have also been

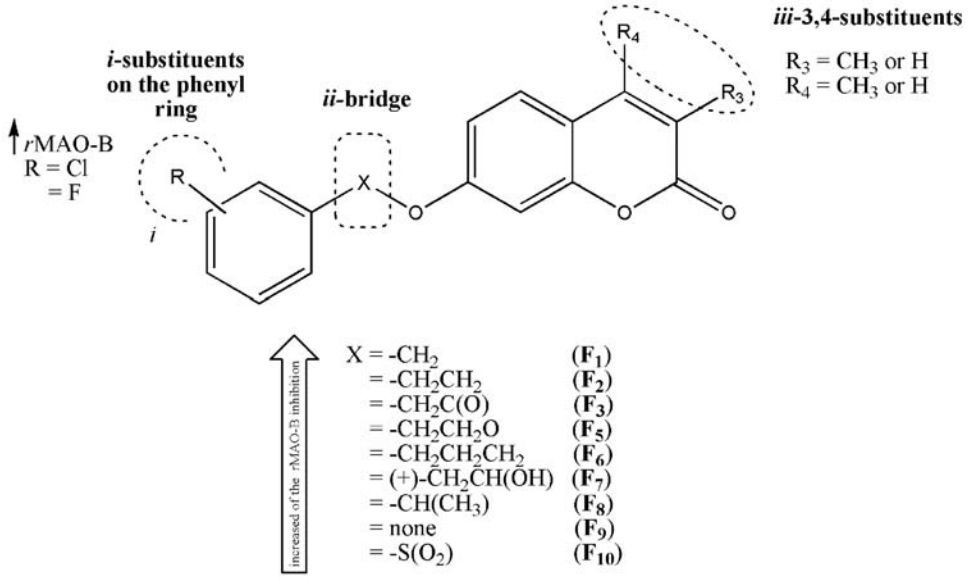


Fig. (7). Summary of the main structural factors responsible for 7-substituted coumarin derivatives MAO-B inhibitory activity.

reported between *h*MAO-B and *b*MAO-B due to an Ile199Phe mutation in *h*MAO-B that has been shown in previous studies to prevent the binding of longer inhibitors (e.g., *trans,trans*-farnesol) into the *b*MAO-B active site [53]. Therefore, although previous SAR studies provided useful information on structural differences between *r*MAO-A and *r*MAO-B active sites in general, the extrapolation of these conclusions directly to the human enzymes could be tenuous [54].

The efforts to obtain a model to predict MAO inhibitory activity have continued along this decade. In fact Santana *et al.* [55] have developed an theoretical study by combining QSAR methodology with complex networks. The model was used to predict the *h*MAO inhibitory activity of 33 coumarins. In that work, the authors explore the influence of new type of substituents at position 7 of coumarin nucleus such as: acetonyloxy, bromoallyloxy, oxocycloalkyloxy (the alkyl is referred to 2, 3 and 4 carbon atoms) and hydroxyl group, while the decoration on position 8 was explored with methoxyl or methyl groups. The *h*MAO inhibition data obtained so far allow to conclude that the majority of the coumarin derivatives obtained by Santana *et al.* [55] act preferentially as *h*MAO-A inhibitors, with IC_{50} values in the micromolar to low nanomolar range, with the exceptions of compounds (**13**) and (**15**) (Fig. **8**). In contrast to the Santana's findings [55], the most part of 7-substituted coumarin derivatives studied by Gnerre *et al.* [30] and Carotti *et al.* [47, 48] act as *r*MAO-B inhibitors. This could mean the substituents at position 7 of coumarin nucleus modulate the MAOs inhibition. The most relevant Santana's findings [55] were: (a) the introduction of bulky groups in the 7-acetonyl substituent increase the MAO-A selectivity; (b) the introduction of bulky groups such as cyclohexyl or phenyl at the 3,4-positions of the 7- acetonyl derivatives increased both the MAO-A and MAO-B inhibitory activities with loss of the selectivity; (c) the replacement of the acetonyl substituent at the position 7 by bromoallyloxy group significantly increase the MAO-B inhibitory activity (Fig. **8**). In addition, the coumarin derivatives (**13**) (pIC_{50} (*h*MAO-B) = 8.93) and (**15**) (pIC_{50} (*h*MAO-B) = 8.83), displayed a higher *h*MAO-B activity, even though with lower selectivity, than the *h*MAO-B reference inhibitor: selegiline (pIC_{50} (*h*MAO-B) =7.71).

Recently, Chimenti *et al.* [56] reported an additional study of the development of *h*MAO inhibitors based on coumarin nucleus in which a large series of 3-carboxamido-7-substituted coumarins was examined. Taking into account all the relevant structural information on *h*MAOs reported in the literature, some changes in the coumarin nucleus were performed and it was concluded that the majority of the compounds display a selective inhibitory activity toward *h*MAO-B. The effect on *h*MAOs activity *versus* selectivity of coumarin derivatives that have *N*-aryl or *N*-alkyl carboxamide substituents in position 3 and benzyloxy or 4′-F-benzyloxy groups at position 7 was studied with particular attention [56]. Substitution at position 7 (with a benzyloxy or a 4′-F-benzyloxy group) of 3-carboxamido coumarins or substitution of both amidic hydrogens appears to be unfavorable. The *h*MAO-B inhibitory activity increases when the nitrogen is substituted with a phenyl group, 3-fluorophenyl or 3-trifluoromethylphenyl, whereas di or poly N-phenyl-substitution leads to poorly active compounds. The most active and selective *h*MAO-B compound has a methanesulfonyl group at the *para* position of the N-phenyl substituent (compound (**16**), Fig. (**9**)).

In 2006, Novaroli *et al.* [54] published an interesting paper about of the impact of species-dependent differences between human and rat MAO-B inhibitors. Their results, based on two classes of compounds, coumarins and 5H-indeno[1,2-c]pyridazin-5-one derivatives [54] suggested that results obtained using rat enzyme should not be extrapolated to human. Accordingly, careful must be taken in the selection of a hit compound for lead generation since data from biological assays could be quite different. In the study Gnerre *et al.* [30] of 17 out of 33 coumarin derivatives were 7-X-substituted benzyloxy-3,4-dimethylcoumarins. All of them were tested towards *r*MAO-B. Later on Novaroli *et al.* [54] have acquired data using *h*MAO-B. In general, the 7-X-

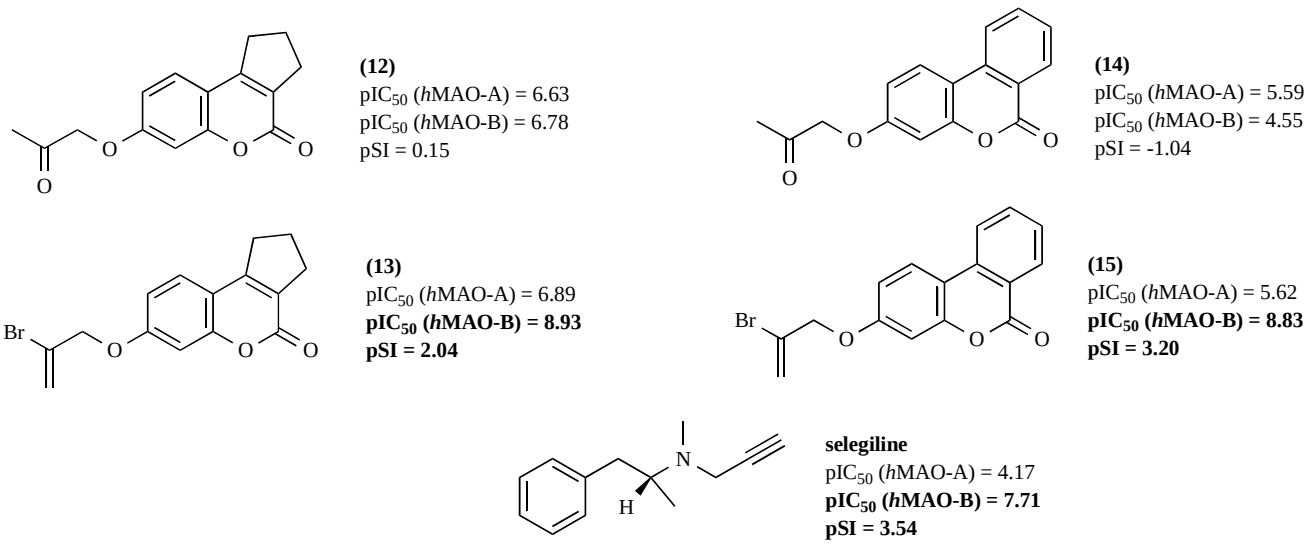
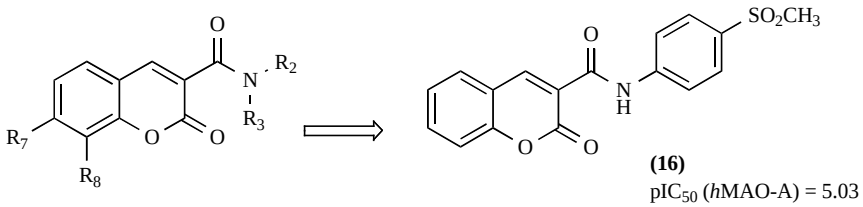


Fig. (8). The most *h*MAO-B active coumarin derivatives (12 and 14) described by Santana *et al.* [55].

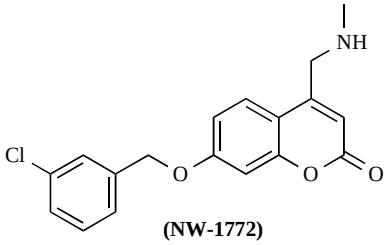


(16)
pIC_{50} (*h*MAO-A) = 5.03
pIC_{50} (*h*MAO-B) = 8.85
pSI = 3.82

Fig. (9). General structure of the 3-carboxamido-7-substituted coumarins studied by Chimenti *et al*. that generates the most active and selective hMAO-B compound (**16**) [56].

substituted benzyloxy-3,4-dimethylcoumarins displayed a noteworthy activity toward both enzymes (*r*MAO-A and *h*MAO-B), although an increment on the inhibitor potency toward *h*MAO B was observed.

On the basis of the information on structure-activity and structure selectivity relationships, Pisani *et al.* [35] developed a series of novel MAO-B inhibitors, maintaining the benzyloxy substituents at position 7 of the coumarin nucleus. To improve the pharmacokinetic profile and druggability of the new inhibitors adequate polar moieties (e.g. cyano, amido, amidoamino, and aliphatic amino groups) at position 4 were introduced. The results of this investigation led to the discovery of an *in vitro* and *in vivo* potent, reversible, and selective MAO-B inhibitor, a methanesulfate derivative of 7-[(3-chlorobenzyl)oxy]-4-[(methylamino)methyl]-2H-chromen-2-one (NW-1772, Fig. (**10**)), exhibiting appropriate pharmacologic features to be progressed to clinical trials.



(NW-1772)

Fig. (10). Chemical structure of the MAO-B inhibitor NW-1772.

Chromones

Chromone scaffold [(4*H*)-1-benzopyran-4-one] has been recognized as a pharmacophore of a large number of bioactive molecules either of natural or synthetic origin. Until now, numerous biological effects, especially in the popular medicine, have been ascribed to this benzo-γ-pyrone nucleus such as anti-inflammatory, antitumoral and antimicrobial activities. Enzymatic inhibition properties towards different systems, such as oxidoreductases, kinases, tyrosinases, cyclooxygenases have also been recognized [34, 57-59].

Recent results obtained by Borges and coworkers [34, 60] revealed that chromones derivatives act as potent *h*MAO-B inhibitors. Fig. (**10**) summarizes the two series of novel chromone derivatives that were synthesized and investigated for their ability to inhibit the *h*MAO activity (*h*MAO-A and *h*MAO-B). Preliminary studies performed with the chromones carboxylic acid ((**21**) and (**26**), Fig. (**11**)) allow disclosing the importance of the location of a carboxylic moiety in the γ-pyrone nucleus. In fact, when the –COOH substituent is in position 3- of the heterocyclic scaffold ((**26**), Fig. (**11**)) it binds to the *h*MAO-B exerting a selective inhibition (pIC_{50} *h*MAO-B 7.32; pSI > 3.32). As the inhibition is of irreversible type, and in an attempt to develop novel reversible and selective *h*MAO-B inhibitors, the synthesis of 2- and 3-carboxamide chromone derivatives and still capable of establishing hydrogen interactions with the enzyme was then performed [60, 61]. The overall data suggests that chromones with substituents at the position 3 of γ-pyrone nucleus act preferably as *h*MAO-B inhibitors (with IC_{50} values in the micromolar to nanomolar range), while the same type of substitutions at the position 2 of γ-pyrone nucleus in general gives rise to a total loss of activity (Fig. **11**) [60, 61]. The inhibitory *h*MAO-B activity of chromone 3-carboxamide series is dependent on the type of substituent present in the exocyclic nucleous, increasing with the presence of hydroxyl (**24**) and chloro (**25**) groups in *para* position of the N-phenyl moiety [60, 61]. However, the chromone (**25**) exhibits better selectivity than the chromone (**24**) (Fig. **11**).

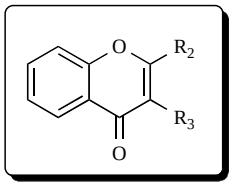
From the data reported so far, and considering the isomeric nature of the oxacycles rings and that the compounds were assayed following the same *in vitro h*MAO assay protocol, an important relationship between the same types of substituents on isomeric nucleus can be established: γ-benzopyrone nucleus *versus* α-benzopyrone nucleus (Fig. (**11**) chromone *versus* coumarin) [62]. This SAR assessment suggests an interesting correlation data set that in a near future could allow the optimization of the lead compounds towards MAO (potency *versus* selectivity).

Chalcones

Chalcones (*trans*-1,3-diphenyl-2-propen-1-ones) are biogenic precursors of all known flavonoids and are abundant in edible plants [63]. Chemically, they are open-chain flavonoids in which the two aromatic rings are joined by a three carbon α,β-unsaturated carbonyl system. They present a broad spectrum of biological activities such as anticancer, anti-inflammatory, antimalarial, antifungal and antiviral activities [64].

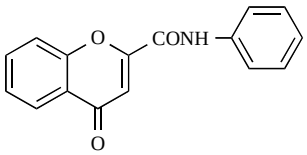
To date, the information on chalcones as MAO inhibitors reported in the literature is limited to behavioral tests and assays on rat mitochondrial MAOs [65-67], with the

2-substituted chromone series
R_2 when R_3 = H
COOH
CONH-Ph
CONH-(4'-OH-Ph)
CONH-(3'-OH-4'-OCH_3-Ph)
CONH-(3',4'-OH-Ph)
CONH-(3',4'-OCH_3-Ph)

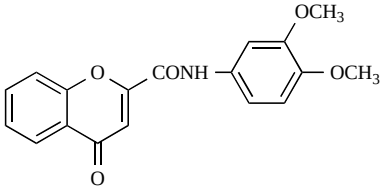


3-substituted chromone series
R_3 when R_2 = H
COOH
CONH-Ph
CONH-(4'-OH-Ph)
CONH-(3'-OH-4'-OCH_3-Ph)
CONH-(3',4'-OH-Ph)
CONH-(3',4'-OCH_3-Ph)

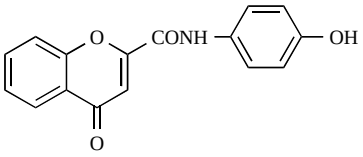
2-substituted chromone series



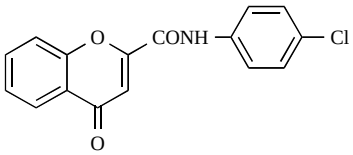
(17)
pIC_{50} (*h*MAO-A) < 4.00
pIC_{50} (*h*MAO-B) < 4.00



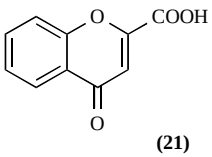
(18)
pIC_{50} (*h*MAO-A) = 6.72
pIC_{50} (*h*MAO-B) = 5.58
pSI = -1.14



(19)
pIC_{50} (*h*MAO-A) = 4.19
pIC_{50} (*h*MAO-B) = 4.38
pSI = 0.19

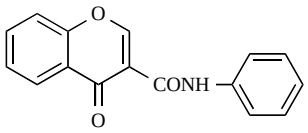


(20)
pIC_{50} (*h*MAO-A) = 4.00
pIC_{50} (*h*MAO-B) = 4.00

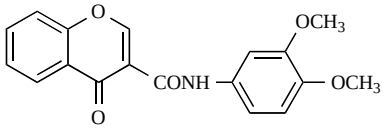


(21)
pIC_{50} (*h*MAO-A) < 4.00
pIC_{50} (*h*MAO-B) < 4.00

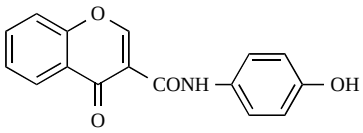
3-substituted chromone series



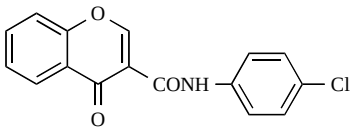
(22)
pIC_{50} (*h*MAO-A) < 4.00
pIC_{50} (*h*MAO-B) = 6.40
pSI > 2.40



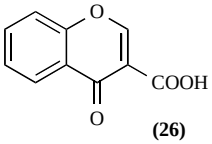
(23)
pIC_{50} (hMAO-A) = 6.37
pIC_{50} (hMAO-B) = 6.80
pSI = 0.43



(24)
pIC_{50} (*h*MAO-A) = 5.32
pIC_{50} (*h*MAO-B) = 7.19
pSI = 1.87

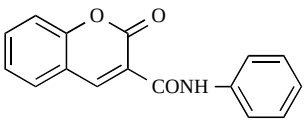


(25)
pIC_{50} (*h*MAO-A) = 4.00
pIC_{50} (*h*MAO-B) = 7.20
pSI > 3.20

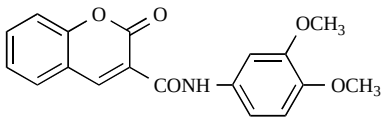


(26)
pIC_{50} (*h*MAO-A) < 4.00
pIC_{50} (*h*MAO-B) = 7.32
pSI > 3.32

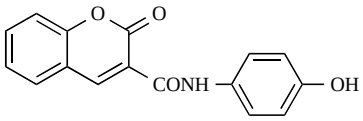
3-substituted coumarin series



(27)
pIC_{50} (*h*MAO-A) = 4.88
pIC_{50} (*h*MAO-B) = 6.12
pSI = 1.24



(28)
pIC_{50} (*h*MAO-A) = 5.02
pIC_{50} (*h*MAO-B) = 6.07
pSI = 1.05



(29)
pIC_{50} (*h*MAO-A) < 4.00
pIC_{50} (*h*MAO-B) = 6.10
pSI > 2.10

Fig. (11). Series of novel chromone derivatives that were synthesized and investigated by Borges and coworkers [34, 60, 61]. A comparison between chromone [60] and coumarin [56] scaffolds *h*MAO-activities have been also included.

exception of the investigation recently reported by Chimenti *et al.* [31] related to the synthesis, and the inhibitory activity against the A and B isoforms of *h*MAO, of a number of chalcone derivatives. The gathered results allow concluding that chalcone derivatives displayed a selective inhibitory activity towards *h*MAO-B in the micro to nanomolar range. The presence of hydroxyl and methoxyl groups (compound (**30**), Fig. (**12**)) or two hydroxyls substituents (compound (**31**), Fig. (**12**)) in the *ortho* and *para* position of the A aromatic moiety as well as a chlorine atom in the *para* position of the B aromatic moiety is found to be a fundamental requisite for activity. On the other hand chalcones substituted with isoprenyloxyl substructures ($-OCH_2CH=C(CH_3)_2$) have shown a lower activity.

4. DEVELOPMENT OF MULTI-TARGET DRUGS FOR PARKINSON'S DISEASE: THE ROLE OF MAO-B INHIBITORS

Recent data have disclosed a paradigm shift in the development and design of drugs to treat diseases with complex etiological pathways. The current tendency claims that a complex pathway disease should be treated with one molecule that possesses "promiscuous" activity acting on different pathways (multiple-mechanism drugs) [68, 69]. Drug therapy with multi-target compounds embracing diverse biological targets, with single bioavailability and pharmacokinetic metabolism, will have pronounced advantage over individual-target drugs or cocktail of drugs [22, 70-75]

Accordingly, due to the complex cascade of molecular events involved in the etiology of PD, an innovative approach towards neuroprotection or neurorescue may entail the use of multifunctional drugs that target an array of pathological pathways, each of which is believed to contribute to events that ultimately lead to neuronal cell death. Therefore this type of compounds may add a new dimension to PD treatment options as an adjunct to current drugs. In this context some multi-target MAO-B inhibitors have been by this time described in the literature. In this review the multi-target compounds with MAO-B properties developed so far will be briefly described (Fig. **13**).

Ladostigil (TV3326)

Structure-activity studies provide evidence that neuroprotection is associated with some intrinsic pharmacological action of the propargylamine moiety in the selective MAO-B inhibitors, selegiline and rasagiline [23, 76, 77]. This indication has stimulated the development of multifunctional chimeric propargylamine-derivatives, *e.g* ladostigil (TV3326, [(N-propargyl-(3R) 1-(R)-aminoindan-5yl)-ethyl methyl carbamate)], which combines the pharmacophore of rasagiline, with the carbamate moiety of the cholinesterase inhibitor rivastigmine. The resulting molecule is a novel cholinesterase and brain-selective MAO-B inhibitor, intended for the treatment of dementia comorbid with extra pyramidal disorders (Parkinsonism) and depression (presently in Phase IIa studies in man).

Rasagiline Derivatives

A second derivative of rasagiline, 5-[(N-methyl-N-propargylaminomethyl)-8-hydroxyquinoline], known as M30, was developed by amalgamating the propargylamine moiety of rasagiline that was embedded into the backbone of the neuroprotective and brain permeable iron chelator 8-hydroxyquinoline derivative. M30 was designed to inhibit the formation of reactive hydroxyl radical from H_2O_2 generated by MAO and potentiate the pharmacological action of accumulated dopamine formed from L-DOPA. Several multifunctional propargylamine-derivatives (M30, VK28 and HLA20) were found to possess neuroprotective and anti-apoptotic properties [69, 74].

Safinamide

A recently developed MAO-B inhibitor; safinamide, combine dual mechanism of action based on the enhancement of the dopaminergic function through potent reversible inhibition of MAO-B and of DA uptake and inhibition of excessive release of glutamate [78].

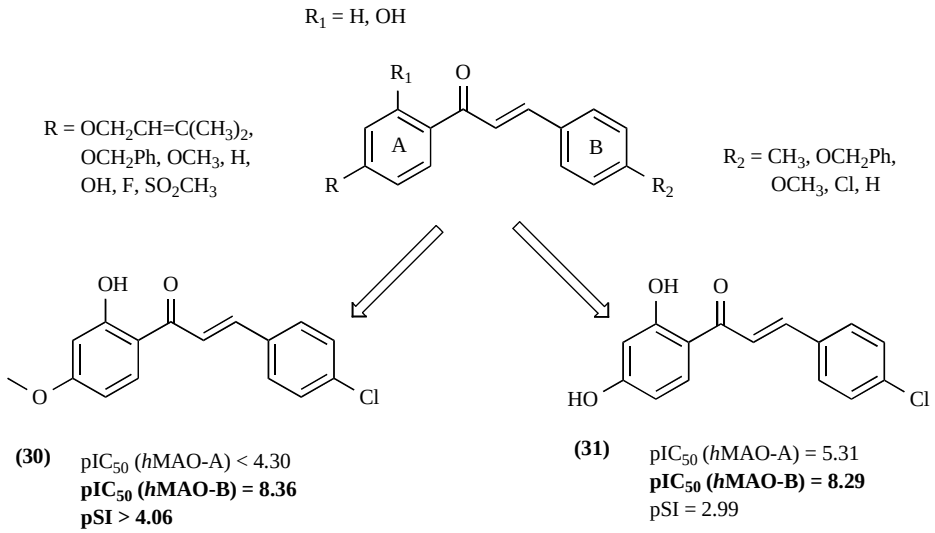


Fig. (12). SAR strategy followed by Chimenti *et al.* [31] to generate selective chalcone hMAO-B inhibitors.

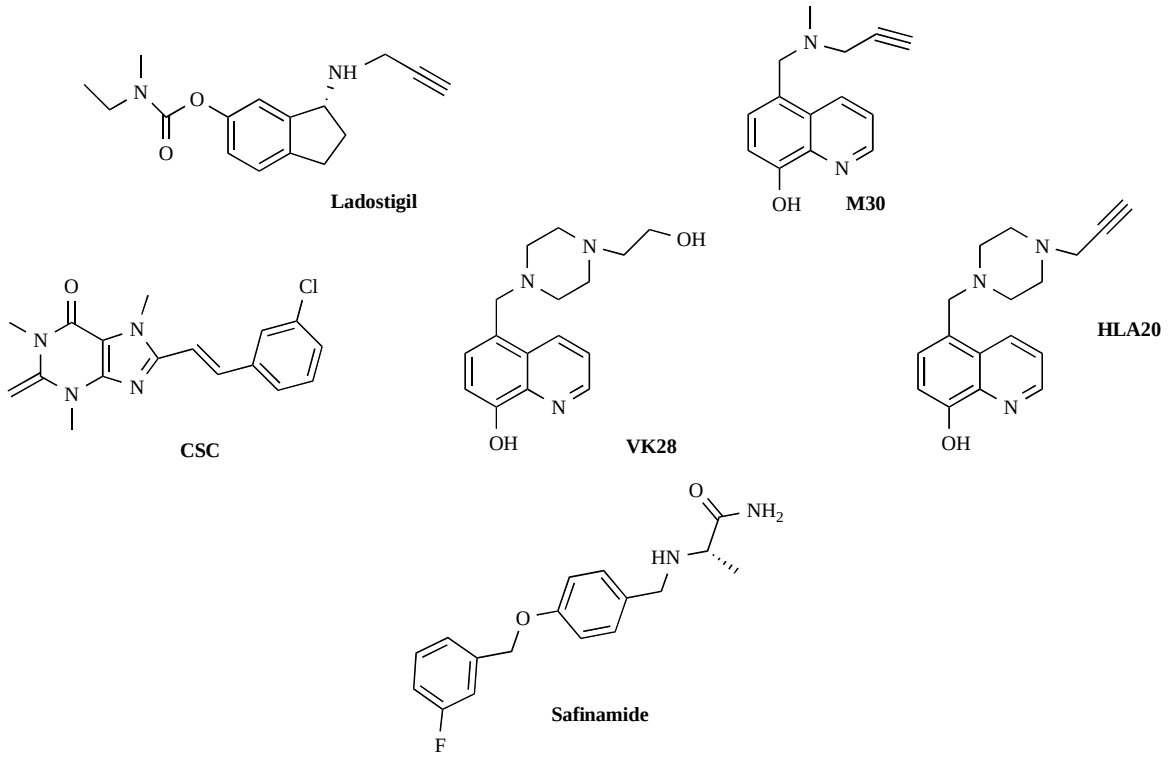


Fig. (13). Multi-target MAO-B inhibitors.

(E)-8-(3-Chlorostyryl) Caffeine (CSC) and Analogs

Several authors reported the effects of caffeine and their derivatives, on behavior and biochemical alterations in 6-OHDA-lesioned rats. The most potent among these was (E)-8-(3-chlorostyryl) caffeine (CSC), which alone or mainly combined with l-DOPA reversed these alterations [79-82].

The results of a SAR study established that the potency of MAO-B inhibition by (E)-8-styrylcaffeinyl analogs depends upon the van der Waals volume, lipophilicity, and the Hammett constant of the substituents attached to change of the phenyl ring of the styryl moiety. The potency also change with substituents attached to C-4 with bulkiness and lipophilicity being the principal substituent descriptors [83]. Using CSC as a lead compound, Pretorius *et al.* studied a series of (E,E)-8-(4-phenylbutadien-1-yl)caffeine analogs, which were identified as dual-acting compounds. The 8-phenyl- and 8-benzylcaffeinyl analogs exhibited relatively weak MAO-B inhibition potencies while selected (E,E)-8-(4-phenylbutadien-1-yl)caffeinyl analogs were found to be exceptionally potent reversible MAO-B inhibitors [84]

However, more detailed studies should be undertaken in the future, in both experimental animals and human to clarify whether antagonism and inhibition on A_2AR and MAO-B at the same time may be used effectively in the treatment of PD.

CONCLUDING REMARKS

The use of MAO-B inhibitors for the symptomatic treatment of PD is widespread. In early PD, these medications are safe and very well tolerated, though their efficacy is only modest.

In spite of the considerable progresses in the understanding of the interactions of MAO isoforms with their specific substrates or inhibitors no available rules for the rational design of new potent and selective MAO inhibitors are developed yet.

On the other hand neurodegenerative diseases remain a huge unmet pharmaceutical need. Since multiple pathogenetic factors are implicated in neurodegenerative diseases, the current hitting-one-target therapeutic strategy has proven to be inefficient. Despite the diverse pathogenetic factors involved, the current neurodegenerative strategies depend largely on single-targeted drugs. The development of multitarget drugs for the treatment of PD is in its infancy. Thanks to the continuing effort of medicinal chemists in the past decade, some multipotent agents have been rationally designed by incorporating two or more pharmacophores in one scaffold. Given the current embryonic state of the art, considerable challenges exist for medicinal chemists on the road to discovering drug-like multiple ligands.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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PATIENT CONSENT

Declared none.

ABBREVIATIONS

A2AAR	=	A2A adenosine receptor
AA2ARA	=	A2AAR antagonist
AChE	=	Acetylcholinesterase
CNS	=	Central Nervous System
CoMFA	=	Comparative molecular field analysis
COMT	=	Catechol-O-methyltransferase inhibitors
DA	=	Dopamine
FDA	=	Food and Drug Administration
IC50	=	Half maximal inhibitory concentration
pIC50	=	-logIC50
5-HT	=	5-Hydroxytryptamine
l-DOPA	=	Levodopa
MAO	=	Monoamine oxidase
MAO-A	=	Monoamine oxidase isoform A
MAO-B	=	Monoamine oxidase isoform B
bMAO	=	bovine MAO
hMAO	=	human MAO
rMAO	=	rat MAO
MlogP	=	Moriguchi octanol-water partition coefficient
PD	=	Parkinson's disease
QSAR	=	Quantitative structure activity relationships
3D-QSAR	=	Tridimensional QSAR
SAR	=	Structure-Activity Relationship
SI	=	Selectivity Index.

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