Bioorganic & Medicinal Chemistry Letters 20 (2010) 2709–2712

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Chromone-2- and -3-carboxylic acids inhibit differently monoamine oxidases A and B

Stefano Alcaro [a,*], Alexandra Gaspar [b,c], Francesco Ortuso [a], Nuno Milhazes [c], Francisco Orallo [d], Eugenio Uriarte [e], Matilde Yáñez [d], Fernanda Borges [b,*]

[a] Dipartimento di Scienze Farmacobiologiche, Facoltà di Farmacia, Università "Magna Græcia" di Catanzaro, Campus Universitario "S. Venuta", Viale Europa, 88100 Catanzaro, Italy
[b] CIQUP/Departamento de Química, Faculdade de Ciências, 4169-007, Universidade do Porto, Porto, Portugal
[c] Instituto Superior de Ciências de Saúde-Norte, 4585-116 Gandra PRD, Portugal
[d] Departamento de Farmacologia, Facultad de Farmacia, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain
[e] Departamento de Química Orgânica, Facultad de Farmacia,Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain

ARTICLE INFO

Article history:
Received 24 February 2010
Revised 19 March 2010
Accepted 23 March 2010
Available online 27 March 2010

Keywords:
Chromones
Docking
Monoamine oxidase
Inhibitors

ABSTRACT

Chromone carboxylic acids were evaluated as human monoamine oxidase A and B (*h*MAO-A and *h*MAO-B) inhibitors. The biological data indicated that only chromone-3-carboxylic acid is a potent *h*MAO-B inhibitor, with a high degree of selectivity for *h*MAO-B compared to *h*MAO-A. Conversely the chromone-2-carboxylic acid resulted almost inactive against both MAO isoforms. Docking experiments were performed to elucidate the reasons of the different MAO IC_{50} data and to explain the absence of activity versus selectivity, respectively.

Monoamine oxidases (MAOs; EC 1.4.3.4) are widely distributed enzymes that contain a flavin adenine dinucleotide (FAD) covalently bounded to a cysteine residue.[1] Many living organisms possess MAOs and in mammals two isoforms are present, MAO-A and MAO-B, located in the outer membrane of the mitochondria. These two isoforms are involved in the oxidative deamination of exogenous and endogenous amines, including neurotransmitters, thus modulating their concentrations in the brain and peripheral tissues. Physiologically, MAOs oxidize biogenic neurotransmitters such as dopamine, norepinephrine, 5-hydroxytryptamine (5-HT, serotonin) and β-phenethylamine, dietary, and xenobiotic amines such as tyramine and benzylamine (Fig. 1).[2]

Although MAO-A and MAO-B have an amino acid sequence similarity up to 70% they exhibit different substrate specificity, inhibitor sensitivity, and tissue distribution. MAO-A is located predominantly in catecholaminergic and serotonergic neurons. Consequently, this MAO isoform has a higher affinity for serotonin, epinephrine and norepinephrine and it is more sensitive to inhibition by clorgyline and moclobemide.[1] On the other hand MAO-B is present in dopaminergic neurons and glia and, therefore, has a preferential action on dopamine, β-phenethylamines, benzylamines and sterically hindered amines. This isoform is selectively inhibited by low concentrations of selegiline (L-deprenyl) and rasagiline.[1,3]

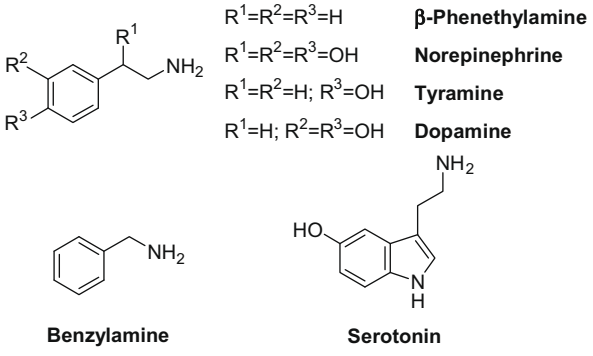

Figure 1. Endogenous, dietary, and xenobiotic amines.

MAO-A inhibitors are frequently used as antidepressants and antianxiety agents while MAO-B inhibitors, alone or combined with L-Dopa, are relevant tools in the therapy of Alzheimer's and Parkinson's diseases.[1,4] Development of MAO inhibitors is important not only from the standpoint of symptomatic treatment (i.e., by increasing the biological half-life of monoamine neurotransmitters),

* Corresponding authors. Tel.: +39 0961 3694197; fax: +39 0961 391490 (S.A.), tel.: +351 220402560; fax: +351 220402659 (F.B).
E-mail addresses: alcaro@unicz.it (S. Alcaro), fborges@fc.up.pt (F. Borges).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.03.081

but also with regard to the neuroprotective effects (i.e., prevention or delay of neurodegeneration itself).

All of these aspects have led to an intensive search for novel MAO inhibitors and this effort has increased considerably in recent years. However, a large number of MAOs inhibitors introduced into clinical practice were abandoned due to adverse effects, such as hepatotoxicity, orthostatic hypotension, and the so-called 'cheese effect', which is characterized by hypertensive crises.[1]

In spite of the considerable progresses in the understanding of the interactions of MAO isoforms with their specific substrates or inhibitors, 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 considered useful in drug discovery. Different families of nitrogen and oxygen heterocycles, such as xanthones, coumarins and their precursors (chalcones) have also been extensively used as scaffolds in medicinal chemistry programs searching for novel MAO-B inhibitors.[4–6]

In order to include other scaffolds in the series of MAO inhibitors the chromone scaffold [(4*H*)-1-benzopyran-4-one] has been considered, since it is common to 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, antitumor and antimicrobial activities.[7] Enzymatic inhibition properties towards different systems, such as oxidoreductases, kinases, tyrosinases, cyclooxygenases have also been recognized.[8]

In this work, we focus our attention on two chromone isomers (Fig. 2) that were screened towards MAO-A and MAO-B (Table 1). Details about the enzymatic evaluations are reported in the Supplementary data.

The results of the enzymatic experiments of the two chromones under study, with respect to the reference compounds, disclose a significant chemical feature related with the location of carboxylic moiety in the γ-pyrone nucleus. In fact, when the acidic substituent is in position 3 of the heterocyclic scaffold the compound **2** acted as MAO-B inhibitor with IC_{50} values in the nanomolar range. Its inhibition effect onto the MAO-A isoform resulted three orders of magnitude lower with a interesting selectivity ratio. Surprisingly the isomer **1**, with the acid moiety in position 2, gave no inhibition on both MAO isoforms. Compound **2** revealed to bind the *h*MAO-B exerting a selective inhibition with respect to the A isoform (see Supplementary data).

This curious appraisal attracted our attention and led us to investigate at molecular level the recognition mechanisms of compounds **1** and **2** within both enzymatic clefts by means of molecular modeling experiments. These studies were performed using available *h*MAO-A and *h*MAO-B structures deposited into the Protein Data Bank (PDB) as receptor models to understand the enzyme–inhibitor interactions and explain the biological data. Two recently determined MAO crystallographic structures were adopted as targets after a preliminary treatment (see Supplementary data). Ligand flexible docking, followed by fully geometry optimization of the generated complexes, reported the capability of compounds **1** and **2** to fit within both *h*MAOs catalytic sites, but only the latter compound revealed strong energy favoured binding modes into the *h*MAO-B (Table 2). Such an observation was in qualitative agreement to the experimental IC_{50} previously discussed.

Then the most stable optimized complexes were graphically inspected and the key enzyme–ligand interacting residues were highlighted (Table 3).

In *h*MAO-A both compounds **1** and **2** showed a similar orientation (see Supplementary data), with their aromatic and carboxylate moieties respectively directed towards the FAD and the outer side. The latter compound was able to perform a deeper recognition with respect to the former one. Actually, compound **1** did not establish direct contacts to the cofactor with the lack of strong productive interactions into the active site. Moreover, compound **1** recognition was penalized by electrostatic repulsions between its sp^2 oxygen atom and Ile180-Asn181 backbone. These unfavorable interactions, coupled to the lack of the few productive contacts highlighted by compound **2**, could explain the poor complexation energy of **1** within the *h*MAO-A binding cleft. A similar scenario can be addressed to **2**, but it showed stacking contacts to the Tyr407 and, moderately, to Tyr444 and its ether oxygen, partially negative charged, favorably interacted to FAD C4 that, in the used force field, resulted partially positive charged.

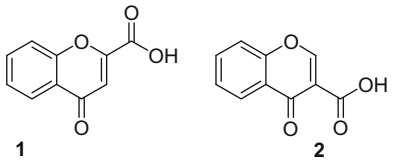

Figure 2. Chemical structures of the chromone isomers **1** and **2**.

Table 1
IC_{50} values for the inhibition of *h*MAO (in μM) of chromone carboxylic acids and reference inhibitors

Compounds	*h*MAO-A	*h*MAO-B	SI
1	**	**	—
2	**	0.048 ± 0.0026	>2083[b]
Clorgyline	0.0052 ± 0.00092[a]	63.41 ± 1.20	0.000082
***R*-(−)-Deprenyl**	68.73 ± 4.21[a]	0.017 ± 0.0019	4043
Iproniazide	6.56 ± 0.76	7.54 ± 0.36	0.87
Moclobemide	361.38 ± 19.37[a]	***	<0.36[b]

[a] $P < 0.01$ versus the corresponding IC_{50} values obtained against MAO-B.
[b] Values obtained under the assumption that the corresponding IC_{50} against MAO-A or MAO-B is the highest concentration tested; SI: *h*MAO-B selectivity index = $IC_{50\ (hMAO\text{-}A)}/IC_{50\ (hMAO\text{-}B)}$.
** Inactive at 100 μM (highest concentration tested).
*** Inactive at 1 mM (highest concentration tested).

Table 2
MM-GBSA interaction energies in kcal/mol of compounds **1** and **2** into the *h*MAOs

Compounds	*h*MAO-A	*h*MAO-B
1	31.11	−1.67
2	−2.86	−22.91

Table 3
Relevant *h*MAO-A and -B interacting residues with respect to **1** and **2** binding modes

Corresponding residues		Compounds	
*h*MAO-A	*h*MAO-B	**1**	**2**
Tyr69	Tyr60	*b*	*a*
Ile180	Leu171	*ab*	*ab*
Asn181	Cys172	*ab*	*ab*
Tyr197	Tyr188	*a*	*ab*
Ile207	Ile198	*ab*	*ab*
Phe208	Ile199	*ab*	*b*
Ser209	Ser200	*a*	—
Gln215	Gln206	*ab*	*ab*
Ile335	Tyr326	*ab*	*ab*[+]
Leu337	Leu328	*a*	*ab*
Met350	Met341	—	*a*
Phe352	Phe343	*b*	*ab*
Tyr407	Tyr398	*ab*	*ab*
Gly443	Gly434	—	*ab*
Tyr444	Tyr435	*ab*	*ab*
FAD	FAD	*b*	*ab*

a: *h*MAO-A interaction; *b*: *h*MAO-B interaction; [+]: hydrogen bond.

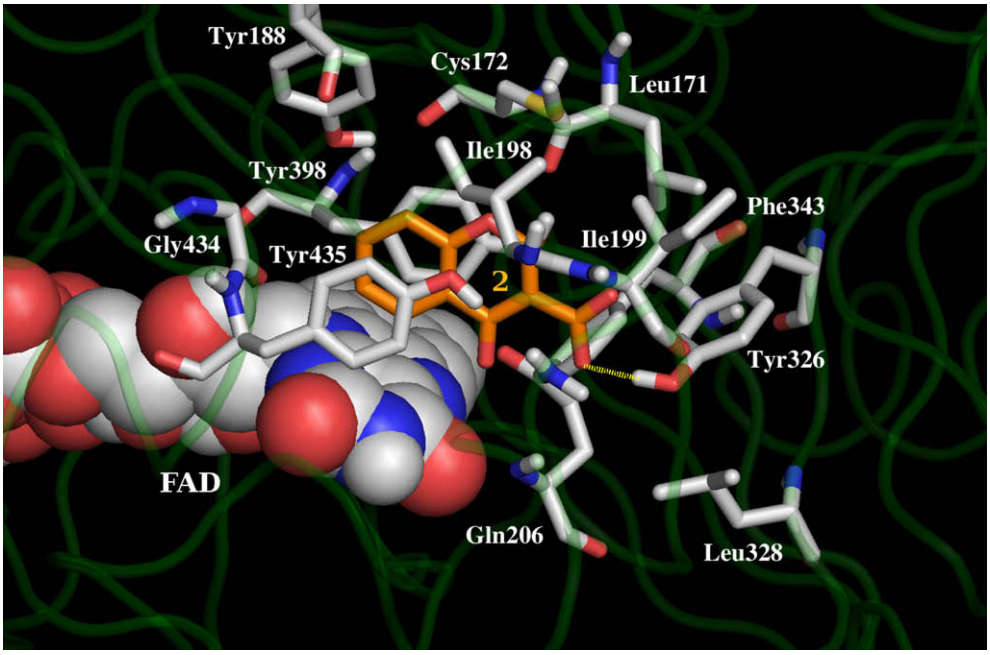

Figure 3. Global minimum energy configuration of compound **2** into the *h*MAO-B catalytic site. The ligand is reported as orange carbon polytube. The interacting residues are in white carbon polytube and the FAD cofactor is displayed in spacefill rendering. Yellow dotted line indicates the intermolecular hydrogen bond.

Conversely, into the *h*MAO-B cleft compounds **1** and **2** gave best poses in opposite configurations. The former chromone reported its carboxylate moiety directed toward the FAD electrostatically interacting to the C4 cofactor atom. No stacking contacts were highlighted by the aromatic pyrone ring that hydrophobically interacted to Ile171, Ile199, Tyr326 and Phe343. The sp^2 oxygen atom of **1** electrostatically recognizes the Tyr435 hydroxyl group but, due to a distance larger than 3.0 Å, was not able to establish hydrogen bond (see Supplementary data). Compound **2** showed, into the *h*MAO-B catalytic site, its aromatic moiety directed toward the inner side and the carboxylate substituent was involved in hydrogen bond to the Tyr326. The pyrone ring perfectly fits between Tyr398 and Tyr435, suggesting a strong stacking interaction, the sp^2 oxygen was located in front of the FAD C4 (Fig. 3).

The compounds **1** and **2** binding modes analysis provided interesting information to rationalize the experimental biological data. The most relevant issue could be addressed to the hydrogen bond established by **2** into the *h*MAO-B recognition site. Such a productive interaction was allowed by the presence of the Tyr326, substituted, in the *h*MAO-A, by the Ile335. Taking into account the position of the carboxylate moiety onto the pyrone ring, the hydrogen bond to Tyr326 allowed the perfect fit of **2** aromatic moieties with respect to Tyr398 and Tyr435 and the favorable electrostatic interaction of the sp^2 oxygen to the C4 FAD atom.

Compound **1**, reporting the carboxylate in position 2, was not able to concurrently perform all these productive interactions. In order to verify this observation, we manually built a configuration of **1** starting from the global minimum energy complex of **2** and after fully optimization of the resulting structure we found that the hydrogen bond was conserved, but the stacking to catalytic tyrosines and the electrostatic contact to the C4 FAD was completely lost. Moreover the internal energy of such biased optimized structure was about 13 kcal/mol higher than **1** *h*MAO-B global minimum configuration, suggesting it as improbable.

In conclusion chromone appears to be an interesting scaffold for the design of selective MAO inhibitors. The easy synthetic accessibility, the potentially low toxicity and especially the versatile binding properties of chromones make them as 'privileged' scaffolds. Our findings pointed out a crucial, undisclosed role of the presence of a hydrogen donor group in position 3 of the pyrone ring that is able to establish hydrogen bond interactions with active site residues. This discovery opens a new avenue to obtain highly potent and selective MAO-B inhibitors. The docking technique provided new insights on the inhibition mechanism and the rational drug design of this type of inhibitors. The molecular modeling studies highlighted that the most structurally simple chromone derivatives **1** and **2** can fit into the *h*MAO binding clefts. The hydrogen donor moiety should be located in position 3 of the pyrone ring for obtaining favoured energy and selective recognition of the *h*MAO-B isoform.

Additional studies are warranted for a systematic lead optimization, modulated by appropriate modifications of length, size, and chemical nature of the substituents, process that can lead to in the future to a drug candidate.

Acknowledgments

The authors dedicate this manuscript to Professor Francisco Orallo Camberio, who left us prematurely, for his outstanding human and professional qualities.

The authors acknowledge for financial support Fundação para a Ciência e a Tecnologia (FCT) (project PTDC/QUI/70359/2006), A.G. doctoral Grant (SFRH/BD/43531/2008) and Ministerio de Sanidad y Consumo (Spain; FISS PI061537 and PI061457)

Supplementary data

Supplementary data (pharmacology, determination of *h*MAO isoform activity, reversibility and irreversibility experiments, data presentation and statistical analysis, molecular modeling and additional figures) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.081.

References and notes

1. (a) Reyes-Parada, M.; Fierro, A.; Iturriaga-Vásquez, A. P.; Cassels, B. K. *Curr. Enzyme Inhib.* **2005**, *1*, 85; (b) Pacher, P.; Kecskeméti, V. *Curr. Med. Chem.* **2004**, *11*, 925.

2. Bertoni, J.; Elmer, L. In *The Role of MAO-B Inhibitors in the Treatment of Parkinson's Disease*; Ebadi, M., Pfeiffer, R. F., Eds.; CRC Press: Florida, 2005; pp 691–704.
3. Lang, A. E.; Lees, A. *Mov. Disord.* **2002**, *17*, 38.
4. (a) Deeb, O.; Alfalah, S.; Clare, B. W. *J. Enzyme Inhib. Med. Chem.* **2007**, *22*, 277; (b) Thull, U.; Kneubühler, S.; Testa, B.; Borges, M. F. M.; Pinto, M. M. *Pharm. Res.* **1993**, *10*, 1187.
5. (a) Santana, L.; González-Díaz, H.; Quezada, E.; Uriarte, E.; Yáñez, M.; Viña, D.; Orallo, F. *J. Med. Chem.* **2008**, *51*, 6740; (b) Binda, C.; Wang, J.; Pisani, L.; Caccia, C.; Carotti, A.; Salvati, P.; Edmondson, D. E.; Mattevi, A. *J. Med. Chem.* **2007**, *50*, 5848; (c) Catto, M.; Nicolotti, O.; Leonetti, F.; Carotti, A.; Favia, A. D.; Soto-Otero, R.; Mendez-Alvarez, E.; Carotti, A. *J. Med. Chem.* **2006**, *49*, 4912; (d) Borges, F.; Roleira, F.; Milhazes, N.; Santana, L.; Uriarte, E. *Curr. Med. Chem.* **2005**, *12*, 887.
6. Chimenti, F.; Fioravanti, R.; Bolasco, A.; Chimenti, P.; Secci, D.; Rossi, F.; Yañez, M.; Orallo, F.; Ortuso, F.; Alcaro, S. *J. Med. Chem.* **2009**, *52*, 2818.
7. Ellis, G. P., Ed.The chemistry of heterocyclic compounds, chromenes, chromanones and chromones; J. Wiley & Sons: New York, 2007; Vol. 31,.
8. (a) Ishar, M. P. S.; Singh, G.; Singh, S.; Sreenivasan, K. K.; Singh, G. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1366; (b) Peixoto, F.; Barros, A. I. R. N. A.; Silva, A. M. S. *J. Biochem. Mol. Toxicol.* **2002**, *16*, 220; (c) Ellis, G. P.; Barker, G. *Prog. Med. Chem.* **1972**, *9*, 65.