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Chromone 3-phenylcarboxamides as potent and selective MAO-B inhibitors

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ABSTRACT

Monoamine oxidase (MAO) is an enzyme, present in mammals in two isoforms MAO-A and MAO-B. These isoforms have a crucial role in neurotransmitters metabolism, representing an attractive drug target in the therapy of neurodegenerative diseases (MAO-B) and depression (MAO-A). In this context, our work has been focused on the discovery of new chemical entities (NCEs) for MAO inhibition, based on the development of chromone carboxamides. Chromone derivatives with a carboxamide function located in position 2- and 3- of the benzo-γ-pyrone core, (compounds **2–6** and **8–12**) were synthesized, with moderate/good yields, by a one-pot condensation reaction using phosphonium salts as coupling reagents. The synthetic compounds were screened towards human MAO isoforms (*h*MAO) to evaluate their potency and selectivity. The chromone-3-carboxamides show high selectivity to *h*MAO-B, with compounds **9** and **12** displaying IC_{50} values at nanomolar range.

Parkinson's disease (PD) is a neurodegenerative disorder characterized by a myriad of symptoms that gradually decreases the quality of life of the patient. The first line of treatment is a dopamine replacement therapy with Levodopa.[1] Among other therapeutic strategies monoamine oxidase B (MAO-B) inhibitors have also been extensively used in PD. In fact, selective MAO-B inhibitors (i.e., deprenyl and rasagiline) are currently used, alone or in combination with Levodopa, in the symptomatic treatment of Parkinson's disease. The side effects associated with the use of deprenyl and, to a lesser extent, rasagiline, likely due to their irreversible mechanism of inhibition, and the potential application of MAO-B inhibitors as anti-Alzheimer's agents are at the moment the driving forces for the discovery of novel potent and selective MAO-B inhibitors.[2,3]

Privileged structures, such as benzopyranes, are currently ascribed as supportive approaches in drug discovery. In fact, different families of natural nitrogen and oxygen heterocycles, such as xanthones and coumarins, have also been used as scaffolds in medicinal chemistry programs for searching novel MAO-B inhibitors.[4,5]

Chromones (benzopyran-4-one) are one of the most abundant groups of naturally occurring heterocyclic compounds.[4] Because of their structural features they occupy an important place in the realm of natural products and synthetic organic chemistry. In addition, remarkable antioxidant, anticancer and enzymatic inhibition properties have been ascribed to these systems.[5]

Noteworthy evidences have been already acquired to reinforce the interest of simple coumarins (benzopyran-2-one) as potent and selective monoamine oxidase inhibitors (IMAO) and that their affinity and selectivity can be efficiently modulated by appropriate substitution patterns in the heterocyclic moiety.[6] However, in which concerns the benzopyran-4-one scaffold, only few works were found in the literature about their putative potential as IMAO.[7]

Accordingly, our project has been focused on the discovery of new chemical entities for MAO inhibition with benzopyran-4-one substructure (Scheme 1). Preliminary studies performed with chromones (**1**) and (**7**) allow disclosing a significant chemical feature: 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 (**7**) binds to *h*MAO-B, exerting a selective inhibition with respect to A isoform (IC_{50} *h*MAO-B 0.048 ± 0.0026 nM; SI >2083). The inhibition is of irreversible type (data not shown). Its isomer (**1**), with the carboxylic function in position 2, doesn't present activity for both MAO isoforms.[8] Molecular modeling studies performed with the chromone carboxylic acids reveal a crucial, undisclosed role of the presence of an hydrogen donor group in position 3 of the pyrone ring that could be able to establish hydrogen bond interactions with active site residues.[8]

In this context, and in an attempt to develop novel reversible and selective MAO-B inhibitors, the synthesis of 2- and 3-carboxamide chromone derivatives capable of establishing hydrogen interactions with the enzyme was performed. In this work, several 2- and 3-phenylcarboxamide chromones with or without different

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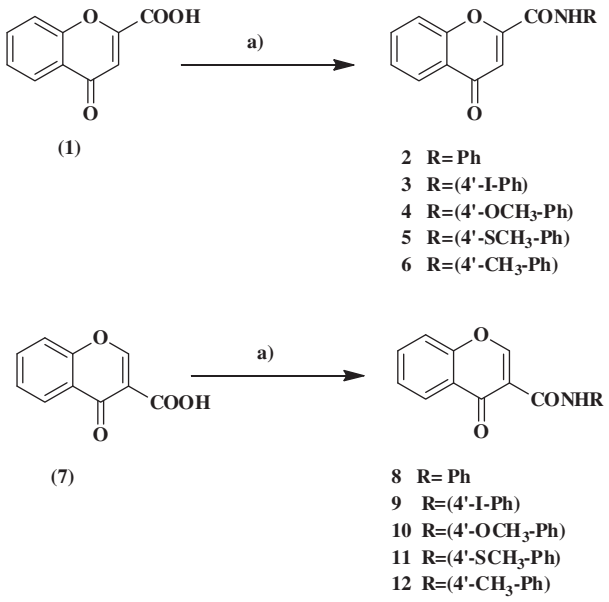

Scheme 1. Structure of the chromones under study. Reagents and conditions: (a) BOP or PyBOP, R-C_6H_4–NH_2, CH_2Cl_2, DMF, DIPEA.

type of substituents in *para*-position of the exocyclic aromatic nucleus (Scheme 1) were obtained by an expedite synthetic strategy and screened towards human MAO isoforms (*h*MAO) to evaluate their potency/selectivity ratio.

The chromone derivatives **2–6** and **8–12** were efficiently synthesized according to the synthetic protocol outlined in Scheme 1.[9] Chromone carboxamide derivatives were synthesized straightforward by a one-pot condensation reaction that occurs, in equimolar amounts, between the corresponding chromone carboxylic acid (compound **1** or **7**) and aniline or its ring-substituted derivatives. The reaction was clean and the compounds were obtained with moderate/high yields (50%–80%). After the reaction, the crude products were purified by flash column chromatography and crystallization.

The MAO inhibitory activity of compounds **2–6** and **8–12** was evaluated in vitro by the measurement of the enzymatic activity of human recombinant MAO isoforms in BTI insect cells infected with baculovirus.[10] Then, the IC_{50} values and MAO-B selectivity ratios (SI) [IC_{50} (MAO-A)]/[IC_{50} (MAO-B)] for inhibitory effects of both new compounds and reference compounds (*R*-(−)-deprenyl and iproniazide) were calculated.

The results of the inhibitory potencies and selectivities of the chromones under study towards MAO isoforms, and reference compounds, are depicted in Table 1. From the data one can conclude that 'chromones bearing carboxamide substituents in position 3 of the γ-pyrone nucleus' act preferably as MAO-B inhibitors (IMAO-B) with IC_{50} values in micro to nanomolar range (**8–12**). The same tendency was found with the chromone carboxylic acids.[8] In addition, one can conclude that the type of substituents in the *para*-position of the exocyclic aromatic ring can modulate the affinity and selectivity of the chromones-3-carboxamides as IMAO-B. The introduction of a methoxyl group (**10**) seems to have no effect in IMAO-B potency when compared with the activity found for compound (**8**). Nevertheless it is important to point out that the introduction of a thiomethyl group (**11**), a bioisostere of the methoxyl function, has improved the potency of the compound 3–4-fold relatively to the compounds **8** and **10**. The most promissory compounds as IMAO-B, with an IC_{50} <75 nm and a SI of >1440, are the compounds **9** and **12**, which are substituted in *para*-position by iodo and methyl groups, respectively, representing an improvement of potency of six fold relatively to compounds **8** and **10**. From the overall data it was concluded that the positive hydrophobicity (+π) of the substituent, besides inductive and mesomeric effects, located on the phenyl exocyclic moiety markedly influence the potency and selectivity of the chromone carboxamides.

Table 1
*h*MAO-A and *h*MAO-B inhibitory activity results for compounds **2–6**, **8–12** and reference compounds

Compound	*h*MAO-A IC_{50} (μM)	*h*MAO-B IC_{50} (μM)	SI
2 R = Ph	[a]	[a]	—
3 R = (4′-I-Ph)	[a]	[a]	—
4 R = (4′-OCH_3-Ph)	[a]	[a]	—
5 R = (4′-SCH_3-Ph)	[a]	[a]	—
6 R = (4′-CH_3-Ph)	[a]	[a]	—
8 R = Ph	[a]	0.40 ± 0.022	>250[c]
9 R = (4′-I-Ph)	[a]	0.069 ± 0.003	>1449[c]
10 R = (4′OCH_3-Ph)	[a]	0.45 ± 0.029	>222[c]
11 R = (4′SCH_3-Ph)	[a]	0.12 ± 0.0080	>833[c]
12 R = (4′-CH_3-Ph)	[a]	0.068 ± 0.003	>1471[c]
Deprenyl	68.73 ± 4.21[b]	0.017 ± 0.002	4043
Iproniazide	6.56 ± 0.76	7.54 ± 0.36	0.87

[a] Inactive at 100 μM (highest concentration tested). At higher concentrations the compounds precipitate.
[b] P <0.01 versus the corresponding IC_{50} values obtained against *h*MAO-B, as determined by ANOVA/Dunnett's.
[c] Values obtained under the assumption that the corresponding IC_{50} against *h*MAO-A is the highest concentration tested (100 μM).

Noteworthy to mark out that the chromones bearing the same type of substituents in position 2 of γ-pyrone nucleus (chromones **2–6**) present a total loss of MAO activity.

Preliminary studies on the type of *h*MAO inhibition were performed revealing that chromone 3-phenylcarboxamides behave as quasi-reversible MAO-B inhibitors (data not shown).

In the present Letter, the effect of the introduction of a methyl or iodo substituent in *para*-position of the exocyclic aromatic ring of chromone 3-phenylcarboxamides was outlined. In fact, the introduction of this type of groups improves the pharmacologic potential of chromone 3-phenylcarboxamides confirming that this lead could be effectively optimized in a candidate for the treatment of neurodegenerative diseases. These findings have encouraged us to continue the efforts towards the optimization of the lead compound.

In conclusion chromone appears to be an interesting scaffold for the design of selective IMAO. The easy synthetic accessibility and especially the versatile binding properties of chromones make them as 'privileged' scaffolds. These discoveries open a new avenue to obtain highly potent and selective MAO-B inhibitors structurally based on chromone scaffold.

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General procedure for amide obtention. 2-Carboxychromone (**1**) or 3-carboxychromone (**7**) (2.63 mmol) was dissolved in 6 mL of DMF and 0.37 mL of diisopropylethylamine (DIPEA). The solution was then cooled to 0 °C and a BOP (2.63 mmol) or PyBOP (2.63 mmol) solution in CH_2Cl_2 (6 mL) was added and the mixture was stirred for 30 min. After, the corresponding amine was added in equimolar amount and the temperature was let to gradually increase to room temperature. The reaction was stirred for additional 4–6 h.
10. *Evaluation of human monoamine oxidase (hMAO) isoform activity*. The effects of the tested compounds on *h*MAO isoform enzymatic activity were evaluated by a fluorimetric method following the experimental protocol previously described (Santana, L.; Uriarte, E.; González-Díaz, H.; Quezada, E.; Uriarte, E.; Yáñez, M.; Viña, D.; Orallo, F. *J. Med. Chem.* **2008**, *51*, 75). Briefly, 0.1 mL of sodium phosphate buffer (0.05 M, pH 7.4) containing the test drugs in various concentrations and adequate amounts of recombinant *h*MAO-A or *h*MAO-B required and adjusted to obtain in our experimental conditions the same reaction velocity. The reaction was started by adding (final concentrations) 200 μM Amplex® Red reagent, 1 U/mL horseradish peroxidase and 1 mM *p*-tyramine. The production of H_2O_2 and, consequently, of resorufin was quantified at 37 °C in a microplate fluorescence reader (excitation: 545 nm, emission: 590 nm) over a 15 min period, in which the fluorescence increased linearly. Control experiments were carried out simultaneously. The control activity of *h*MAO-A and *h*MAO-B (using *p*-tyramine as a common substrate for both isoforms) was 165 ± 2 pmol of *p*-tyramine oxidized to *p*-hydroxyphenylacetaldehyde/min ($n = 20$).
All IC_{50} values shown in the table are expressed as means ± SEM from five experiments.