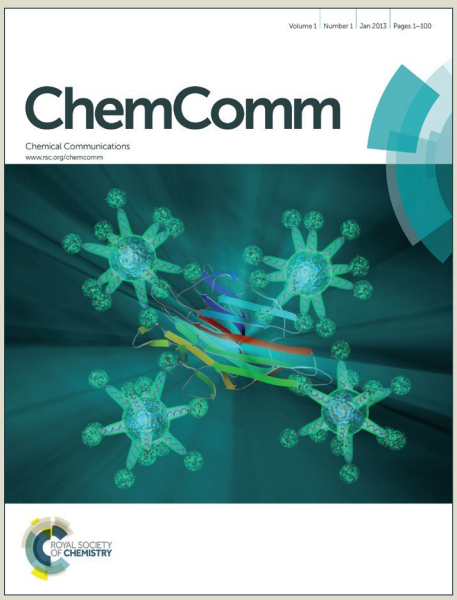
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

View Article Online
DOI: 10.1039/C4CC08798D

Journal Name

RSCPublishing

COMMUNICATION

Discovery of two new classes of potent monoaminoxidase-B inhibitors by tricky chemistry

Cite this: DOI: 10.1039/x0xx00000x

F. Cagide,[a¥] T. Silva[a¥], J. Reis[a], A. Gaspar[a], F. Borges[a*], L.R. Gomes[b] and J.N. Low[c]

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

The discovery of potent and selective monoamine oxidase-B inhibitors for the management of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease is still a challenging endeavor. Herein, we report the discovery of two new classes of potent and selective MAO-B inhibitors based on chromane-2,4-dione and chromone-3-carboxamide scaffolds.

Monoamine oxidase (MAO) is an enzyme present in mammals in two isoforms: MAO-A and MAO-B. These isoforms have a crucial role in the metabolism of neurotransmitters, representing strategic and important drug targets in the therapy of neurodegenerative diseases (MAO-B) and depression (MAO-A). Selective MAO-B inhibitors (IMAO-B) as deprenyl and rasagiline are currently used in the symptomatic treatment of Parkinson's disease (PD), either alone or in combination with levodopa. Currently, the side effects associated with the use of deprenyl and rasagiline (Figure **1A**) as well as their potential application for Alzheimer's disease (AD) are the main factors driving the discovery of new IMAO-B with increased potency and selectivity.[1-3] Over the past years, the privileged structure concept has emerged as a stimulus for the acceleration of drug discovery and development processes, as diversity-oriented synthesis is a fast-track practice. In this context, the chromone (1,4-benzopyrone) scaffold has been selected by our research group as the core for the design and development of new IMAO-B.[4] In this framework, a function-oriented library based on chromone-3-carboxylic acid, a promising IMAO-B,[5] has been synthesized to accomplish structure-activity-relationship (SAR) studies. The effect of the incorporation of an amide bond, a long-established biological chemical connection, in the chromone core on the activity was also assessed.[6,7] Consequently, a set of chromone carboxamide derivatives have been synthesized and their IMAO activity evaluated. In addition, a combined theoretical and experimental study has been carried out to explain the superior activity of chromone 3-carboxamide versus chromone 2-carboxamide as IMAO-B.[7,8] As a result, chromone-3-carboxamide emerged as a new scaffold for the development of IMAO-B (Fig. **1B**).

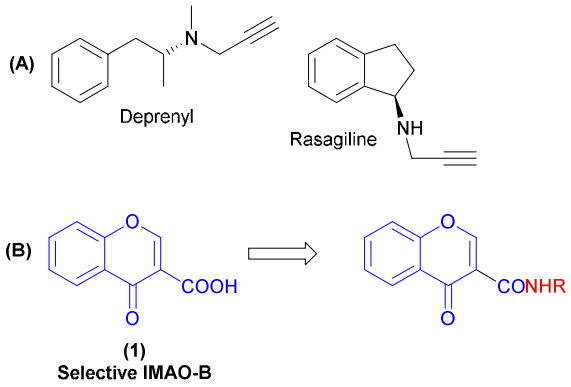
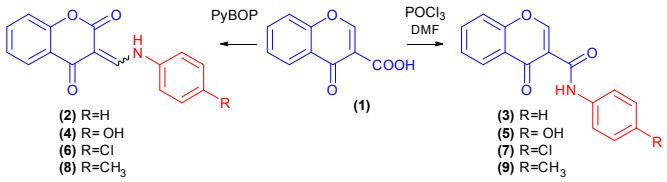


Figure 1. (A) MAO-B inhibitors used in single or combined therapy; (**B**) Discovery of IMAO-B based on chromone scaffold.

However, some queries arising from the previous studies still remain open, namely those related to spectroscopic data and the putative presence of tautomeric forms in solution.[8] To provide further insight, NMR experiments at variable temperatures were performed along with theoretical studies using NMR spectral predictors, but no spectral changes were observed, even at low temperatures. Thus, additional efforts had to be carried to address and clarify the remaining issues. Accordingly, the chromone-3-carboxamide derivatives were synthesized by two different amidation reactions using the same starting materials (chromone-3-carboxylic acid and the appropriate amine). The selected reactions involve different methods of activating the carboxylic acid function and have in common the subsequent addition of an arylamine: a) the use of phosphonium salt (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) as coupling agent and b) the *in situ* generation of an acyl chloride with phosphorus (V) oxychloride

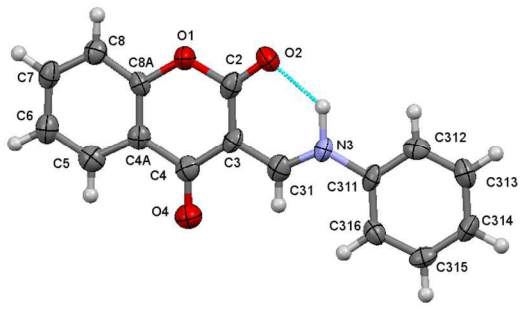
View Article Online
DOI: 10.1039/C4CC08798D
Journal Name

($POCl_3$) in DMF. From the array of coupling reagents described so far, PyBOP was selected since it can minimize some of the well-known drawbacks of this type of reactions, such as laborious purification steps due to the presence of by-products, namely those from dicyclohexylcarbodiimide type coupling reagents. [9-11] In addition, PyBOP operates in mild and slightly basic conditions and presents less toxicity constraints as compared to other phosphonium coupling agents, such as BOP. Accordingly, our first attempt to obtain chromone-3-carboxamides was performed using a one-pot condensation procedure and PyBOP as coupling reagent. Briefly, to a solution of the chromone-3-carboxylic acid in dimethylformamide (DMF) in the presence of N,N-diisopropylethylamine (DIPEA), PyBOP was added at 0ºC. This step led to the activation of the chromone-3-carboxylic acid and *in situ* formation of an activated ester intermediate. Then, the amine with the appropriate aromatic substitution pattern was added to the mixture. In the PyBOP-assisted experiments, the structural elucidation (NMR and EM/IE) data of the main reaction products was consistent with the previous reported results. [6-8] (see Supplementary Information). In parallel, and as mentioned before, a different methodology using acidic conditions ($POCl_3$ in DMF) was performed. Briefly, the synthetic strategy involved the *in situ* generation of the chromone-3-acyl chloride, and subsequent addition of aniline, or its derivatives. This procedure led to the formation of chromone type compounds that were fully characterized by NMR and EM/IE (see Supplementary Information). The spectroscopic data were divergent from the aforementioned. From the data gathered, it was concluded that two types of chemical entities have been synthesized from chromone-3-carboxylic acid (Scheme **1**): one based on chromane-2,4-dione and the other on the chromone-3-carboxamide core.

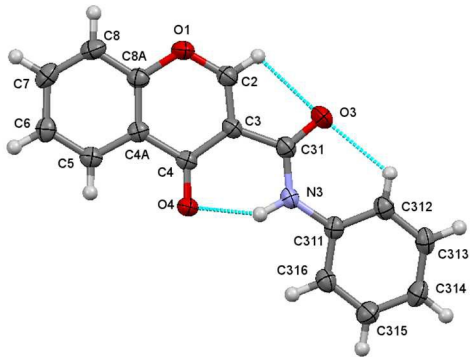


Scheme 1. Synthesis of chromane-2,4-dione (**2**, **4**, **6** and **8**) and chromone-3- carboxamide (**3**, **5**, **7** and **9**) based derivatives from chromone -3-carboxylic acid (**1**).

A complete characterization of compounds **2** and **3** (Scheme **1**) obtained in PyBOP and $POCl_3$/DMF reactional conditions, respectively, was made by single crystal X-Ray diffractometry (Fig. **2**). Details for hydrogen bonding geometries as well as the discussion of molecular geometries are given in *Supplementary Information*. In both cases the X-Ray structural analysis corroborate the spectroscopic data.



(2)



(3)

Figure 2. X Ray structure of 3-((phenylamino)methylene)chromane-2,4-dione (**2**) and N-phenyl-4-oxo-4H-chromene-3-carboxamide (**3**). Details for data acquisition, structural solving and refinement are given in files deposited in the Cambridge Structural Database: CCDC 895638 for 2 and CCDC 932188 for 3.

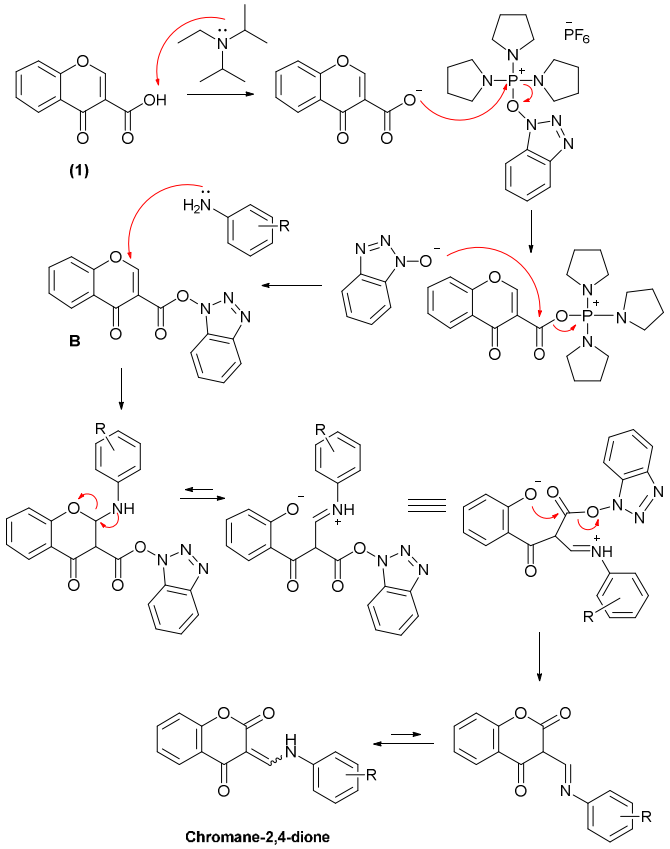
After the identification of chromane-2,4-dione and chromone-3-carboxamide, and their derivatives, other studies were implemented to acquire evidence to explain their formation from the same starting material. As the reactivity of chromone-3-carboxylic acid (**1**) towards primary and secondary amines has been recently reported, [12, 13] the first step was to check if the arylamine could promote a nucleophilic attack at the C2 position of the starting material. To test this hypothesis, chromone-3-carboxylic acid (**1**) and aniline were mixed in dichloromethane, in the presence of DIPEA but in the absence of PyBOP, and stirred for the same reactional period of time (Scheme **2**).

Scheme 2. Formation of (Z)-1-(2-hydroxyphenyl)-3-(phenylamino)prop-2-en-1-one from chromone-3-carboxylic acid (**1**).

Upon completion a new compound was detected by TLC analytical control and it was identified as (*Z*)-1-(2-hydroxyphenyl)-3-(phenylamino)prop-2-en-1-one (Scheme **2**). Its formation is in accordance with previously published data. [12, 13] Hence, it can be concluded that in these conditions the amidation of chromone-3-carboxylic acid (**1**) does not take place. The formation of chromane-2,4-dione nucleus must then be directly connected to the PyBOP activation process and the presence of aniline, or its derivatives. From the experiments performed with chromone-3-carboxylic acid (**1**) in the presence of PyBOP and DIPEA, and in the absence of the aromatic amines, only the benzotriazolyl ester derivative (compound **B**, Scheme **3**) resultant from the activation of –COOH function was detected. Up to now, it seems that a nucleophilic attack by the amine at the C2 position occurs after *in situ* formation of the ester intermediate, causing ring-opening and ring closing processes that at the end lead to the formation of chromane-2,4-dione derivatives. Moreover, two main compounds were identified, either in the presence or absence of the non-nucleophilic base DIPEA : (*Z*)-1-(2-hydroxyphenyl)-3-(phenylamino)prop-2-en-1-one and the chromane-

View Article Online
DOI: 10.1039/C4CC08798D COMMUNICATION

2,4-dione based derivatives (Schemes **1** and **3**). Chromone-3-carboxamide based compounds were only detected in vestigial amounts. As the same process does not occur in the same conditions with chromone-2-carboxylic acid, one must conclude that the C2 position of chromone-3-carboxylic acid (**1**) is more activated towards nitrogen nucleophiles. It is worth noting that when chromone-2-carboxylic acid was used as starting material only chromone-2-carboxamides were obtained in both methods. [13, 14]



Scheme 3. Mechanistic proposal for the formation of chromane-2,4-dione and its derivatives from chromone-3-carboxylic acid (**1**).

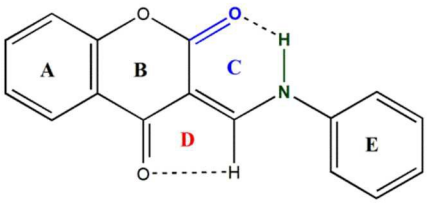
After the chemical findings, the next step involved MAO-B inhibition studies of chromane-2,4-dione derivatives (Scheme **1**, compounds **2**, **4**, **6** and **8**) and chromone-3-carboxamide derivatives (Scheme **1**, compounds **3**, **5**, **7** and **9**). Our previous studies [6-8] point out that, in general, chromone-3-carboxamide derivatives have superior inhibitory activity towards MAO-B than the chromone-2-carboxamide counterparts. The inhibitory activity of the compounds on human MAO (hMAO-A and hMAO-B isoforms) was evaluated by measuring their effect on the production of hydrogen peroxide (H_2O_2) from *p*-tyramine, which was detected using 10-acetyl-3,7-dihydroxyphenoxazine (Amplex Red reagent), a non-fluorescent and highly sensitive probe that reacts with H_2O_2 in the presence of horseradish peroxidase to produce a fluorescent product, resorufin (see *Supplementary Information*). The data of the hMAO *in vitro* studies, inhibitory potencies and selectivity indexes (SI) for the compounds under study and reference compounds is shown in Table **1**. The results show that regardless of the functionalization of the dihydropyran-2,4-dione and pyrone ring the majority of the compounds display potent and selective hMAO-B inhibitory activity (micromolar to nanomolar range). The presence of C3-substituents in the scaffold seems to be important for MAO-B inhibition. For each chromane-2,4-dione/ chromane-3-carboxamide pair (see Table **1**) bearing the same substitution pattern, the chromone-3-carboxamide counterparts generally display a higher activity towards hMAO-B, except for compounds with the 4'-hydroxyaryl substituent (Scheme **1**, compounds **4** and **5**).

Table 1. MAO inhibitory activities of chromone carboxamides and reference inhibitors.

Compound	IC_{50} (nM)[a]		S.I.[a]
	*h*MAO-A	*h*MAO-B	
2	*b*	267.9 ± 7.8	> 37
3	*b*	70.7 ± 1.7	> 140
4	*b*	49.5 ± 1.6	>202
5	*b*	1382.4 ± 2.5	> 7.2
6	*b*	64.9 ± 1.4	> 154
7	*b*	2.9 ± 1.2	> 3448
8	*b*	42.3 ± 0.3	> 236
9	*b*	8.0 ± 3.2	> 1250
(*R*)-(−)-Deprenyl	68734 ± 4.2	19.44 ± 0.7	3536
Clorgyline	6.27 ± 0.3	63410 ± 1.1	0.000099

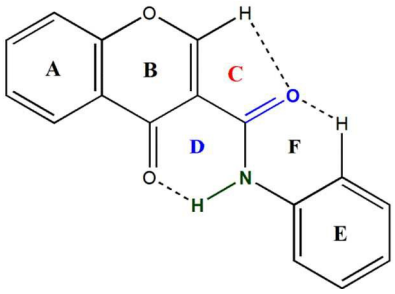
[a] All IC_{50} values are the mean ± SD from three experiments. SI: *h*MAO-B selectivity index = IC50(*h*MAO-A)/IC50(*h*MAO-B); [b] Inactive at 10 µM (highest concentration tested).

These results point out that the presence of the amide spacer between the heterocyclic and the exocyclic ring is of utmost importance for MAO-B inhibitory activity. The presence of a p-substituent in the exocyclic ring (Cl or CH_3) favors the activity and selectivity, with the exception for the 4'-hydroxyaryl chromone (compound **5**). In fact, compounds **7** and **9** are potent and selective IMAO-B, being more potent than the standard inhibitor deprenyl 6.7- and 2.4-fold, respectively. The differences in IMAO-B activity found for **2** and **3** sparked a closer look into the geometrical differences between them, as determined by single crystal X-Ray diffractometry, which are schematically depicted in Fig. **3**.



(2)

View Article Online
DOI: 10.1039/C4CC08798D Journal Name



(3)

Figure 3. Schematic diagram of the geometrical differences of 3-(phenylamino)methylene)chromane-2,4-dione **(2)** and N-phenyl-4-oxo-4H-chromene-3-carboxamide **(3)**.

Apart from the slight differences found in molecular conformation with respect to planarity, see supplementary information for values , both compounds 3-(phenylamino)methylene)chromane-2,4-dione (2) and N-phenyl-4-oxo-4H-chromene-3-carboxamide (3) present a framework consisting of 5 similiar rings: A and B of the benzopyran moiety, the E benzyl ring and two extra pseudo-cyclic rings: a six-membered S(6) ring C and a five-member S(5) ring [15] identified as D, both resulting from the formation of intramolecular hydrogen interactions. Moreover, in N-phenyl-4-oxo-4H-chromene-3-carboxamide (3) an additional six-membered S(6) ring [15], identified as F was projected. The geometric molecular differences may help in the understanding of the enzyme-inhibitor interactions taking place within the substrate pocket and they seem to be fundamentally related with: (i) the relative positions of the rings; compound 2 presents a framework of three fused "anthracene like" 6 member rings while compound 3 displays a skeleton of three fused "phenantrene like" 6 member rings: (ii) the relative positions of the hydrogen bonding donors and acceptors; the 4-keto oxygen atom of the pyran type ring is identically positioned in both molecules but the nitrogen donors and the other carbonyl acceptors are placed differently; iii) the position of the phenyl substituent differs as a consequence of the assumed configurations around the enamine and amide spacers between the aromatic residues.

Conclusions

In summary, chromane-2,4-dione and chromone-3-carboxamide derivatives were obtained from chromone-3-carboxylic acid by a process that is dependent on the nature of the activating reagents and not reliant on the type of aniline used in the study. Although chromane-2,4-dione and chromane-3-carboxamide derivatives are potent and selective IMAO-B, chromone-3-carboxamide counterparts generally display higher activity The geometrical differences found between the two chemical frameworks can justify the biological data as they can hinder or assist the ligand-enzyme interactions with the enzyme pocket.

Notes and references

[a] CIQUP/Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, 4169-007Porto, Portugal.
[b] FP-ENAS-Faculdade de Ciências de Saúde, Escola Superior de Saúde da UFP, Universidade Fernando Pessoa, P-4200-150 Porto, Portugal
[c] Department of Chemistry, University of Aberdeen, Meston Walk, Old Aberdeen, AB24 3UE, Scotland
[¥] These authors contributed equally to this work

† Electronic supplementary information (ESI) available: Experimental chemical and biological details, characterization data and X-ray data collection, structure solution and refinement.

The authors thank the Foundation for Science and Technology (FCT) of Portugal (PEst-C/QUI/UI0081/2013). F. Cagide (SFRH/BPD/74491/2010), T. Silva (SFRH/BD/79671/2011), J. Reis (SFRH/BD/96033/2013) and A. Gaspar (SFRH/BPD/93331/2013) grants are supported by FCT. Thanks are also due to the staff at the National Crystallographic Service, University of Southampton for the data collection, help and advice. [16]

1 J. Reis, I. Encarnação, A. Gaspar, A. Morales, N. Milhazes and F. Borges, *Curr. Top. Med. Chem.*, 2012, **12**, 2116-2130.
2 A.M. Helguera, G. Perez-Machado, M.N. Cordeiro and F. Borges, *Mini Rev. Med. Chem.*, 2012, **12**, 907-919.
3 T. Thomas, *Neurobiol. Aging*, 2000, **21**, 343-348.
4 A. Gaspar, M.J. Matos, J. Garrido, E. Uriarte and F. Borges, *Chem. Rev.*, 2014, **114**, 4960-4992.
5 S. Alcaro, A. Gaspar, F. Ortuso, N. Milhazes, F. Orallo, E. Uriarte, M. Yáñez and F. Borges, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 2709-2712.
6 A. Gaspar, J. Reis, A. Fonseca, N. Milhazes, D. Viña, E. Uriarte and F. Borges, *Bioorg Med Chem Lett*, 2011, **21**, 707-709.
7 A. Gaspar, T. Silva, M. Yáñez, D. Vina, F. Orallo, F. Ortuso, E. Uriarte, S. Alcaro and F. Borges, *J. Med. Chem.*, 2011, **54**, 5165-5173.
8 A. Gaspar, F. Teixeira, E. Uriarte, N. Milhazes, A. Melo, M.N. Cordeiro, F. Ortuso, S. Alcaro and F. Borges, *ChemMedChem,* 2011, **6**, 628-632.
9 C.A.G.N. Montalbetti and V. Falque, *Tetrahedron*, 2005, **61**, 10827-10852.
10 E. Valeur and M. Bradley, *Chem Soc Rev*, 2009, **38**, 606-631.
11 M.M. Joullie and K.M. Lassen, *Arkivoc,* 2010, **viii**, 189-250.
12 M.A. Ibrahim, *Tetrahedron*, 2009. **65**, 7687-7690.
13 M.A. Ibrahim, *Arkivoc*, 2008, **xvii,** 192-204.
14 F. Cagide, J. Reis, A. Gaspar and F. Borges, *Tetrahedron Lett.*, 2011, **52**, 6446-6449.
15 A. Gaspar, F. Cagide, E. Quezada, J. Reis, E. Uriarte and F. Borges, *Magn. Reson. Chem.*, 2013, **51**, 251-254.
16 S.J. Coles and P.A. Gale, *Chem. Sci.*, 2012, **3**, 683-689