Supplementary information

A. Chemistry

1. Apparatus

Microwave-assisted synthesis was performed in a Biotage® Initiator Microwave Synthesizer.

NMR was recorded on a Bruker AMX 400 NMR spectrometer. ^{1}H and ^{13}C NMR spectra of samples were recorded at room temperature in 5 mm outside diameter (o.d.) tubes. Tetramethylsilane (TMS) was used as internal standard, chemical shifts are expressed in ppm (δ) and J in Hz. For the DEPT sequence, the width of the 90◦ pulse for ^{13}C was 4 μs, and that of the 90º pulse for ^{1}H was 9.5 μs; the delay $2J_{C,H}{}^{-1}$ was set to 3.5 ms (underlined values).

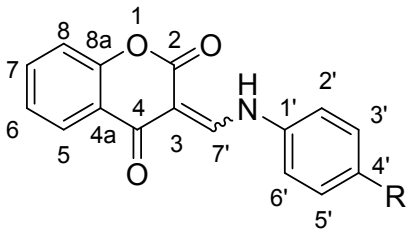
Electron impact mass spectra (EIMS) were carried out on a VG AutoSpec Fison, Ipswich, United Kingdom) instrument; the data are reported as m/z (percentage of relative intensity of the most important fragments).

2. Materials

Chromone-3-carboxylic acid, phosphorus(V) oxychloride, (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP), N,N-diisopropylethylamine (DIPEA), dimethylformamide (DMF) and the different anilines were purchased from Sigma-Aldrich Química S.A. (Sintra, Portugal). All other reagents and solvents were pro analysis grade and used without additional purification. Thin-layer chromatography (TLC) was carried out on precoated silica gel 60 F254 (Merck) with layer thickness of 0.2 mm. For analytical control the following systems were used: ethyl acetate/petroleum ether, ethyl acetate/methanol, chloroform/methanol in several proportions. The spots were visualized under UV detection (254 and 366 nm) and iodine vapour. Flash column chromatography was performed using silica gel 60 0.2-0.5 or 0.040-0.063 mm (Carlo Erba Reagents).Following the workup and after extraction, the organic phases were dried over Na_2SO_4. Solvents were evaporated in a Buchi Rotavapor.

3. Synthetic procedures

3.1 Synthesis of the chromane-2,4-dione derivatives



R	
H	**2**
OH	**4**
Cl	**6**
CH_3	**8**

General procedure

To a solution of the chromone-3-carboxylic acid (1mmol) in DMF (2.5 mL) at 0ºC was added a solution of PyBOP (1mmol) in CH_2Cl_2 (2.5 mL). The mixture was kept in an ice bath and stirred for half hour. After this period aniline, or its derivatives, was added and the mixture was allowed to warm up to room temperature. The reaction was kept with stirring by 4 hours. The working up of the crude material encloses a liquid-liquid extraction (CH_2Cl_2/water) followed by flash chromatography and/or crystallization.

(E/Z)-3-((Phenylamino)methylene)chromane-2,4-dione (**2**)

Yield 60%. Purified by flash column chromatography with a hexane/ethyl acetate (5:5). ^{1}H NMR (400 MHz, $CDCl_3$) δ : 7.40 – 7.26 (5H, *m*, H2, H3', H4', H5', H6'), 7.52 – 7.45 (2H, *m*, H6, H8), 7.64 – 7.57 (1H, *m*, H7), 8.14 (0.7H, *dd*, *J* = 7.8, 1.6 Hz, H5), 8.08 (0.3H, *dd, J* = 7.8, 1.7 Hz, H5), 8.91 (0.7H, *d*, *J* = 13.6 Hz, H7), 9.05 (0.3H, *d*, *J* = 14.5 Hz, H7), 11,94 (0.3H, *d*, *J* = 13.8 Hz, NH), 13.70 (0.7H, *d*, *J* = 12.2 Hz, NH). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 99.0/98.9 (C3), 117.6/117.5 (C8), 118.6/118.7 (C2', C6'), 120.9/120.5 (C4a), 124.6/124.4 (C6), 126.7/126.0 (C5), 127.7/127.5 (C4'), 130.4/130.3 (C3', C5'), 135.0/134.9 (C7), 137.9 (C1'), 154.9/153.6 (C8a), 155.2/155.1 (C7'), 165.4/163.8 (C2), 182.0/178.8 (C4), EI/ME (*m/z*): 266 (6), 265($M^{+\bullet}$, 61), 173 (100), 144 (16), 121 (35), 117 (36).

(E/Z)-3-(((4-hydroxyphenyl)amino)methylene)chromane-2,4-dione (**4**)

Yield 55%. Purified by flash column chromatography with a ethyl eter/petroleum ether gradient, starting ratio 5:5. ^{1}H NMR (400 MHz, DMSO) δ: 6.96 (2H, *m*, H3', H5'), 7.38-7.32 (2H, *m*, H6, H8), 7.50 (2H, *d*, *J*=8.6 Hz H2', H6'), 7,98 (1H, *d*, *J*=7,3 Hz, H5), 7.73-7.68 (1H, *m*, H7), 9.87/9.83 (1H, *s*, OH), 8.71/8.76 (1H, *s*, H7'), 11.85 (0.3H, *d*, *J*=14.2 Hz, NH), 13.53 (0.7H, *d*, *J*=14.6 Hz, NH). ^{13}C NMR (101 MHz, DMSO) δ: 97.2 (C3), 116.2/116.1 (C3', C5'), 117.1 (C8), 120.1 (C4a), 121.3/120.9 (C2', C6'), 124.2 (C6), 125.4 (C5), 129.7 (C1'), 134.9 (C7), 154.6/154.3 (C8a), 154.6 (C7'), 156.9 (C4'), 162.4 (C2), 179.9 (C4). EI/ME (m/z): 282 (19), 281 ($M^{+\bullet}$, 100), 208 (5), 207 (29), 174 (7), 173 (67), 133 (12), 121 (15), 93 (2), 58 (21).

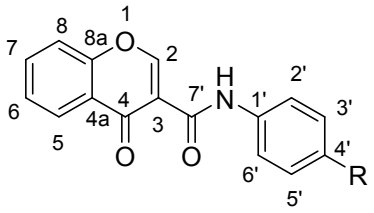
(E/Z)-3-(((4-chlorophenyl)amino)methylene)chromane-2,4-dione (**6**)

Yield 47%. Purified by crystallization from dichloromethane. ^{1}H NMR (400 MHz, DMSO) δ: 7.34 (1H, *d*, *J* = 7.8 Hz, H8), 7.38 (1H, *dd*, *J* = 7.6, 1.3 Hz, H6), 7.54 (2H, *d*, *J* = 8.8 Hz, H3', H5'), 7.70-7.73 (3H, m, H2', H6', H7), 7.99 (1H, *dd*, *J* = 7.8, 1.5 Hz , H5), 8.85 (0.7H, *d*, *J* = 13.8, H7'), 8.88 (0.3H, d, *J* = 14.8 Hz , H7'), 11.84 (0.3H, d, *J* = 14.8, NH), 11.84 (0.3H, *d*, *J* = 14.8 Hz, NH), 13.39 (0.7H, *d*, *J* = 13.8 Hz, NH). ^{13}C NMR (101 MHz, CDCl3) δ: 98.3 (C3), 117.2 (C8), 119.9 (C4a), 121.6/121.3 (C2', C6'), 124.3 (C5), 125.5 (C6), 129.6/129.1 (C3', C5'), 131.2 (C4'), 135.1, 137.1 (C1'), 154.5 (C8a) 155.8/154.5 (C7'), 162.2 (C2), 180.3 (C4). EI/ME (m/z): 301 (37), 300 (21), 299 ($M^{+\bullet}$, 88), 174 (16), 173(100), 151 (18), 121 (37), 92 (11), 89 (10)

(E/Z)-3-((p-tolylamino)methylene)chromane-2,4-dione (**8**)

Yield 44%. Purified by flash column chromatography with hexane/ethyl acetate (5:5). ^{1}H NMR (400 MHz, DMSO) δ : 2.39 (3H, *s*, CH_3), 7.23-7.32 (6H, *m*, H6, H8, H2', H3', H5', H6'), 7.60 (1H, *ddd*, *J* = 8.6, 7.0, 1.6 Hz, H7), 8.07 (0.7H, *dd*, *J* = 7.9, 1.6 Hz, H5), 8.14 (0.3H, *dd*, *J* = 7.9, 1.6 Hz H5), 8.86 (0.7H, *d*, *J* = 13.7 Hz, H7'), 9,00 (0.3H, *d*, *J* = 14.6 Hz, H7'), 11..92 (0,3H, *d*, *J* = 14.8 Hz, NH), 13.69 (0,7H, *d, J* = 13.1 Hz, NH). ^{13}C NMR (101 MHz, DMSO) δ: 21.9 (CH_3), 99.3 (C3), 118.3 (C8), 119.3 (C2', C6'), 121.1 (C4a), 125.1 (C6), 126.5 (C5), 131.6 (C3', C5'), 135.6 (C7), 136.2 (C1'), 138.6 (C4'), 155.6/154.2 (C7'), 155.6 (C8a), 164.6 (C2), 182.6/179.7 (C4). EI/ME (m/z): 280 (57), 279 ($M^{+\bullet}$, 99), 278 (21), 250 (10), 174 (24), 173 (100), 159 (16), 158 (23), 131 (37), 130 (44), 121 (44), 92 (11), 91 (24), 77 (12), 65 (20).

3.2. Synthesis of chromone-3-carboxamide derivatives



R	
H	**3**
OH	**5**
Cl	**7**
CH_3	**9**

General procedure

To a solution of chromone-3-carboxylic acid (500 mg, 2.6 mmol) in DMF (4 mL), $POCl_3$ (241 µl, 2.6 mmol) was added. The mixture was stirred at room temperature for 30 min, with the formation *in situ* of the corresponding acyl chloride. Then the aromatic amine with the pretended aromatic pattern was added. After 1- 5 hours, the mixture was then diluted with dichloromethane (20 mL), washed with H_2O (2X10 mL) and with saturated $NaHCO_3$ solution (2X10 mL). The organic phase was dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography and/or crystallization.

N-(phenyl)-4-oxo-4H-chromene-3-carboxamide (**3**)

Yield 39%, purified by flash chromatography (CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.19 – 7.12 (1H, m, H4'), 7.42 – 7.34 (2H, m, H3', H5'), 7.55 (1H, *ddd, J* = 8.1, 7.2, 1.1 Hz, H6), 7.61 (1H, *dd, J* = 8.5, 0.6 Hz, H8), 7.77 – 7.71 (2H, *m*, H2', H6'), 7.80 (1H, *ddd*, *J* = 8.7, 7.2, 1.7 Hz, H7'), 8.35 (1H, *dd*, *J* = 8.0, 1.7 Hz, H8), 9.09 (1H, *s*, H2), 11.41 (1H, *s*, NH). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 116.2 (C3), 118.7 (C8), 120.7 (C2', C6'), 124.2 (C4a), 124.6 (C6), 126.4 (C5), 126.7 (C4'), 129.2 (C3', C5'), 135.0 (C7), 138.1 (C1'), 156.3 (C8a), 160.9 (C7'), 163.0 (C2), 177.6 (C4). EI/ME (*m/z*): 265 (M^+, 92), 173 (100), 146 (15), 121 (68).

N-(4-hydroxyphenyl)-4-oxo-4H-chromene-3-carboxamide (**5**)

Yield 34%, purified by washing the formed solid with H_2O, MeOH and CH_2Cl_2. ^{1}H NMR (400 MHz, DMSO) δ: 6.83 – 6.73 (2H, *m*, H3', H5'), 7.56 – 7.47 (2H, *m*, H2',

H6'), 7.68 – 7.59 (1H, *m*, H6'), 7.81 (1H, *d*, *J* = 8.5 Hz, H8), 7.94 (1H, *ddd*, *J* = 8.6, 7.1, 1.7 Hz, H7), 8.24 (1H, *dd*, *J* = 8.0, 1.5 Hz, H5), 9.15 (1H, *s*, H2), 9.35 (1H, *bs*, *J* = 1.4 Hz, OH), 11.08 (1H, *s*, NH). ^{13}C NMR (101 MHz, DMSO) δ: 115.4 (C3', C5'), 115.7 (C3), 118.8 (C8), 121.5 (C2', C6'), 123.4 (C4a), 125.5 (C6), 126.7 (C5), 129.7 (C1'), 135.4 (C7), 154.0 (C4'), 155.7 (C8a), 159.7 (C7'), 163.1 (C2), 176.7 (C4). EI/ME (*m/z*): 281 (M^+, 45), 173 (100), 121 (40).

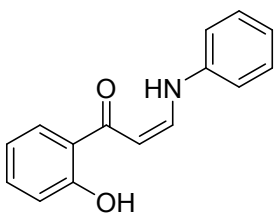
N-(4-chlorophenyl)-4-oxo-4H-chromene-3-carboxamide (**7**)

Yield 38%, purified by flash chromatography (20% AcOEt/CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.39 – 7.30 (2H, *m*, H3', H5'), 7.55 (1H, *ddd*, *J* = 8.1, 7.3, 1.0 Hz, H6), 7.61 (1H *d*, *J* = 8.1 Hz, H8), 7.74 – 7.67 (2H, *m*, H2', H6'), 7.81 (1H, *ddd*, *J* = 8.7, 7.2, 1.7 Hz, H7), 8.34 (1H, *dd*, *J* = 8.0, 1.6 Hz, H5), 9.07 (1H, *s*, H2), 11.47 (1H, *s*, NH). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 115.9 (C3), 118.7 (C8), 121.8 (C2'), 121.9 (C6'), 124.2 (C4a), 126.4 (C6), 126.8 (C5), 129.2 (C3', C5'), 129.6 (C4'), 135.1 (C7'), 136.6 (C1'), 156.3 (C8a), 160.9 (C7'), 163.1 (C2), 177.6 (C4). EI/ME (*m/z*): 302 (M^+ +3, 25), 301 (M^+ +2, 62), 300 (M^+ +1, 50), 299 (M^+, 87), 174 (56) 173 (100), 172 (21), 146 (24), 121 (68).

N-(p-tolyl)-4-oxo-4H-chromene-3-carboxamide (**9**)

Yield 19%, purified by flash chromatography (50% AcOEt/n-hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.34 (3H, *s*, CH_3), 7.21 – 7.14 (2H , *m*, H2', H6'), 7.54 (1H, *ddd*, *J* = 8.1, 7.2, 1.1 Hz, H6'), 7.59 (1H, *dd*, *J* = 8.5, 0.6 Hz, H8), 7.65 – 7.61 (2H, *m*, H3', H5'), 7.79 (1H, *ddd*, *J* = 8.7, 7.2, 1.7 Hz, H7), 8.34 (1H, *dd*, *J* = 8.0, 1.7 Hz, H5), 9.07 (1H, *s*, H2), 11.32 (1H, s, NH). ^{13}C NMR (101 MHz, DMSO) δ: 21.1 (CH_3), 116.3 (C3), 118.7 (C8), 120.7 (C2', C6'), 124.3 (C4a), 126.4 (C6), 126.6 (C5), 129.7 (C3', C5'), 134.2 (C1'), 135.0 (C7), 135.6 (C4'), 156.3 (C8a), 160.7 (C7'), 162.9 (C2), 177.6 (C4) EI/ME (*m/z*): 280 (M^+ +1, 10), 279 (M^+, 60), 118 (100), 91 (25).

3.3. Synthesis of (Z)-1-(2-hydroxyphenyl)-3-(phenylamino)prop-2-en-1-one



To a solution of chromone-3-carboxylic acid (250 mg, 1.3 mmol) in DMF (3 ml) and DCM (3 mL) aniline (120 µl, 1.3 mmol) and DIPEA (120 µl, 1.3 mmol) were added The mixture was stirred at room temperature for 9 hours and then was diluted with dichloromethane (20 mL), washed with H_2O (2X10 mL), HCl 1M (2X10 mL) and with saturated $NaHCO_3$ solution (2X10 mL). The organic phase was dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was washed with MeOH, dried (Yield 62%) and spectroscopic data acquired. The data was in accordance with the literature. [1,2]

B. X-Ray structural analysis

1. Data collection, structure solution and refinement for 2 and 3

Details for crystal data, data collection and refinement can be obtained consulting the following information:

	Compound (**2**)	Compound (**3**)
Crystal data		
Chemical formula	$C_{16}H_{11}NO_3$	$C_{16}H_{11}NO_3$
M_r	265.26	265.26
Crystal system, space group	Orthorhombic, *Pna*2$_1$	Monoclinic, *P*2$_1$/*n*
Temperature (K)	100	100
a, *b*, *c* (Å)	16.539 (3), 6.2596 (12), 11.472 (2)	6.6795 (2), 7.3003 (3), 24.6020 (17)
α, β, γ (°)	90, 90, 90	90, 94.306 (7), 90
V (Å^3)	1187.7 (4)	1196.27 (10)
Z	4	4
Radiation type	Mo *K*α	Mo *K*α
µ (mm^{-1})	0.10	0.10
Crystal size (mm)	0.22 × 0.04 × 0.01	0.34 × 0.14 × 0.11
Data collection		
Diffractometer	Rigaku Saturn724+ (2x2 bin mode) diffractometer	Rigaku Saturn724+ (2x2 bin mode) diffractometer
Absorption correction	Multi-scan *CrystalClear*-SM Expert 2.0 r13 (Rigaku, 2011)	Multi-scan *CrystalClear*-SM Expert 3.1 b18 (Rigaku, 20112)
T_{min}, T_{max}	0.978, 0.999	0.966, 0.989
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	6335, 2573, 1943	15473, 2728, 2464

R_{int}	0.061	0.022
$(\sin\theta/\lambda)_{max}$ (Å^{-1})	0.649	0.649
Refinement		
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.058, 0.131, 1.04	0.034, 0.095, 1.08
No. of reflections	2573	2728
No. of parameters	181	185
No. of restraints	1	0
H-atom treatment	H-atom parameters constrained	H atoms treated by a mixture of independent and constrained refinement
$\Delta\rangle_{max}$, $\Delta\rangle_{min}$ (e Å^{-3})	0.25, -0.21	0.36, -0.18
Absolute structure	Not Determined	–
Absolute structure parameter	-	–

Computer programs: *CrystalClear*-SM Expert 2.0 r13 (3), *SHELXS* (4), *OSCAIL* (5), ShelXle (6) *SHELXL* (4), *Mercury* (7), *OSCAIL* (5).

Compound (**2**) crystallised in the non-centrosymmetric spacegroup $Pna2_1$, the polarity of the structure could not be determined since the molecule did not contain any atom with a higher atomic number than O. H atoms were treated as riding atoms with C—H(aromatic), 0.95 Å, with U_{iso} = 1.2Ueq(C).

In (**2**) the H attached to N3 was placed at a calculated position with the N—H distance of 0.88Å and with U_{iso} = 1.2Ueq(C). Its position checked on a final difference Fourier map. Table 1 lists the hydrogen bonding for (**2**)

In (**3**) the H attached to N3 was located on a difference map and refined and its position checked on a final difference Fourier map. Table 1 lists the hydrogen bonding for (**3**).

2. H bond parameters for 2 and 3.

Table 1. Hydrogen-bond geometry (Å, º) for (**2**)

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
N3—H3···O2	0.88	2.04	2.703 (5)	131
C316—H316···O2^{i}	0.95	2.57	3.453 (6)	155

Symmetry code: (i) -*x*+1, -*y*+2, *z*+1/2.

Table 2. Hydrogen-bond geometry (Å, º) for (**3**)

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
N3—H3···O4	0.892 (16)	1.921 (16)	2.7062 (11)	145.9 (14)
C2—H2···O3	0.95	2.31	2.7066 (13)	104
C312—H312···O3	0.95	2.30	2.8915 (13)	120
C2—H2···O4^{i}	0.95	2.35	3.1452 (12)	141
C5—H5···O1^{ii}	0.95	2.44	3.3894 (13)	174
C316—H316···O3^{ii}	0.95	2.44	3.3392 (13)	159

Symmetry codes: (i) *x*+1, *y*, *z*; (ii) *x*-1, *y*, *z*.

3. Discussion of structural data

3.1. Molecular structures

From the X Ray data several important structural features exhibited by the lead compounds: 3-((phenylamino)methylene)chromane-2,4-dione (**2**) and *N*-phenyl-4-oxo-4*H*-chromene-3-carboxamide (**3**) can be highlighted. Molecule **2** is built by a chromane -2,4-dione ring and a benzyl ring connected to the C3 position of the central core by an enamine spacer while molecule **3** is based on a 4-chromene ring and have a phenylamide substituent on C3 position. In **2** there is an electronic delocalization thought the enamine group as suggested by the C—N and C—C bond lengths of 1.315(2) and 1.386(2), respectively. [8] The phenyl substituent is in a *cis* position in relation to the 2-carbonyl group of the chromane ring. This geometry permits the establishment of two intramolecular hydrogen bonds that are schematically depicted in Fig 2 see manuscript) : in one the enamine nitrogen acts as a donor to the oxygen of the carbonyl function in position 2 giving a 6-member S(6) ring [9] and the oxygen atom of the carbonyl group located in position 4 acts as acceptor for the carbon atom of the enamine spacer, making a S(5) ring. [9] This cooperative intramolecular hydrogen pattern probably contributes to the relative planarity of the molecule, the highest dihedral angle is 8.06(16)º between the best planes formed by the chromane atoms and the phenyl ring.

Regarding compound **3** the structural analysis revealed that the amide moiety is practically planar with the chromone ring: the best plane formed by all non-H atoms of the chromone has maximum deviation of -0.0168(9)Å for carbon atom in the C7 position of the chromone and it makes a dihedral angle of 4.30(18)° with the plane defined by the O—C—N atoms of the amide residue. The loss of planarity for the

overall molecule is probably as result of the slightly twist of the phenyl substituent around the amidic N—C bond, that is the main factor affecting the value for the dihedral angle, 9.49(17)°, between the best plane of the benzyl ring and the O—C—N amidic plane. The dihedral angle between the mean plane of the chromone ring and that of the phenyl ring is 10.77(4)°. The molecule exhibits and *anti* configuration in respect to the C—N rotamer of the amide. Nevertheless, due to the asymmetry of the chromone residue, the *anti* configuration can assume a *cis* or *trans* geometry in which concerns the relative position of the amide carbonyl group and the carbonyl group of the chromone. Compound 3 exhibits a *trans* relation between these groups. This molecular configuration allows the establishment of three intramolecular H bonds. Another intramolecular hydrogen bond between amide nitrogen and the 4–oxo oxygen atom of the chromone ring is also shaped this bond allows the formation of 6-member S(5) ring. [4] In addition there are two possible H interaction involving the oxygen atom of the amide group, a C—H(ortho-chromene) interaction and a C—H(ortho-phenyl) with the oxygen atom of the amide group forming S(5) and S6 rings, respectively. [9]

C. Biological evaluation

The effect of the test compounds on both *h*MAO isoforms were evaluated by a fluorimetric assay, following a previously described method.[10] The inhibitory activity of the test compounds on *h*MAO was evaluated by measuring their effects on the production of hydrogen peroxide (H_2O_2) from *p*-tyramine, using the Amplex Red MAO assay kit (Molecular Probes, Inc., Eugene, OR, U.S.) and microsomal MAO isoforms prepared from insect cells (BTI-TN-5B1-4) infected with recombinant baculovirus containing cDNA inserts for *h*MAO-A or *h*MAO-B (Sigma-Aldrich Química S.A). The production of H_2O_2 catalyzed by MAO isoforms was detected using 10-acetyl-3,7-dihydroxyphenoxazine (Amplex Red reagent), a nonfluorescent and highly sensitive probe that reacts with H_2O_2 in the presence of horseradish peroxidase to produce a fluorescent product, resorufin. Briefly, 100 µL of sodium phosphate buffer (0.05 M, pH 7.4) containing various concentrations of the test compounds or reference inhibitors 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, i.e., to oxidize (in the control group) 21 pmol of *p*-tyramine/min (*h*MAO-A, 0,63 µg protein; specific activity, 47.97 nmol of p-tyramine oxidized to (p-hydroxyphenylacetaldehyde/min)/mg protein; *h*MAO-B, 1.26 µg of protein; specific activity, 16.62 nmol of (*p*-tyramine

transformed/min)/mg protein), was incubated for 15 min at 37˚C in a flat-black-bottom 96-well microplate (BRANDplates®, pureGrade™, BRAND GMBH, Wertheim, Germany) placed in a dark multimode microplate reader chamber. After this incubation period, the reaction was started by adding (final concentrations) 200 μM Amplex Red reagent, 1 U/mL horseradish peroxidase, and 1 mM ptyramine. The production of H_2O_2 and consequently of resorufin was quantified at 37˚C in a multimode microplate reader (Biotek Synergy HT), based on the fluorescence generated (excitation, 545 nm, emission, 590 nm) over a 15 min period, in which the fluorescence increased linearly. Control experiments were carried out simultaneously by replacing the test drugs (new compounds and reference inhibitors) with appropriate dilutions of the vehicles. In addition, the possible capacity of the above test drugs to modify the fluorescence generated in the reaction mixture due to nonenzymatic inhibition (*e.g.,* for directly reacting with Amplex Red reagent) was determined by adding these drugs to solutions containing only the Amplex Red reagent in a sodium phosphate buffer. The specific fluorescence emission (used to obtain the final results) was calculated after subtraction of the background activity, which was determined from wells containing all components except the MAO isoforms (replaced by a sodium phosphate buffer solution).

To determine the kinetic parameters of *h*MAO-A and *h*MAO-B (*Km* and *Vmax*), the corresponding enzymatic activity of both isoforms was evaluated (under the experimental conditions described above) in the presence of different *p*-tyramine concentrations. Under our experimental conditions, *h*MAO-A displayed a Michaelis constant (*Km*) of 449.08 ± 28.42 μM and a maximum reaction velocity (*Vmax*) of 30.03 ± 0.6529 nmol/min whereas *h*MAO-B showed a *Km* of 58.76 ± 11.67 μM and a *Vmax* of 22.60 ± 1.018 nmol/min.

References

1 CK Ghosh and S Khan, *Synthesis*, 1981, 719-721

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