Supporting Information

Synthesis, Molecular Modelling and Selective Inhibitory Activity against Human Monoamine Oxidases of 3-Carboxamido-7-substituted Coumarins

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Contents

Chemistry

N-(prop-2-ynyl)-2-oxo-2*H*-chromene-3-carboxamide (4). *Anal.* ($C_{13}H_9NO_3$) Calcd.: C, 68.72; H, 3.99; N, 6.16. Found: C, 68.74; H, 3.99; N, 6.17.

N-(2-benzylphenyl)-2-oxo-2H-chromene-3-carboxamide (7). *Anal.* ($C_{22}H_{15}NO_3$) Calcd.: C, 77.41; H, 4.43; N, 4.10. Found: C, 77.39, H, 4.43; N, 4.11.

N-(3-fluorophenyl)-2-oxo-2H-chromene-3-carboxamide (10). *Anal.* ($C_{16}H_{10}FNO_3$) Calcd.: C, 67.84; H, 3.56; N, 4.94. Found: C, 67.86; H, 3.55; N. 4.95.

N-(3-trifluoromethylphenyl)-2-oxo-2H-chromene-3-carboxamide (11). *Anal.* ($C_{17}H_{10}F_3NO_3$) Calcd.: C, 61.27; H, 3.02; N, 4.20. Found: C, 61.25; H, 3.03; N, 4.21.

N-(4-ethylphenyl)-2-oxo-2H-chromene-3-carboxamide (13). *Anal.* ($C_{18}H_{15}NO_3$) Calcd.: C, 73.71; H, 5.15; N, 4.78. Found: C, 73.68; H, 5.16; N, 4.79.

N-(4-isopropylphenyl)-2-oxo-2H-chromene-3-carboxamide)-amide (14). *Anal.* ($C_{19}H_{17}NO_3$) Calcd.: C, 74.25; H, 5.58; N, 4.56. Found: C, 74.23; H, 5.59; N, 4.57.

N-(4-fluorophenyl)-2-oxo-2H-chromene-3-carboxamide (18). *Anal.* ($C_{16}H_{10}FNO_3$) Calcd.: C, 67.84; H, 3.56; N, 4.94. Found: C, 67.81; H, 3.55; N, 4.96.

N-(4-methanesulfonylphenyl)-2-oxo-2H-chromene-3-carboxamide (21). *Anal.* ($C_{17}H_{13}NO_5S$) Calcd.: C, 59.47; H, 3.82; N, 4.08. Found: C, 59.45; H, 3.83; N, 4.09.

N-(2-chloro-6-methylphenyl)-2-oxo-2H-chromene-3-carboxamide (23). *Anal.* ($C_{17}H_{12}ClNO_3$) Calcd.: C, 65.08; H, 3.86; N, 4.46. Found: C, 65.10; H, 3.86; N, 4.47.

N-(2,4-dimethylphenyl)-2-oxo-2H-chromene-3-carboxamide (25). *Anal.* ($C_{18}H_{15}NO_3$) Calcd.: C, 73.71; H, 5.15; N, 4.78. Found: C, 73.73; H, 5.14; N, 4.79.

N-(2,3-dimethylphenyl)-2-oxo-2H-chromene-3-carboxamide (26). *Anal.* ($C_{18}H_{15}NO_3$) Calcd.: C, 73.71; H, 5.15; N, 4.78. Found: C, 73.69; H, 5.16; N, 4.79.

N-(2,6-dimethylphenyl)-2-oxo-2H-chromene-3-carboxamide (27). *Anal.* ($C_{18}H_{15}NO_3$) Calcd.: C, 73.71; H, 5.15; N, 4.78. Found: C, 73.72; H, 5.14; N, 4.77.

N-(2,6-difluorophenyl)-2-oxo-2H-chromene-3-carboxamide (28). *Anal.* ($C_{16}H_9F_2NO_3$) Calcd.: C, 63.79; H, 3.01; N, 4.65. Found: C, 63.81; H, 3.01; N, 4.64.

N-(3,4-dimethoxyphenyl)-2-oxo-2H-chromene-3-carboxamide (30). *Anal.* ($C_{18}H_{15}NO_5$) Calcd.: C, 66.46; H, 4.65; N, 4.31. Found: C, 66.45; H, 4.66; N, 4.31.

N-(3,5-dimethylphenyl)-2-oxo-2H-chromene-3-carboxamide (31). *Anal.* ($C_{18}H_{15}NO_3$) Calcd.: C, 73.71; H, 5.15; N, 4.78. Found: C, 73.69; H, 5.14; N, 4.78.

N-(3,5-dimethoxyphenyl)-2-oxo-2H-chromene-3-carboxamide (32). *Anal.* ($C_{18}H_{15}NO_5$) Calcd.: C, 66.46; H, 4.65; N, 4.31. Found: C, 66.48; H, 4.66; N, 4.32.

N-methyl-N-phenyl-2-oxo-2H-chromene-3-carboxamide (38). *Anal.* ($C_{17}H_{13}NO_3$) Calcd.: C, 73.11; H, 4.69; N, 5.02. Found: C, 73.13; H, 4.70; N, 5.03.

N-methyl-N-benzyl-2-oxo-2H-chromene-3-carboxamide (39). *Anal.* ($C_{18}H_{15}NO_3$) Calcd.: C, 73.71; H, 5.15; N, 4.78. Found: C, 73.69; H, 5.16; N, 4.79.

N-cyclohexyl-7-benzyloxy-2-oxo-2H-chromene-3-carboxamide (41). *Anal.* ($C_{23}H_{23}NO_4$) Calcd.: C, 73.19; H, 6.14; N, 3.71. Found: C, 73.22; H, 6.13; N, 3.71.

N-isopropyl-7-benzyloxy-2-oxo-2H-chromene-3-carboxamide (42). *Anal.* ($C_{20}H_{19}NO_4$) Calcd.: C, 71.20; H, 5.68; N, 4.15. Found: C, 71.18; H, 5.69; N, 4.16.

N-isobutyl-7-benzyloxy-2-oxo-2H-chromene-3-carboxamide (43). *Anal.* ($C_{21}H_{21}NO_4$) Calcd.: C, 71.78; H, 6.02; N, 3.99. Found: C, 71.76; H, 6.03; N, 4.00.

N-(4-isopropylphenyl)-7-benzyloxy-2-oxo-2H-chromene-3-carboxamide (44). *Anal.* ($C_{26}H_{23}NO_4$) Calcd.: C, 75.53; H, 5.61; N, 3.39. Found: C, 75.55; H, 5.60; N, 3.40.

N-(4-cyano-2,3,5,6-tetrafluorophenyl)-7-benzyloxy-2-oxo-2H-chromene-3-carboxamide (45). *Anal.* ($C_{24}H_{12}F_4N_2O_4$) Calcd.: C, 61.55; H, 2.58; N, 5.98. Found: C, 61.53; H, 2.59; N, 5.99.

N-(2-benzylphenyl)-7-benzyloxy-2-oxo-2H-chromene-3-carboxamide (46). *Anal.* ($C_{30}H_{23}NO_4$) Calcd.: C, 78.07; H, 5.02; N, 3.03. Found: C, 78.09; H, 5.01; N, 3.04.

N-(2-methyl-6-chlorophenyl)-7-benzyloxy-2-oxo-2H-chromene-3-carboxamide (47). *Anal.* ($C_{24}H_{18}ClNO_4$) Calcd.: C, 68.66; H, 4.32; N, 3.34. Found: C, 68.68; H, 4.32; N, 3.35.

N-(3,4-dimethoxyphenyl)-7-benzyloxy-2-oxo-2H-chromene-3-carboxamide (48). *Anal.* ($C_{25}H_{21}NO_6$) Calcd.: C, 69.60; H, 4.91; N, 3.25. Found: C, 69.58; H, 4.92; N, 3.26.

N-(3,5-dimethoxyphenyl)-7-benzyloxy-2-oxo-2H-chromene-3-carboxamide (49). *Anal.* ($C_{25}H_{21}NO_6$) Calcd.: C, 69.60; H, 4.91; N, 3.25. Found: C, 69.59; H, 4.90; N, 3.26.

N-benzyl-7-benzyloxy-2-oxo-2H-chromene-3-carboxamide (50). *Anal.* ($C_{24}H_{19}NO_4$) Calcd.: C, 74.79; H, 4.97; N, 3.63. Found: C, 74.81; H, 4.96; N, 3.64.

N'-phenyl-7-benzyloxy-2-oxo-2*H*-chromene-3-carbohydrazide (51). *Anal.* ($C_{23}H_{18}N_2O_4$) Calcd.: C, 71.49; H, 4.70; N, 7.25. Found: C, 71.51; H, 4.71; N, 7.26.

N-(4-cyano-2,3,5,6-tetrafluorophenyl)-7-(4-fluorobenzyloxy)-2-oxo-2*H*-chromene-3-carboxamide (52). *Anal.* ($C_{24}H_{11}F_5N_2O_4$) Calcd.: C, 59.27; H, 2.28; N, 5.76. Found: C, 59.25; H, 2.29; N, 5.77.

N-(2-benzylphenyl)-7-(4-fluorobenzyloxy)-2-oxo-2*H*-chromene-3-carboxamide (53). *Anal.* ($C_{30}H_{22}FNO_4$) Calcd.: C, 75.15; H, 4.62; N, 2.92. Found: C, 75.13; H, 4.63; N, 2.93

N-(2-methyl-6-chlorophenyl)-7-(4-fluorobenzyloxy)-2-oxo-2*H*-chromene-3-carboxamide (54). *Anal.* ($C_{24}H_{17}ClFNO_4$) Calcd.: C, 65.84; H, 3.91; N, 3.20. Found: C, 65.86; H, 3.92; N, 3.21.

N-(3,4-dimethoxyphenyl)-7-(4-fluorobenzyloxy)-2-oxo-2*H*-chromene-3-carboxamide (55). *Anal.* ($C_{25}H_{20}FNO_6$) Calcd.: C, 66.81; H, 4.49; N, 3.12. Found: C, 66.83; H, 4.50; N, 3.11.

N-(3,5-dimethoxyphenyl)-7-(4-fluorobenzyloxy)-2-oxo-2*H*-chromene-3-carboxamide (56). *Anal.* ($C_{25}H_{20}FNO_6$) Calcd.: C, 66.81; H, 4.49; N, 3.12. Found: C, 66.82; H, 4.50; N, 3.11.

N-(4-isopropylphenyl)-7-(4-fluorobenzyloxy)-2-oxo-2*H*-chromene-3-carboxamide (57). *Anal.* ($C_{26}H_{22}FNO_4$) Calcd.: C, 72.38; H, 5.14; N, 3.25. Found: C, 72.40; H, 5.15; N, 3.26.

N-methyl-N-phenyl-7-benzyloxy-2-oxo-2*H*-chromene-3-carboxamide (58). *Anal.* ($C_{24}H_{19}NO_4$) Calcd.: C, 74.79; H, 4.97; N, 3.63. Found: C, 74.81; H, 4.98; N, 3.64.

N-(3,4-dimethoxyphenyl)-7-benzyloxy-8-methyl-2-oxo-2*H*-chromene-3-carboxamide (59). *Anal.* ($C_{26}H_{23}NO_6$) Calcd.: C, 70.10; H, 5.20; N, 3.14. Found: C, 70.11; H, 5.21; N, 3.15.

Pharmacological studies

Drugs and chemicals

The drugs, vehicle and chemicals used in the experiments were the new compounds, moclobemide (a generous gift from F. Hoffmann-La Roche Ltd., Basel, Switzerland), dimethyl sulfoxide (DMSO), *R*-(-)-deprenyl hydrochloride, iproniazid phosphate (purchased from Sigma-Aldrich, Spain), resorufin sodium salt, clorgyline hydrochloride, *p*-tyramine hydrochloride, sodium phosphate and horseradish peroxidase (supplied in the Amplex® Red MAO assay kit from Molecular Probes).

Appropriate dilutions of the above drugs were prepared every day immediately before use in deionized water from the following concentrated stock solutions kept at –20 °C: the new compounds (0.1 M) in DMSO; *R*-(-)-deprenyl, moclobemide, iproniazid, resorufin, clorgyline, p-tyramine and horseradish peroxidase (0.1 M) in deionized water. Due to the photosensitivity of some chemicals (e.g., Amplex® Red reagent), all experiments were performed in the dark. In all assays, neither deionized water (Milli-Q®, Millipore Ibérica S.A., Madrid, Spain) nor appropriate dilutions of the vehicle used (DMSO) had significant pharmacological effects.

Data presentation and statistical analysis

Unless otherwise specified, results shown in the text and tables are expressed as mean ± standard error of the mean (S.E.M.) from *n* experiments. Significant differences between two means ($P < 0.05$ or $P < 0.01$) were determined by one-way analysis of variance (ANOVA) followed by the Dunnett's *post-hoc* test.

To study the possible effects of the test drugs (new compounds or reference inhibitors) on MAO isoform enzymatic activity, we evaluated the variation of fluorescence per unit of time (fluorescence arbitrary U/min) and indirectly the rate of hydrogen peroxide (H_2O_2) production, and therefore the pmol/min of resorufin produced in the reaction between H_2O_2 and Amplex® Red reagent. For this purpose, several concentrations of resorufin were used to prepare a standard curve with X = pmol resorufin and Y = fluorescence arbitrary U. Note that the value of resorufin production is similar to the pmol of *p*-tyramine oxidized to *p*-hydroxyphenylacetaldehyde/min, since the stoichiometry of the reaction (*p*-tyramine oxidized by MAO isoforms/resorufin produced) is 1:1.

In these experiments, the inhibitory activity of the tested drugs (new compounds and reference inhibitors) is expressed as IC_{50}, i.e. the concentration of these compounds required for a 50% reduction of the control MAO isoform enzymatic activity, estimated by least-squares linear regression, using the program Origin™ 5.0 (Microcal Software, Inc., Northampton, MA, USA), with X = log of tested compound molar concentration and Y = the corresponding percentage of inhibition of control resorufin production obtained with each concentration. This regression was performed using data obtained with 4-6 different concentrations of each tested compound which inhibited the control MAO isoform enzymatic activity by between 20 and 80%. In addition we calculated the corresponding MAO-B selectivity index [IC_{50} (MAO-A)]/[IC_{50} (MAO-B)].