Synthesis and evaluation of a new series of 6-methylcoumarins as potent and selective Monoamine Oxidase B inhibitors

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Experimental section

General

All common reagents and solvents were obtained from commercial suppliers and used without further purification. Reaction progress was monitored using analytical thin layer chromatography (TLC) on precoated silica gel GF254 plates (Qingdao Haiyang Chemical Plant, Qingdao, China) plates and the spots were detected under UV light (254 nm). Column chromatography was performed on silica gel (90-150 μm; Qingdao Marine Chemical Inc.) Melting point was measured on an XT-4 micromelting point instrument and uncorrected. $^1$H NMR and $^{13}$C NMR spectra were measured on a Bruker ACF-500 spectrometer at 25 °C and referenced to TMS. Chemical shifts are reported in ppm (δ) using the residual solvent line as internal standard. Mass spectra were obtained on a MS Agilent 1100 Series LC/MSD Trap mass spectrometer (ESI-MS) and a Mariner ESI-TOF spectrometer (HRESI-MS), respectively.

General procedures for the preparation of 4a-c

Compounds 3a-c (1.25 mmol) was added to a 10% solution of HCl (15 mL) and ethanol (5 mL). The mixture was heated to 80 °C and stirred for 5-12 h. After cooling, it was poured into ice water, and the precipitated solid was filtered, washed with water, dried, and recrystallized from ethyl acetate to give the compounds 4a-c.

3-(2-Hydroxyphenyl)-6-methylcoumarin (4a)

Yield 90%, $^1$H NMR (500 MHz, DMSO-$d_6$) δ 9.58 (s, 1H), 7.98 (s, 1H), 7.55 (br s, 1H), 7.45 (dd, $J = 8.5$, 2.0 Hz, 1H), 7.35 (d, $J = 8.5$ Hz, 1H), 7.30-7.22 (m, 2H),
6.93 (d, $J = 8.5$ Hz, 1H), 6.90-6.86 (m, 1H), 2.40 (s, 3H); MS (ESI) $m/z$ 253.1 [M+H]$^+$.  

3-(3-Hydroxyphenyl)-6-methylcoumarin (4b)  
Yield 95%, $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.56 (s, 1H), 8.15 (s, 1H), 7.59 (br s, 1H), 7.45 (dd, $J = 8.5, 1.5$ Hz, 1H), 7.35 (d, $J = 8.5$ Hz, 1H), 7.27 (t, $J = 7.9$ Hz, 1H), 7.19-7.16 (m, 1H), 7.14 (d, $J = 7.5$ Hz, 1H), 6.84 (dd, $J = 8.5, 1.5$ Hz, 1H), 2.40 (s, 3H); MS (ESI) $m/z$ 253.1 [M+H]$^+$.  

3-(4-Hydroxyphenyl)-6-methylcoumarin (4c)  
Yield 92%, $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.73 (s, 1H), 8.09 (s, 1H), 7.64-7.59 (m, 2H), 7.56 (d, $J = 1.5$ Hz, 1H), 7.42 (dd, $J = 8.5, 2.0$ Hz, 1H), 7.33 (d, $J = 8.5$ Hz, 1H), 6.90-6.83 (m, 2H), 2.40 (s, 3H); MS (ESI) $m/z$ 253.1 [M+H]$^+$.  

Biological assay  
Determination of hMAO isoform activity.  
Human MAO-A and MAO-B were purchased from Sigma-Aldrich. The capacity of the test compounds to inhibit MAO-A and MAO-B activities was assessed by reported assay. Briefly, 0.1 mL of sodium phosphate buffer (0.05 M, pH 7.4) containing the test drugs at various concentrations and adequate amounts of recombinant hMAO-A or hMAO-B required and adjusted to obtain in our experimental conditions the same reaction velocity, i.e., to oxidize (in the control group) the same concentration of substrate: 165 pmol of $p$-tyramine/min (hMAO-A: 1.1 μg protein; specific activity: 150 nmol of $p$-tyramine oxidized to $p$-hydroxyphenylacetaldehyde/min/mg protein; hMAO-B: 7.5 μg protein; specific activity: 22 nmol of $p$-tyramine transformed/min/mg protein) were incubated for 15
min at 37 °C in a flat-black-bottom 96-well microtest plate placed in a dark fluorimeter chamber. After this incubation period, the reaction was started by adding 200 μM (final concentrations) Amplex Red reagent, 1 U/mL horseradish peroxidase, and 1 mM p-tyramine. The production of H₂O₂ and consequently, of resorufin, was quantified at 37 °C in a SpectraMax Paradigm (Molecular Devices, Sunnyvale, CA) muti-mode detection platform reader based on the fluorescence generated (excitation, 545 nm; emission, 590 nm). The specific fluorescence emission was calculated after subtraction of the background activity. The background activity was determined from wells containing all components except the hMAO isoforms, which were replaced by a sodium phosphate buffer solution (0.05 M, pH 7.4). The percent inhibition was calculated by the following expression: \((1 – \text{IFi}/\text{IFc}) \times 100\) in which IFi and IFc are the fluorescence intensities obtained for hMAO in the presence and absence of inhibitors after subtracting the respective background.

**Molecular docking study**

All calculations and analyses were carried out with Molecular Operating Environment (MOE) program (Chemical Computing Group, Montreal, Canada). The X-ray crystal structure of the MAO-B complex with a coumarin inhibitor (PDB ID: 2V61) was applied to build the starting model, which was obtained from the Protein Data Bank (www.rcsb.org). Ligands and water molecules in the PDB files were removed and all hydrogen atoms were subsequently added to the proteins. Compound 5n was drawn in MOE. The compound was then protonated using the protonate 3D protocol and energy was minimized using the MMFF94x force field in MOE. After
the enzymes and compound 5n were ready for the docking study, 5n was docked into
the active site of the protein by the “Triangle Matcher” method. The Dock scoring in
MOE software was done using ASE scoring function and Forcefield was selected as
the refinement method. The best 10 poses of molecules were retained and scored.
After docking, the geometry of resulting complex was studied using the MOE’s pose
viewer utility.

**Cell toxicity**

The toxicity effect of test compound on the human normal cell-HUVEC, neural cell-
PC12 and neural cell-(SH-SY5Y) was examined according to the previous methods.
The cells were cultured in Eagle’s minimum essential medium (EMEM)/ham’s F-12
(1:1) medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin
and 100 μg/mL streptomycin, at 37 °C in a humidified atmosphere containing 5%
CO₂. Cells were subcultured in 96-well plates at a seeding density of 10,000 cells per
well and allowed to adhere and grow. When cells reached the required confluence,
they were placed into serum-free medium and treated with compound 5n. Forty-eight
hours later the survival of cells was determined by MTT assay. Briefly, after
incubation with 20 μL of MTT at 37 °C for 4 h, living cells containing MTT formazan
crystals were solubilized in 200 μL DMSO. The absorbance of each well was
measured using a microculture plate reader with a test wavelength of 570 nm and a
reference wavelength of 630 nm. Results are expressed as the mean ± SD of three
independent experiments.