# Supplementary Date

**Chromanones: selective and reversible Monoamine Oxidase B inhibitors with nanomolar potency**

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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.) The purity of all compounds used for biological evaluation was confirmed to be higher than 95% through analytical HPLC performed with Agilent 1200 HPLC System. $^{1}$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 ($\delta$) 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.

**Synthesis of 7-Hydroxy-chroman-4-one (2).**

Resorcinol (9.00 g, 82 mmol) was acylated by the equimolar amount of 3-chloropro-pionic acid in the presence of trifluoromethanesulphonic acid (3 equiv.), stirred at 75-80 °C. After 1 h the solution was cooled to room temperature and poured into $CH_2Cl_2$ (200 mL). This solution was slowly poured into water (200 mL), and the phases were separated. The aqueous phase was partitioned with 3 × 200 mL of $CH_2Cl_2$. The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under vacuum to give semisolid intermediate 2', 4'-

Dihydroxy-3-chloro-propiophenone (7.24 g). The intermediate (7.24 g) was added to 400 mL NaOH (2 mol/L) at 5 °C and stirred for 20 min. The solution was allowed to reach room temperature for 2 h and again cooled to 5 °C. The pH of the solution was adjusted to 2 with 6 mol/L $H_2SO_4$. The acidic mixture was extracted with 3 × 200 mL EtOAc, washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under vacuum to give crude solid, which was purified by chromatography (hexanes -EtOAc = 75:25) on *silica gel* to afford **2** (3.50 g).

**Synthesis of 7-hydroxy-2, 2-dimethylchroman-4-one (3).**

To a stirred suspension of resorcinol (13.28 g, 121 mmol), 3, 3-dimethylacrylic acid (12.1 g, 121 mmol) and phosphorous oxychloride (96.5 mL, 1050 mmol) was added unfused $ZnCl_2$ (22.9 g, 168 mmol). The mixture was heated at 50 °C for 2 h. The resulting deep-red, homogeneous solution was then poured onto 600 g of crushed ice and allowed to sit for 12 h. The precipitate was collected by vacuum filtration, washed with $H_2O$ (3 × 100 mL) and dried in air, which was purified by chromatography (hexanes-EtOAc = 70:30) on *silica gel* to afford **3** (12.8 g).

**Biological assay.**

***In vitro* inhibition of Monoamine oxidase**

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 Amplex Red MAO 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 *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) the same concentration of substrate: 165 pmol of *p*-tyramine/min (*h*MAO-A: 1.1 μg protein; specific activity: 150nmol of *p*-tyramine oxidized to *p*-hydroxyphenylacetaldehyde/min/mg protein; *h*MAO-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 $H_2O_2$ 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 *h*MAO 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: $(1 - IFi/IFc) \times 100$ in which IFi and IFc are the fluorescence intensities obtained for *h*MAO in the presence and absence of inhibitors after subtracting the respective background.

**Reversibility and irreversibility study**

To determine whether the inhibition of MAO-B by the chromanone derivatives is reversible or irreversible, the time-dependence of inhibition of a selected inhibitor, **4f**, was examined. Compound **4f** was allowed to preincubate with recombinant human

MAO-B for various periods of time (0, 15, 30, 60 min) at 37 °C in potassium phosphate buffer (0.05 M, pH 7.4). The concentration of **4f** was equal to twofold the measured $IC_{50}$ value for the inhibition of MAO-B. The reactions were subsequently diluted twofold to yield a final enzyme concentration of 0.015 mg/mL and concentrations of the **4f** that are equal to the $IC_{50}$ values. The reactions were incubated at 37 °C for a further 15 min. All measurements were carried out in triplicate and are expressed as mean ± SD.

**Kinetic study of MAO-B inhibition**

To obtain of the mechanism of action **4f**, reciprocal plots of 1/velocity versus 1/substrate were constructed at different concentrations of the substrate *p*-tyramine (50–500 μM) by using Ellman's method. Four different concentrations of **4f** (0, 4.3, 8.6 and 17.2 nM) were selected for the kinetic analysis of MAO-B inhibition. The plots were assessed by a weighted least-squares analysis that assumed the variance of velocity (v) to be a constant percentage of v for the entire data set. Slopes of these reciprocal plots were then plotted against the concentration of **4f** in a weighted analysis. Data analysis was performed with Graph Pad Prism 4.03 software (Graph Pad Software Inc.).

**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 MAO-B (PDB code 2V61) were applied to build the starting model, which were obtained from the Protein Data Bank (www.rcsb.org).

Heteroatoms and water molecules in the PDB files were removed and all hydrogen atoms were subsequently added to the proteins. Compound **4f** 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 **4f** were ready for the docking study, **4f** 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.

**Cytotoxicity assay of compounds 4f and 5d**

The toxicity effect of compounds **4f** and **5d** on the SH-SY5Y cells was examined. The SH-SY5Y cells were routinely grown at 37 °C in a humidified incubator with 5% $CO_2$ in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% bovine calf serum, 100 units/mL penicillin, and 100 units/mL of streptomycin. 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 compounds **4f and 5d**. 48 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.