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

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Abstract

A new series of C7-substituted chromanones has been designed, synthesized and evaluated for *h*MAO-B inhibitory activity *in vitro*. Most of the studied compounds were remarkably potent and selective MAO-B inhibitors, and showed weak or no inhibition of MAO-A. Especially, compound **4f** (IC_{50} = 8.62 nM) was the best MAO-B inhibitor and exhibited the highest selectivity for MAO-B (SI > 11627.9-fold). In addition, the structure-activity relationships for MAO-B inhibition indicated that substitutions at the C7 of the chromanone moiety, particularly with the halogen substituted benzyloxy, were more favorable for MAO-B inhibition. Molecular docking studies have been done to explore the interaction modes of C7-substituted chromanones with MAO-B. Furthermore, the representative compounds **4f** and **5d** showed low neurotoxicity in SH-SY5Y cells *in vitro*. So the C7-substituted chromanones could be used to develop promising drug candidates for the therapy of neurodegenerative diseases.

Keywords

Monoamine Oxidase; chromanones; neurodegenerative diseases; Molecular docking.

Abbreviations

MAO, Monoamine oxidase; CNS, central nervous system; 5-HT, 5-hydroxy-tryptamine; NE, norepinephrine; DA, dopamine; FAD, flavin adenine dinucleotide; SI, selectivity index; BBB, blood-brain barrier; MTT, methyl thiazolyl tetrazolium.

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Introduction

Monoamine oxidases (MAOs) are flavoenzymes that can regulate and metabolize amine neurotransmitters, such as serotonin, dopamine (DA) and epinephrine in the peripheral tissues and central nervous system (CNS).[1] In animals MAOs exist as two isoforms, namely MAO-A and MAO-B, which were recognized on account of differences in immunological properties, inhibitor specificity, amino acid sequences, substrate preference and tissue distribution.[2-6] Catecholaminergic neurons predominantly contain MAO-A, while MAO-B is present in astrocytes and serotonergic neurons.[7] MAO-A is selectively inhibited by clorgyline and preferentially metabolizes epinephrine, 5-hydroxytryptamine (5-HT) and norepinephrine (NE). MAO-B specifically deaminates *β*-phenethylamine and benzylamine and is selectively inhibited by selegiline and rasagiline. Both enzymes can metabolize tryptamine, DA and tyramine.[8-9]

Recent X-ray crystal structures of the two MAO isoforms supply information about the pharmacophoric requirements and the selective interactions, which are useful to design potent and selective inhibitors.[10-12] The active sites of *h*MAOs are thought to be the main structural differences due to the different volume and shape of the inhibitors/substrate binding pockets. *h*MAO-A has a single hydrophobic cavity, which is nearly 550 $Å^3$ volume. In contrast, the active site of *h*MAO-B consists of two distinct cavities: one is the so-called “entrance cavity” of 290 $Å^3$ volume, which is located towards the outside of the protein and another larger cavity called “substrate cavity” is connected to the flavine adenine dinucleotide cofactor (FAD), which is

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about 420 $Å^3$ volume. There are narrow pockets in these cavities of both isoenzymes, and the Tyr326 and Ile199 residues in *h*MAO-B act as a bottleneck and thus form a gate, which separates the region as the two cavities.[13]

MAOs are important targets for discovering and developing drugs. Selective inhibitors of MAO-A have been applied to treat depression and anxiety;[14] while selective MAO-B inhibitors have been applied alone or in combination to treat Parkinson's and Alzheimer's diseases.[15] Based on these aspects, novel MAO inhibitors are needed.

To search for novel MAO inhibitors, the chromone scaffold [(4*H*)-1-benzopyran-4-one] has been developed as a principally promising scaffold.[16-21] The chromones from both natural and synthetic origin are important bioactive molecules. Recently, chromones and differently substituted chromones have been synthesized and evaluated for their potential as MAO inhibitors, and display selective inhibition of MAO-B from the micromolar to the nanomolar range (A-F, Fig. 1). Especially chromone derivatives with the benzyloxy substituents at C7 are particularly potent and selective MAO-B inhibitors.[19] Because the structures of chromanone and chromone are similar, we explore the probability that C7-substituted chromanones may have the potent MAO inhibitory properties. For this purpose, alkyloxy substituents were selected for substitution at C7 of the chromanone ring, such as benzyloxy, phenylethoxy and phenylpropoxy. To further examine the structure–activity relationships (SARs) against MAO, different substitutions (F, Cl, Br, CN and CH_3) were introduced to the benzyloxy ring and C2 of the chromanone nucleus, and

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the effects on MAO inhibition were studied.

Results and discussion

Chemistry

The C7-substituted chromanone derivatives (**4** and **5**) and C6-substituted benzofuran-3(2H)-one derivatives **7** were efficiently synthesized along with the pathway shown in Scheme 1. The commercially available resorcinol was reacted with 3-chloropropionic acid in the presence of trifluoromethanesulphonic acid to give the chloride. Intramolecular cyclization of the chloride afforded the intermediate **2**.[22] On the other hand, the intermediate **3** was obtained by reacting resorcinol with 3, 3-dimethylacrylic acid in a $ZnCl_2/POCl_3$ mixture.[23] Finally the intermediates **2**, **3** and **6** reacted with the appropriate benzyl bromides in the presence of K_2CO_3 to give the target molecules in good yields (75-93%).

Prediction of BBB penetration of compounds 4, 5 and 7

With the aim of developing the central nervous system (CNS) drugs, the ability of compounds to cross the blood-brain barrier (BBB) is very important. So in the drug discovery process, BBB permeability properties should be determined as early as possible.[24] To cross BBB, molecules should meet the limiting terms of Lipinski's rules[25]: molecular weight (MW) less than 500, the number of hydrogen bond acceptor atoms (HBA) less than 10, the number of hydrogen bond donor atoms (HBD) less than 5, the calculated logarithm of the octanol-water partition coefficient (Clog P) less than 5, and the small polar surface area less than 90 $Å^2$. The log BB is calculated as the following equation: $\log BB = -0.0148 \times PSA + 0.152\ ClogP + 0.139$.[25] Calculated

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log BB for potential applications in brains and defined by the restrictive terms of Lipinski's rules, as shown in Table 1, compounds **4**, **5** and **7** satisfied possible brain penetration and drug-like standards.

Inhibition of hMAO activity

For target compounds **4**, **5** and **7**, the MAO inhibitory activities were explored by measuring the effects on the production of hydrogen peroxide from *p*-tyramine, according to the reported assay,[26] with iproniazid and pargyline as references. The corresponding IC_{50} values and MAO-B selectivity ratios were shown in Table 2. Based on the screening data, it could be seen that most of the tested compounds were selective inhibitors toward MAO-B and the IC_{50} values in the nanomolar range. MAO-A inhibition was very weak and no apparent structure-activity relationship existed. Among the synthesized compounds, **4f** was the most potent and selective inhibitor against MAO-B (IC_{50} = 8.62 nM, SI > 11627.9), being more active than iproniazid and pargyline.

Initially, to introduce groups with different sizes at C7 of chromanones, compounds **4a-c** were synthesized. As shown in Table 2, the 7-benzyloxychromanone derivative **4a**, exhibited an IC_{50} value of 57.37 nM for MAO-B. Extending the length of the C7 side chain of **4a** with methylene and ethylene units to yield the phenylethoxy (**4b**, IC_{50}= 251.14 nM for MAO-B) and phenylpropoxy (**4c**, IC_{50}= 21.45 μM for MAO-B) substituted homologues, resulted in an equal or a drastic loss of MAO-B inhibition potency. From these results, it might be concluded that the length of the linker of **4a** was more suitable for volume of the substrate/inhibitors binding pockets than that of

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phenylethoxy and phenylpropoxy. Then we planned to introduce substituents with varying electronic properties to benzyloxy substitution for studying possible effects on MAO-B inhibition potency. Compared to **4a**, **4d-k** bearing electron-withdrawing groups exhibited an increase in MAO-B inhibition. However, **4l-m** with an electron-donating group showed a slight decrease in MAO-B inhibition. For example, **4f** (IC_{50} = 8.62 nM for MAO-B) possessing electron-withdrawing substituent F was about 7-fold more active than **4a,** while **4m** (IC_{50} = 91.29 nM for MAO-B) substituted with CH_3, decrease the MAO-B inhibition potency of **4a** by 2-fold. Furthermore, among **4d-k,** compounds with *para* substitution of the benzyloxy phenyl ring were more portent for MAO-B inhibition than *ortho* and *meta* substitution.

Moreover, to introduce substituents with different sizes to the 2-position of chromanone ring for studying possible effects on MAO inhibition, compounds **5a-k** were synthesized. Compared to compounds **4a-m**, an apparent decrease of MAO-B inhibition was observed for **5a-k**. For example, **4a** (IC_{50} = 57.37 nM) possessing small substituent H was almost two-fold than **5a** (IC_{50} = 97.47 nM) with the CH_3 substituent. Like **4a-m**, a similar trend against MAO inhibition was noted for **5a-k**.

When the chromanone ring was substituted by the benzofuranone ring, compounds **7a-c** were synthesized. Compound **7b** (IC_{50} = 2.13 μM) was the best MAO-B inhibitory activity among them. And the size of substitution at C6 of benzofuranone also affected the MAO-B inhibitory activity, but was not pivotal for MAO-A inhibition. Compared to compounds **4** and **5**, compounds **7a-c** remarkably decreased in MAO-B inhibitory activities. These results implied that the chromanone ring is

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important for MAO-B inhibitory activity.

Reversibility of MAO-B inhibition

As we know, MAO-B inhibitors could be classified as irreversible or reversible because of the different interactions between the inhibitors and the enzyme. When affecting the enzyme to give covalent complexes, the inhibitors are irreversible. However, irreversible MAO-B inhibitors may display pharmacological side effects and safety issues. For example the side effects of selegiline, which is induced by its amphetamine metabolites, are the major drawbacks.[27] Compared to irreversible MAO-B inhibitors, the enzyme activity of reversible MAO-B inhibitors can be regained when the inhibitors are metabolized. Moreover, when the substrate concentration increases the enzyme activity will relieve. For these reasons, discovering new reversible MAO-B inhibitors may be valuable.

To examine whether C7-substituted chromanone derivatives were reversible or irreversible MAO-B inhibitors, the time dependencies of inhibition were evaluated.[28] Compound **4f** was selected as a representative inhibitor since it displayed the most potent MAO-B inhibitory activity. For a reversible inhibitor, the MAO-B activity would almost be same, when the enzyme is preincubated with a reversible inhibitor over different time periods. In contrast, when the enzyme is preincubated with an irreversible inhibitor over different time periods, the MAO-B activity would show a time-dependent reduction. Compound **4f** was preincubated with MAO-B over different time periods (0-60 min) at a concentration of twofold IC_{50}. As shown in Fig. 2, we could observe that MAO-B activities were 42.4% at 0 min, 44.5% at 15 min,

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45.5% at 30 min and 49.6% at 60 min, and these results demonstrated that **4f** was not a time-dependent inhibitor of MAO-B at a concentration of IC_{50} and over the time period (0-60 min). So these experiments clearly indicated that the chromones were reversible MAO-B inhibitors.

Kinetic study of MAO-B inhibition

Compound **4f** was also used to further investigate the mode of MAO-B inhibition. The type of MAO-B inhibition was determined by Michaelis-Menten kinetic experiments.[29] The catalytic rates were measured at five different *p*-tyramine concentrations (50-500 μM), and each plot was constructed at four different concentrations of **4f** (0, 4.3, 8.6 and 17.2 nM). The overlaid reciprocal Lineweaver-Burk plots (Fig. 3) showed that the plots for different concentrations of **4f** were linear and intersected at the *y*-axis. This pattern indicated that **4f** was a competitive MAO-B inhibitor, and these results further proved that the chromones were reversible MAO-B inhibitors.

Molecular modeling studies

In order to explore the interaction modes of C7-substituted chromanones with MAO-B, we have carried out a structure-based molecular modeling study using *h*MAOs cocrystals deposited into the PDB. Crystallographic structure of MAO-B (PDB code 2V61)[30] was used to dock the derivatives under study. And molecular docking study was performed using software package MOE 2008.10.[31] According to the inhibition results, compound **4f** was selected as a typical ligand. The 3D and 2D pictures of binding were illustrated in Fig. 4. As shown in Fig. 4A and 4B, compound

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4f located in the well-known binding pocket of MAO-B,[32] with the chromanone ring interacting with Tyr 398 and Tyr 60 at bottom of the substrate cavity. A hydrogen bond formed between the carbonyl oxygen of the ligands and Tyr435-OH. Finally, the F-substituted benzyl group occupied the entrance cavity, which was a hydrophobic subpocket existing only in the MAO-B isoform and constituted by Leu 171, Ile 199, Tyr 326, Ile 316, Phe 99, Pro 104 and Phe 168.

Cells toxicity

Based on the screening results above, compounds **4f** and **5d** as the most potent and highly selective inhibitors against MAO-B were selected to further examine the potential toxicity effect on the SH-SY5Y cells.[33] After incubating the cells with compound **4f** and **5d** for 48 h, the cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay. As shown in the Fig. 5, the result revealed that compounds **4f** and **5d** at 3-50 μM did not have neurotoxicity. This suggested that compounds **4f** and **5d** might be used to develop promising drug candidates for the therapy of neurodegenerative diseases.

Conclusions

In conclusion, a new series of C7-substituted chromanones were designed, synthesized and evaluated for *h*MAO inhibitory activity *in vitro*. It was observed that most of the studied compounds were remarkably competitive and reversible MAO-B inhibitors with nanomolar potency, and revealed weak or no inhibition of MAO-A. In particular, compound **4f** was the best MAO-B inhibitor with 892-fold more active than iproniazid. The MAO inhibition data indicated that substitutions at the C7

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position of the chromanone moiety, particularly with the benzyloxy substituent, were more favorable for MAO-B inhibition. Moreover, halogen substituents on the benzyloxy ring further increased MAO-B inhibition. Molecular docking studies of the compound **4f** suggested that the high MAO-B selectivity might be ascribed to the hydrogen bond interaction and the larger set of residues interacting with MAO-B. Due to low neurotoxicity in SH-SY5Y cells *in vitro*, these compounds could be used to develop promising drug candidates for the therapy of neurodegenerative diseases.

Experimental

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. 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),

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respectively.

General procedure for the preparation of C7-substituted 4-Chromanone derivatives (4 and **5).**

7-Hydroxy-chroman-4-one (1.85 mmol) was suspended in acetonitrile (15 mL) containing K_2CO_3 (3.70 mmol). The reaction was treated with an appropriately substituted arylalkyl bromide (2.04 mmol) and heated under reflux for 8 h. The reaction progress was monitored using *silica gel* TLC with hexanes/EtOAc as mobile phase. Upon completion, the acetonitrile was evaporated *in vacuo* and the mixture was then poured into water, which was extracted with 3 × 200 mL of EtOAc, washed with brine, dried over anhydrous Na_2SO_4 and purified by chromatography (hexanes /EtOAc) on *silica gel.*

7-(benzyloxy) chroman-4-one (4a).

Yield 87%, yellow solid; ^{1}H NMR (500 MHz, DMSO) δ 7.69 (d, J = 8.8 Hz, 1H, Ar-H), 7.44 (d, J = 7.2 Hz, 2H, Ar-H), 7.40 (dd, J = 10.1, 4.7 Hz, 2H, Ar-H), 7.37–7.31 (m, 1H, Ar-H), 6.70 (dd, J = 8.8, 2.4 Hz, 1H, Ar-H), 6.62 (d, J = 2.4 Hz, 1H, Ar-H), 5.18 (s, 2H, Ar-CH_2), 4.50 (t, J = 6.4 Hz, 2H, OCH_2), 2.70 (t, J = 6.4 Hz, 2H, $COCH_2$); ^{13}C NMR (125 MHz, DMSO) δ 190.44, 164.93, 163.84, 136.80, 128.99, 128.99, 128.68, 128.53, 128.23, 128.23, 115.54, 110.69, 102.34, 70.22, 67.60, 37.38. HRMS (ESI) *m/z* 531.1774 $[2M+Na]^+$ (calcd for 531.1778, $C_{32}H_{28}NaO_6$).

7-phenethoxychroman-4-one (**4b**).

Yield 81%, colorless solid; ^{1}H NMR (500 MHz, DMSO) δ 7.66 (d, J = 8.8 Hz, 1H, Ar-H), 7.35–7.26 (m, 4H, Ar-H), 7.25–7.19 (m, 1H, Ar-H), 6.61 (dd, J = 8.8, 2.4 Hz,

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1H, Ar-H), 6.54 (d, J = 2.3 Hz, 1H, Ar-H), 4.49 (t, J = 6.4 Hz, 2H, Bn-CH_2), 4.26 (t, J = 6.8 Hz, 2H, OCH_2), 3.03 (t, J = 6.8 Hz, 2H, Ar-CH_2), 2.69 (t, J = 6.4 Hz, 2H, $COCH_2$); ^{13}C NMR (125 MHz, DMSO) δ 190.42, 165.07, 163.90, 138.54, 129.41, 129.41, 128.80, 128.80, 128.67, 126.82, 115.40, 110.41, 101.93, 69.13, 67.58, 37.38, 35.12. HRMS (ESI) *m/z* 559.2093 $[2M+Na]^+$ (calcd for 559.2091, $C_{34}H_{32}NaO_6$).

7-(3-phenylpropoxy) chroman-4-one (**4c**).

Yield 75%, colorless solid; ^{1}H NMR (500 MHz, DMSO) δ 7.68 (d, J = 8.8 Hz, 1H, Ar-H), 7.28 (t, J = 7.5 Hz, 2H, Ar-H), 7.22 (d, J = 7.1 Hz, 2H, Ar-H), 7.18 (t, J = 7.2 Hz, 1H, Ar-H), 6.63 (dd, J = 8.8, 2.3 Hz, 1H, Ar-H), 6.51 (d, J = 2.3 Hz, 1H, Ar-H), 4.50 (t, J = 6.4 Hz, 2H, Bn-CH_2, alkyl chains-H), 4.02 (dd, J = 6.7, 2.3 Hz, 2H, OCH_2), 2.76–2.64 (m, 4H, $COCH_2$), 2.02 (dd, J = 14.8, 6.8 Hz, 2H, Bn-CH_2); ^{13}C NMR (125 MHz, DMSO) δ 190.42, 165.28, 163.90, 141.70, 128.83, 128.83, 128.81, 128.81, 128.68, 126.35, 115.36, 110.38, 101.83, 67.84, 67.58, 37.39, 31.83, 30.56. HRMS (ESI) *m/z* 587.2399 $[2M+Na]^+$ (calcd for 587.2404, $C_{36}H_{36}NaO_6$).

7-((2-fluorobenzyl) oxy) chroman-4-one (**4d**).

Yield 88%, yellow solid; ^{1}H NMR (500 MHz, DMSO) δ 7.72 (d, J = 8.8 Hz, 1H, Ar-H), 7.58 (m, 1H, Ar-H), 7.49–7.43 (m, 1H, Ar-H), 7.32–7.24 (m, 2H, Ar-H), 6.73 (dd, J = 8.8, 2.4 Hz, 1H, Ar-H), 6.69 (d, J = 2.3 Hz, 1H, Ar-H), 5.23 (s, 2H, Ar-CH_2), 4.54 (t, J = 6.4 Hz, 2H, OCH_2), 2.74 (t, J = 6.4 Hz, 2H, $COCH_2$); ^{13}C NMR (125 MHz, DMSO) δ 190.45, 164.72, 163.86 (d, $^1J_{CF}$ = 245.1 Hz), 131.35, 131.16, 128.73, 124.98, 123.57, 116.03, 115.87, 115.69, 110.51, 102.25, 67.63, 64.42, 37.39. HRMS (ESI) *m/z* 567.1588 $[2M+Na]^+$ (calcd for 567.1590, $C_{32}H_{26}NaO_6F_2$).

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7-((3-fluorobenzyl) oxy) chroman-4-one (**4e**).

Yield 90%, colorless solid; ^{1}H NMR (500 MHz, DMSO) δ 7.70 (d, J = 8.8 Hz, 1H, Ar-H), 7.56 (m, 1H, Ar-H), 7.50–7.40 (m, 1H, Ar-H), 7.26 (dd, J = 15.8, 8.0 Hz, 2H, Ar-H), 6.71 (dd, J = 8.8, 2.4 Hz, 1H, Ar-H), 6.67 (d, J = 2.3 Hz, 1H, Ar-H), 5.21 (s, 2H, Ar-CH_2), 4.52 (t, J = 6.4 Hz, 2H, OCH_2), 2.72 (t, J = 6.4 Hz, 2H, $COCH_2$); ^{13}C NMR (125 MHz, DMSO) δ 190.45, 164.72, 163.86 (d, $^{1}J_{CF}$ = 245.0 Hz), 131.35, 131.20, 128.73, 125.07, 123.63, 116.03, 115.87, 115.69, 110.51, 102.25, 67.63, 64.59, 37.39. HRMS (ESI) *m/z* 567.1589 $[2M+Na]^{+}$ (calcd for 567.1590, $C_{32}H_{26}NaO_6F_2$).

7-((4-fluorobenzyl) oxy) chroman-4-one (4f).

Yield 91%, colorless solid; ^{1}H NMR (500 MHz, DMSO) δ 7.69 (d, J = 8.8 Hz, 1H, Ar-H), 7.50 (dd, J = 8.5, 5.6 Hz, 2H, Ar-H), 7.23 (t, J = 8.8 Hz, 2H, Ar-H), 6.70 (dd, J = 8.8, 2.3 Hz, 1H, Ar-H), 6.63 (d, J = 2.3 Hz, 1H, Ar-H), 5.16 (s, 2H, Ar-CH_2), 4.51 (t, J = 6.4 Hz, 2H, OCH_2), 2.71 (t, J = 6.4 Hz, 2H, $COCH_2$); ^{13}C NMR (125 MHz, DMSO) δ 189.86, 164.25, 163.27 (d, $^{1}J_{CF}$ = 242.6 Hz), 162.79, 132.47, 132.45, 130.01, 129.95, 128.12, 115.33, 115.16, 110.09, 101.78, 68.93, 67.04, 36.82. HRMS (ESI) *m/z* 567.1586 $[2M+Na]^{+}$ (calcd for 567.1590, $C_{32}H_{26}NaO_6F_2$).

7-((3-chlorobenzyl) oxy) chroman-4-one (4g).

Yield 85%, colorless solid; ^{1}H NMR (500 MHz, DMSO) δ 7.70 (d, J = 8.8 Hz, 1H, Ar-H), 7.52 (s, 1H, Ar-H), 7.42 (t, J = 4.0 Hz, 3H, Ar-H), 6.72 (dd, J = 8.8, 2.4 Hz, 1H, Ar-H), 6.63 (d, J = 2.3 Hz, 1H, Ar-H), 5.20 (s, 2H, Ar-CH_2), 4.51 (t, J = 6.4 Hz, 2H, OCH_2), 2.71 (t, J = 6.4 Hz, 2H, $COCH_2$); ^{13}C NMR (125 MHz, DMSO) δ 190.43, 164.64, 163.82, 139.39, 133.67, 130.92, 128.73, 128.45, 127.86, 126.72, 115.68,

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110.62, 102.40, 69.23, 67.62, 37.38. HRMS (ESI) *m/z* 311.0443 $[M+Na]^+$ (calcd for 311.0445, $C_{16}H_{13}ClNaO_3$).

7-((4-chlorobenzyl) oxy) chroman-4-one (4h).

Yield 89%, colorless solid; ^1H NMR (500 MHz, DMSO) δ 7.69 (d, J = 8.8 Hz, 1H, Ar-H), 7.47 (s, 4H, Ar-H), 6.70 (dd, J = 8.8, 2.4 Hz, 1H, Ar-H), 6.62 (d, J = 2.4 Hz, 1H, Ar-H), 5.18 (s, 2H, Ar-CH_2), 4.50 (t, J = 6.4 Hz, 2H, OCH_2), 2.70 (t, J = 6.4 Hz, 2H, $COCH_2$); ^{13}C NMR (125 MHz, DMSO) δ 190.44, 164.71, 163.82, 135.86, 133.15, 130.05, 130.05, 129.01, 129.01, 128.71, 115.62, 110.66, 102.38, 69.33, 67.61, 37.37. HRMS (ESI) *m/z* 311.0446 $[M+Na]^+$ (calcd for 311.0445, $C_{16}H_{13}ClNaO_3$).

7-((3-bromobenzyl) oxy) chroman-4-one (4i).

Yield 92%, colorless solid; ^1H NMR (500 MHz, DMSO) δ 7.70 (d, J = 8.8 Hz, 1H, Ar-H), 7.66 (s, 1H, Ar-H), 7.54 (d, J = 7.9 Hz, 1H, Ar-H), 7.45 (d, J = 7.6 Hz, 1H, Ar-H), 7.37 (t, J = 7.8 Hz, 1H, Ar-H), 6.71 (dd, J = 8.8, 2.4 Hz, 1H, Ar-H), 6.63 (d, J = 2.3 Hz, 1H, Ar-H), 5.19 (s, 2H, Ar-CH_2), 4.51 (t, J = 6.4 Hz, 2H, OCH_2), 2.71 (t, J = 6.4 Hz, 2H, $COCH_2$); ^{13}C NMR (125 MHz, DMSO) δ 190.45, 164.64, 163.82, 139.64, 131.36, 131.22, 130.75, 128.74, 127.12, 122.22, 115.68, 110.64, 102.39, 69.18, 67.62, 37.38. HRMS (ESI) *m/z* 354.9939 $[M+Na]^+$ (calcd for 354.9440, $C_{16}H_{13}BrNaO_3$).

7-((4-bromobenzyl) oxy) chroman-4-one (4j).

Yield 91%, colorless solid; ^1H NMR (500 MHz, DMSO) δ 7.69 (d, J = 8.8 Hz, 1H, Ar-H), 7.60 (d, J = 8.4 Hz, 2H, Ar-H), 7.41 (d, J = 8.4 Hz, 2H, Ar-H), 6.70 (dd, J = 8.8, 2.4 Hz, 1H, Ar-H), 6.61 (d, J = 2.4 Hz, 1H, Ar-H), 5.17 (s, 2H, Ar-CH_2), 4.50 (t,

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J = 6.4 Hz, 2H, OCH_2), 2.70 (t, *J* = 6.4 Hz, 2H, $COCH_2$); ^{13}C NMR (125 MHz, DMSO) δ 190.44, 164.69, 163.82, 136.29, 131.94, 131.94, 130.34, 130.34, 128.71, 121.67, 115.63, 110.67, 102.39, 69.37, 67.61, 37.37. HRMS (ESI) *m/z* 354.9938 $[M+Na]^+$ (calcd for 354.9440, $C_{16}H_{13}BrNaO_3$).

4-(((4-oxochroman-7-yl) oxy) methyl) benzonitrile (4k).

Yield 79%, colorless solid; ^1H NMR (500 MHz, DMSO) δ 7.88 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.70 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.64 (d, *J* = 8.2 Hz, 2H, Ar-H), 6.73 (dd, *J* = 8.8, 2.4 Hz, 1H, Ar-H), 6.63 (d, *J* = 2.3 Hz, 1H, Ar-H), 5.30 (s, 2H, Ar-CH_2), 4.51 (t, *J* = 6.4 Hz, 2H, OCH_2), 2.71 (t, *J* = 6.4 Hz, 2H, $COCH_2$); ^{13}C NMR (125 MHz, DMSO) δ 190.44, 164.50, 163.81, 142.59, 132.96, 132.96, 128.78, 128.61, 128.61, 119.14, 115.77, 111.21, 110.59, 102.46, 69.19, 67.63, 37.37. HRMS (ESI) *m/z* 302.0787 $[M+Na]^+$ (calcd for 302.0788, $C_{17}H_{13}NNaO_3$).

7-((3-methylbenzyl) oxy) chroman-4-one (4l).

Yield 77%, colorless solid; ^1H NMR (500 MHz, DMSO) δ 7.69 (dd, *J* = 8.8, 4.8 Hz, 1H, Ar-H), 7.26 (dt, *J* = 17.5, 7.4 Hz, 3H, Ar-H), 7.15 (d, *J* = 7.3 Hz, 1H, Ar-H), 6.70 (dd, *J* = 8.8, 2.3 Hz, 1H, Ar-H), 6.61 (d, *J* = 2.3 Hz, 1H, Ar-H), 5.13 (s, 2H, Ar-CH_2), 4.50 (t, *J* = 6.4 Hz, 2H, OCH_2), 2.70 (t, *J* = 6.4 Hz, 2H, $COCH_2$), 2.32 (s, 3H, Ar-CH_3); ^{13}C NMR (125 MHz, DMSO) δ 190.43, 164.98, 163.83, 138.19, 136.70, 129.17, 128.89, 128.78, 128.67, 125.33, 115.51, 110.68, 102.30, 70.27, 67.60, 37.38, 21.44. HRMS (ESI) *m/z* 559.2089 $[2M+Na]^+$ (calcd for 559.2091, $C_{34}H_{32}NaO_6$).

7-((4-methylbenzyl) oxy) chroman-4-one (4m).

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Yield 80%, colorless solid; ^{1}H NMR (500 MHz, DMSO) δ 7.68 (d, J = 8.8 Hz, 1H, Ar-H), 7.32 (d, J = 7.9 Hz, 2H, Ar-H), 7.20 (d, J = 7.9 Hz, 2H, Ar-H), 6.68 (dd, J = 8.8, 2.3 Hz, 1H, Ar-H), 6.60 (d, J = 2.3 Hz, 1H, Ar-H), 5.12 (s, 2H, Ar-CH_2), 4.49 (t, J = 6.4 Hz, 2H, OCH_2), 2.70 (t, J = 6.4 Hz, 2H, $COCH_2$), 2.30 (s, 3H, Ar-CH_3); ^{13}C NMR (125 MHz, DMSO) δ 190.42, 164.97, 163.83, 137.84, 133.76, 129.54, 129.54, 128.64, 128.33, 128.33, 115.48, 110.72, 102.32, 70.14, 67.59, 37.38, 21.24. HRMS (ESI) *m/z* 559.2094 $[2M+Na]^+$ (calcd for 559.2091, $C_{34}H_{32}NaO_6$).

7-(benzyloxy)-2, 2-dimethylchroman-4-one (5a).

Yield 86%, colorless solid; ^{1}H NMR (500 MHz, DMSO) δ 7.66 (d, J = 8.7 Hz, 1H, Ar-H), 7.44 (d, J = 7.3 Hz, 2H, Ar-H), 7.40 (t, J = 7.5 Hz, 2H, Ar-H), 7.35 (dd, J = 8.3, 6.0 Hz, 1H, Ar-H), 6.67 (dd, J = 8.8, 2.3 Hz, 1H, Ar-H), 6.58 (d, J = 2.3 Hz, 1H, Ar-H), 5.17 (s, 2H, Ar-CH_2), 2.71 (s, 2H, $COCH_2$), 1.39 (s, 6H, $CH(CH_{3)2}$). ^{13}C NMR (125 MHz, DMSO) δ 195.54, 170.06, 166.64, 141.61, 133.74, 133.28, 133.07, 133.02, 132.91, 132.80, 119.08, 114.91, 107.40, 84.91, 74.96, 64.97, 31.38, 31.38. HRMS (ESI) *m/z* 587.2401 $[2M+Na]^+$ (calcd for 587.2404, $C_{36}H_{36}NaO_6$).

7-((2-fluorobenzyl) oxy)-2, 2-dimethylchroman-4-one (5b).

Yield 89%, colorless solid; ^{1}H NMR (500 MHz, DMSO) δ 7.67 (d, J = 8.7 Hz, 1H, Ar-H), 7.56 (m, 1H, Ar-H), 7.45 (m, 1H, Ar-H), 7.26 (dd, J = 15.6, 8.0 Hz, 2H, Ar-H), 6.67 (dd, J = 8.7, 2.4 Hz, 1H, Ar-H), 6.62 (d, J = 2.3 Hz, 1H, Ar-H), 5.20 (s, 2H, Ar-CH_2), 2.72 (s, 2H, $COCH_2$), 1.39 (s, 6H, $CH(CH_{3)2}$). ^{13}C NMR (125 MHz, DMSO) δ 190.82, 165.09, 161.90 (d, $^{1}J_{CF}$ = 103.6 Hz), 131.42, 131.16, 128.15, 125.09, 123.67,

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116.03, 115.87, 114.48, 109.97, 102.57, 80.20, 64.58, 60.03, 26.62, 26.62. HRMS (ESI) *m/z* 623.2211 $[2M+Na]^+$ (calcd for 623.2216, $C_{36}H_{34}F_2NaO_6$).

7-((3-fluorobenzyl) oxy)-2, 2-dimethylchroman-4-one (5c).

Yield 87%, yellow solid; ^{1}H NMR (500 MHz, DMSO) δ 7.67 (d, J = 8.7 Hz, 1H, Ar-H), 7.55 (m, 1H, Ar-H), 7.44 (m, 1H, Ar-H), 7.25 (dd, J = 15.7, 7.9 Hz, 2H, Ar-H), 6.67 (dd, J = 8.7, 2.4 Hz, 1H, Ar-H), 6.62 (d, J = 2.3 Hz, 1H, Ar-H), 5.19 (s, 2H, Ar-CH_2), 2.71 (s, 2H, $COCH_2$), 1.39 (s, 6H, $CH(CH_3)_2$). ^{13}C NMR (125 MHz, DMSO) δ 195.59, 169.85, 166.66 (d, $^1J_{CF}$ = 152.4 Hz), 132.96, 132.86, 129.83, 129.80, 128.40, 128.31, 120.49, 119.22, 114.73, 107.30, 84.97, 69.36, 65.05, 31.38, 31.38. HRMS (ESI) *m/z* 623.2213 $[2M+Na]^+$ (calcd for 623.2216, $C_{36}H_{34}F_2NaO_6$).

7-((4-fluorobenzyl) oxy)-2, 2-dimethylchroman-4-one (5d).

Yield 87%, yellow solid; ^{1}H NMR (500 MHz, DMSO) δ 7.66 (d, J = 8.7 Hz, 1H, Ar-H), 7.50 (dd, J = 8.5, 5.6 Hz, 2H, Ar-H), 7.23 (t, J = 8.8 Hz, 2H, Ar-H), 6.66 (dd, J = 8.8, 2.3 Hz, 1H, Ar-H), 6.58 (d, J = 2.3 Hz, 1H, Ar-H), 5.15 (s, 2H, Ar-CH_2), 2.71 (s, 2H, $COCH_2$), 1.39 (s, 6H, $CH(CH_3)_2$). ^{13}C NMR (125 MHz, DMSO) δ 190.78, 165.18, 163.48 (d, $^1J_{CF}$ = 57.9 Hz), 161.88, 133.09, 130.61, 130.54, 128.10, 115.89, 115.72, 114.29, 110.12, 102.65, 80.16, 69.47, 48.26, 26.62, 26.62. HRMS (ESI) *m/z* 623.2210 $[2M+Na]^+$ (calcd for 623.2216, $C_{36}H_{34}F_2NaO_6$).

7-((3-chlorobenzyl) oxy)-2, 2-dimethylchroman-4-one (5e).

Yield 83%, colorless solid; ^{1}H NMR (500 MHz, DMSO) δ 7.67 (d, J = 8.7 Hz, 1H, Ar-H), 7.51 (s, 1H, Ar-H), 7.44–7.40 (m, 3H, Ar-H), 6.68 (dd, J = 8.8, 2.4 Hz, 1H, Ar-H), 6.58 (d, J = 2.3 Hz, 1H, Ar-H), 5.19 (s, 2H, Ar-CH_2), 2.71 (s, 2H, $COCH_2$),

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1.39 (s, 6H, $CH(CH_{3)2}$). ^{13}C NMR (125 MHz, DMSO) δ 190.77, 165.00, 161.84, 139.42, 133.65, 130.91, 128.43, 128.13, 127.88, 126.73, 114.46, 110.10, 102.69, 80.19, 69.21, 48.24, 26.62, 26.62. HRMS (ESI) *m/z* 655.1623 $[2M+Na]^+$ (calcd for 655.1625, $C_{36}H_{34}Cl_2NaO_6$).

7-((4-chlorobenzyl) oxy)-2, 2-dimethylchroman-4-one (5f).

Yield 85%, colorless solid; ^1H NMR (500 MHz, DMSO) δ 7.66 (d, J = 8.7 Hz, 1H, Ar-H), 7.46 (s, 4H, Ar-H), 6.66 (dd, J = 8.7, 2.3 Hz, 1H, Ar-H), 6.57 (d, J = 2.3 Hz, 1H, Ar-H), 5.17 (s, 2H, Ar-CH_2), 2.70 (s, 2H, $COCH_2$), 1.38 (s, 6H, $CH(CH_{3)2}$). ^{13}C NMR (125 MHz, DMSO) δ 190.79, 165.08, 161.86, 135.91, 133.14, 130.07, 130.07, 129.00, 129.00, 128.12, 114.40, 110.13, 102.67, 80.18, 69.31, 48.24, 26.62, 26.62. HRMS (ESI) *m/z* 655.1621 $[2M+Na]^+$ (calcd for 655.1625, $C_{36}H_{34}Cl_2NaO_6$).

7-((3-bromobenzyl) oxy)-2, 2-dimethylchroman-4-one (5g).

Yield 84%, yellow solid; ^1H NMR (500 MHz, DMSO) δ 7.69–7.63 (m, 2H, Ar-H), 7.55 (d, J = 8.0 Hz, 1H, Ar-H), 7.45 (d, J = 7.7 Hz, 1H, Ar-H), 7.37 (t, J = 7.8 Hz, 1H, Ar-H), 6.67 (dd, J = 8.8, 2.4 Hz, 1H, Ar-H), 6.58 (d, J = 2.3 Hz, 1H, Ar-H), 5.18 (s, 2H, Ar-CH_2), 2.71 (s, 2H, $COCH_2$), 1.38 (s, 6H, $CH(CH_{3)2}$). ^{13}C NMR (125 MHz, DMSO) δ 190.81, 165.01, 161.86, 139.68, 131.36, 131.22, 130.78, 128.15, 127.15, 122.20, 114.46, 110.12, 102.69, 80.20, 69.16, 48.24, 26.62, 26.62. HRMS (ESI) *m/z* 361.0435 $[M+H]^+$ (calcd for 361.0436, $C_{18}H_{18}BrO_3$).

7-((4-bromobenzyl) oxy)-2, 2-dimethylchroman-4-one (5h).

Yield 85%, colorless solid; ^1H NMR (500 MHz, DMSO) δ 7.66 (d, J = 8.7 Hz, 1H, Ar-H), 7.60 (d, J = 8.4 Hz, 2H, Ar-H), 7.40 (d, J = 8.4 Hz, 2H, Ar-H), 6.66 (dd, J =

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8.8, 2.4 Hz, 1H, Ar-H), 6.56 (d, J = 2.3 Hz, 1H, Ar-H), 5.15 (s, 2H, Ar-CH_2), 2.70 (s, 2H, $COCH_2$), 1.38 (s, 6H, $CH(CH_{3)2}$). ^{13}C NMR (125 MHz, DMSO) δ 190.77, 165.06, 161.86, 136.35, 131.93, 131.93, 130.35, 130.35, 128.12, 121.66, 114.42, 110.13, 102.70, 80.18, 69.35, 48.25, 26.62, 26.62. HRMS (ESI) *m/z* 361.0430 $[M+H]^+$ (calcd for 361.0436, $C_{18}H_{18}BrO_3$).

4-(((2, 2-dimethyl-4-oxochroman-7-yl) oxy) methyl) benzonitrile (5i).

Yield 84%, yellow oil; ^{1}H NMR (500 MHz, DMSO) δ 7.88 (d, J = 8.2 Hz, 2H, Ar-H), 7.79 (d, J = 8.2 Hz, 1H, Ar-H), 7.67 (d, J = 8.7 Hz, 1H, Ar-H), 7.64 (d, J = 8.2 Hz, 2H, Ar-H), 7.51 (d, J = 8.1 Hz, 1H, Ar-H), 6.69 (dd, J = 8.8, 2.4 Hz, 1H, Ar-H), 6.59 (d, J = 2.3 Hz, 1H, Ar-H), 5.29 (s, 2H, Ar-CH_2), 2.71 (s, 2H, $COCH_2$), 1.38 (s, 6H, $CH(CH_{3)2}$). ^{13}C NMR (125 MHz, DMSO) δ 195.57, 169.63, 166.62, 147.40, 137.80, 137.33, 133.34, 132.90, 132.02, 123.91, 115.96, 114.84, 107.52, 84.99, 73.93, 67.47, 53.00, 31.37, 31.37. HRMS (ESI) *m/z* 330.1099 $[M+Na]^+$ (calcd for 330.1101, $C_{19}H_{17}NNaO_3$).

2, 2-dimethyl-7-((3-methylbenzyl) oxy) chroman-4-one (5j).

Yield 79%, colorless solid; ^{1}H NMR (500 MHz, DMSO) δ 7.66 (d, J = 8.7 Hz, 1H, Ar-H), 7.26 (m, 3H, Ar-H), 7.16 (d, J = 7.4 Hz, 1H, Ar-H), 6.66 (dd, J = 8.8, 2.3 Hz, 1H, Ar-H), 6.57 (d, J = 2.3 Hz, 1H, Ar-H), 5.12 (s, 2H, Ar-CH_2), 2.70 (s, 2H, $COCH_2$), 2.32 (s, 3H, Ar-CH_3), 1.39 (s, 6H, $CH(CH_{3)2}$). ^{13}C NMR (125 MHz, DMSO) δ 190.78, 165.34, 161.88, 138.17, 136.74, 129.16, 128.88, 128.81, 128.09, 125.35, 114.30, 110.14, 102.60, 80.15, 70.25, 48.26, 26.63, 26.63, 21.38. HRMS (ESI) *m/z* 615.2715 $[2M+Na]^+$ (calcd for 615.2717, $C_{38}H_{40}NaO_6$).

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2, 2-dimethyl-7-((4-methylbenzyl) oxy) chroman-4-one (5k).

Yield 77%, colorless solid; ^{1}H NMR (500 MHz, DMSO) δ 7.65 (d, J = 8.7 Hz, 1H, Ar-H), 7.32 (d, J = 7.9 Hz, 2H, Ar-H), 7.20 (d, J = 7.8 Hz, 2H, Ar-H), 6.64 (dd, J = 8.8, 2.4 Hz, 1H, Ar-H), 6.56 (d, J = 2.3 Hz, 1H, Ar-H), 5.11 (s, 2H, Ar-CH_2), 2.70 (s, 2H, $COCH_2$), 2.31 (s, 3H, Ar-CH_3), 1.38 (s, 6H, $CH(CH_{3)2}$). ^{13}C NMR (125 MHz, DMSO) δ 190.20, 164.77, 161.29, 137.25, 133.23, 128.96, 128.96, 127.78, 127.78, 127.49, 113.69, 109.59, 102.05, 79.56, 69.54, 47.69, 26.06, 26.06, 20.67. HRMS (ESI) *m/z* 615.2713 $[2M+Na]^+$ (calcd for 615.2717, $C_{38}H_{40}NaO_6$).

General procedure for the preparation of C6-substituted benzofuranone derivatives (7).

6-hydroxybenzofuran-3(2*H*)-one (1.85 mmol) was suspended in acetonitrile (15 mL) containing K_2CO_3 (3.70 mmol). The reaction was treated with an appropriately substituted arylalkyl bromide (2.04 mmol) and heated under reflux for 8 h. The reaction progress was monitored using *silica gel* TLC with hexanes/EtOAc as mobile phase. Upon completion, the acetonitrile was evaporated *in vacuo* and the mixture was then poured into water, which was extracted with 3 × 200 mL of EtOAc, washed with brine, dried over anhydrous Na_2SO_4 and purified by chromatography (hexanes /EtOAc) on *silica gel.*

6-(benzyloxy) benzofuran-3(2*H*)-one (7a).

Yield 86%, yellow solid; ^{1}H NMR (500 MHz, DMSO) δ 7.54 (d, J = 8.6 Hz, 1H, Ar-H), 7.47 (d, J = 7.2 Hz, 2H, Ar-H), 7.41 (dd, J = 10.1, 4.7 Hz, 2H, Ar-H), 7.38–7.33 (m, 1H, Ar-H), 6.90 (d, J = 2.0 Hz, 1H, Ar-H), 6.78 (dd, J = 8.6, 2.1 Hz, 1H,

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Ar-H), 5.23 (s, 2H, $COCH_2$), 4.76 (s, 2H, Ar-CH_2). ^{13}C NMR (125 MHz, DMSO) δ 197.58, 176.14, 167.15, 136.55, 129.02, 129.02, 128.63, 128.36, 128.36, 125.16, 114.68, 112.41, 98.22, 75.98, 70.60. HRMS (ESI) *m/z* 263.0680 $[M+Na]^+$ (calcd for 263.0679, $C_{15}H_{12}NaO_3$).

6-phenethoxybenzofuran-3(2*H*)-one (7b).

Yield 87%, yellow solid; ^1H NMR (500 MHz, DMSO) δ 7.51 (d, J = 8.6 Hz, 1H, Ar-H), 7.39–7.27 (m, 4H, Ar-H), 7.23 (m, 1H, Ar-H), 6.83 (d, J = 2.0 Hz, 1H, Ar-H), 6.72–6.64 (m, 1H, Ar-H), 4.75 (s, 2H, $COCH_2$), 4.32 (t, J = 6.8 Hz, 2H, OCH_2), 3.07 (t, J = 6.8 Hz, 2H, Ar-CH_2). ^{13}C NMR (125 MHz, DMSO) δ 197.55, 176.24, 167.32, 138.44, 129.44, 129.44, 128.82, 128.82, 126.86, 125.10, 114.51, 112.21, 97.80, 75.93, 69.52, 35.07. HRMS (ESI) *m/z* 531.1775 $[2M+Na]^+$ (calcd for 531.1778, $C_{32}H_{28}NaO_6$).

6-(3-phenylpropoxy) benzofuran-3(2*H*)-one (7c).

Yield 77%, yellow solid; ^1H NMR (500 MHz, DMSO) δ 7.54 (d, J = 8.6 Hz, 1H, Ar-H), 7.31 (t, J = 7.5 Hz, 2H, Ar-H), 7.25 (d, J = 7.2 Hz, 2H, Ar-H), 7.21 (t, J = 7.2 Hz, 1H, Ar-H), 6.81 (d, J = 1.8 Hz, 1H, Ar-H), 6.73 (dd, J = 8.6, 2.0 Hz, 1H, Ar-H), 4.78 (s, 2H, $COCH_2$), 4.11 (t, J = 6.3 Hz, 2H, OCH_2), 2.80–2.70 (m, 2H, Ar-CH_2), 2.14–1.97 (m, 2H, alkyl chains-H). ^{13}C NMR (125 MHz, DMSO) δ 197.55, 176.24, 167.52, 141.67, 128.84, 128.84, 128.83, 128.83, 126.37, 125.11, 114.47, 112.18, 97.68, 75.94, 68.27, 31.82, 30.51. HRMS (ESI) *m/z* 559.2090 $[2M+Na]^+$ (calcd for 559.2091, $C_{34}H_{32}NaO_6$).

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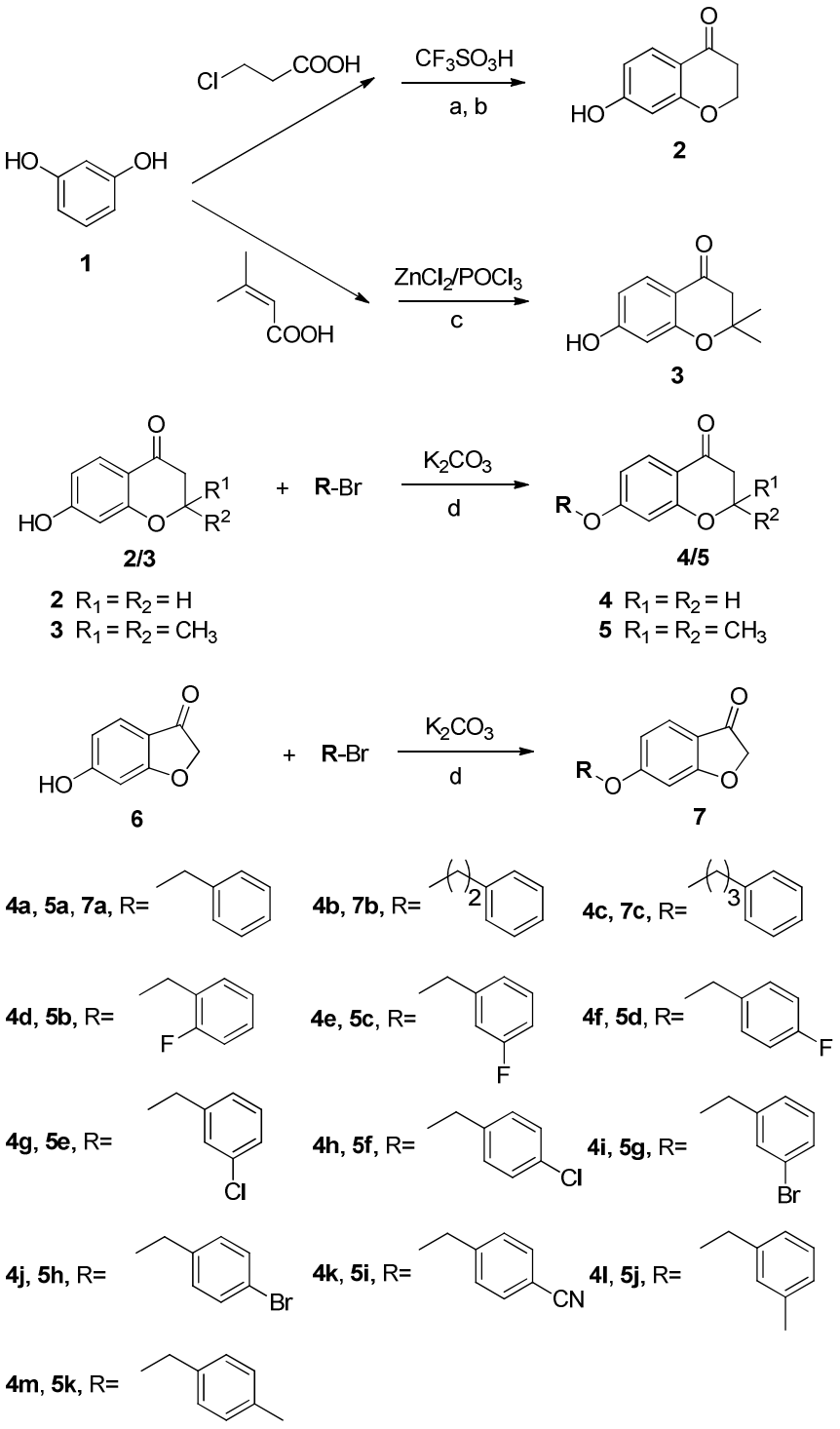

Scheme 1. Synthesis of C7-substituted 4-Chromanone derivatives **4**, **5** and **7**. Reagents and conditions: (a) CF_3SO_3H, 80 °C, 1h; (b) NaOH, rt, 2h; (c) $ZnCl_2/POCl_3$, 50 °C, 2h; (d) K_2CO_3, CH_3CN, reflux, 8h.

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Table 1

Physicochemical properties of compounds **4**, **5** and **7**.

Compounds	MW [a]	Clog P [a]	HBA [a]	HBD [a]	PAS [a]	Log BB [a]
4a	254.09	3.512	3	0	35.53	0.147
4b	268.11	3.841	3	0	35.53	0.225
4c	282.13	4.220	3	0	35.53	0.283
4d	272.08	3.655	3	0	35.53	0.197
4e	272.08	3.655	3	0	35.53	0.197
4f	272.08	3.655	3	0	35.53	0.197
4g	288.06	4.225	3	0	35.53	0.284
4h	288.06	4.225	3	0	35.53	0.284
4i	322.00	4.375	3	0	35.53	0.307
4j	322.00	4.375	3	0	35.53	0.307
4k	279.09	2.945	3	0	59.32	-0.244
4l	268.11	4.011	3	0	35.53	0.251
4m	268.11	4.011	3	0	35.53	0.251
5a	282.13	4.550	3	0	35.53	0.333
5b	300.12	4.693	3	0	35.53	0.355
5c	300.12	4.693	3	0	35.53	0.355
5d	300.12	4.693	3	0	35.53	0.355
5e	316.09	5.263	3	0	35.53	0.442
5f	316.09	5.263	3	0	35.53	0.442
5g	360.04	5.413	3	0	35.53	0.464
5h	360.04	5.413	3	0	35.53	0.464
5i	307.12	3.983	3	0	59.32	-0.086
5j	296.14	5.049	3	0	35.53	0.409
5k	296.14	5.049	3	0	35.53	0.409
7a	240.08	3.153	3	0	35.53	0.121
7b	254.09	3.482	3	0	35.53	0.171
7c	268.11	3.861	3	0	35.53	0.228
Rules	≤450	≤5.0	≤10	≤5	≤90	≥-0.3

[a] MW: molecular weight; C log P: calculated logarithm of the octanol-water partition coefficient; HBA: hydrogen-bond acceptor atoms; HBD: hydrogen-bond donor atoms; PSA: polar surface area; log BB = -0.0148 × PSA + 0.152 ClogP + 0.139.

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Table 2.

*h*MAO inhibitory activities of the synthesized compounds.

Compounds	R	MAO-A inhibition (%)[a]	MAO-B IC_{50} (nM)[b]	Selectivity Index[c]
2	H-	71380 ± 2560[b]	78860 ± 3690	0.9
4a	C_6H_5-CH_2-	18	57.37 ± 2.35	>1742
4b	C_6H_5-$(CH_2)_2$-	58	251.14 ± 3.62	36.2
4c	C_6H_5-$(CH_2)_3$-	65	21450 ± 260	3.9
4d	2-FC_6H_5-CH_2-	35	58.82 ± 1.84	>1700
4e	3-FC_6H_5-CH_2-	26	52.65 ± 3.16	>1898
4f	4-FC_6H_5-CH_2-	43	8.62 ± 0.96	>11628
4g	3-ClC_6H_5-CH_2-	35	35.26 ± 1.56	>2841
4h	4-ClC_6H_5-CH_2-	37	33.45 ± 1.29	>2994
4i	3-BrC_6H_5-CH_2-	28	30.27 ± 2.35	>3311
4j	4-BrC_6H_5-CH_2-	19	21.56 ± 3.61	>4638
4k	4-CNC_6H_5-CH_2-	27	47.28 ± 4.86	>2114
4l	3-$CH_3C_6H_5$-CH_2-	19	51.37 ± 3.27	>1946
4m	4-$CH_3C_6H_5$-CH_2-	26	91.29 ± 2.98	>1095
5a	C_6H_5-CH_2-	25	97.47 ± 1.56	>1026
5b	2-FC_6H_5-CH_2-	28	68.78 ± 2.25	>1454
5c	3-FC_6H_5-CH_2-	18	65.48 ± 1.79	>1527
5d	4-FC_6H_5-CH_2-	25	10.26 ± 0.98	>9709
5e	3-ClC_6H_5-CH_2-	31	55.37 ± 2.59	>1085
5f	4-ClC_6H_5-CH_2-	28	44.18 ± 1.94	>2262
5g	3-BrC_6H_5-CH_2-	16	52.23 ± 1.67	>1916
5h	4-BrC_6H_5-CH_2-	21	45.36 ± 2.35	>2203
5i	4-CNC_6H_5-CH_2-	23	69.51 ± 1.67	>1439
5j	3-$CH_3C_6H_5$-CH_2-	18	98.32 ± 2.56	>1017
5k	4-$CH_3C_6H_5$-CH_2-	25	113.21 ± 3.12	>883
7a	C_6H_5-CH_2-	38150 ± 1240[b]	15210 ± 510	2.5
7b	C_6H_5-$(CH_2)_2$-	46360 ± 1690[b]	2130 ± 120	21.8
7c	C_6H_5-$(CH_2)_3$-	39270 ± 2130[b]	20510 ± 490	1.9
Pargyline	-	nt.[e]	198.5 ± 5.28	-
Iproniazid	-	6550 ± 240[b]	7690 ± 280	0.8

[a] Test concentration is 100 μM.

[b] IC_{50}: 50% inhibitory concentration (means ± SEM of three experiments).

[c] Selectivity Index = IC_{50} (MAO-A)/IC_{50} (MAO-B).

[e] nt. = not tested.

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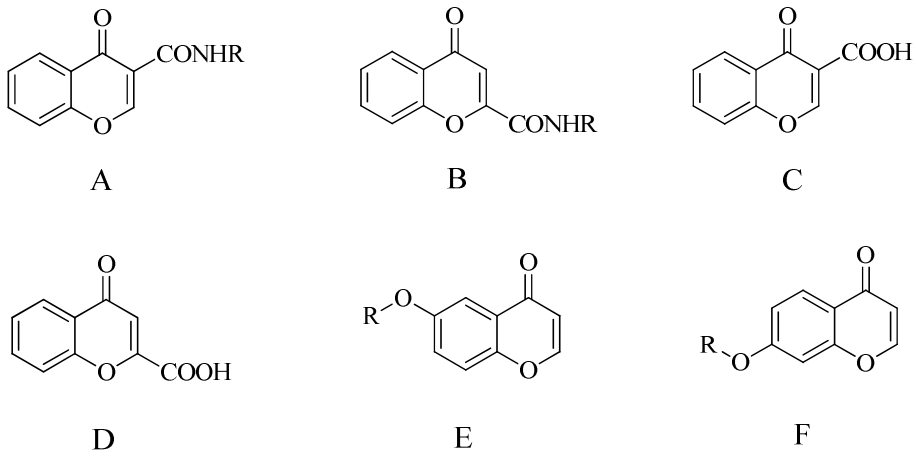

Figure 1. Structures of known chromone-based MAO inhibitors.

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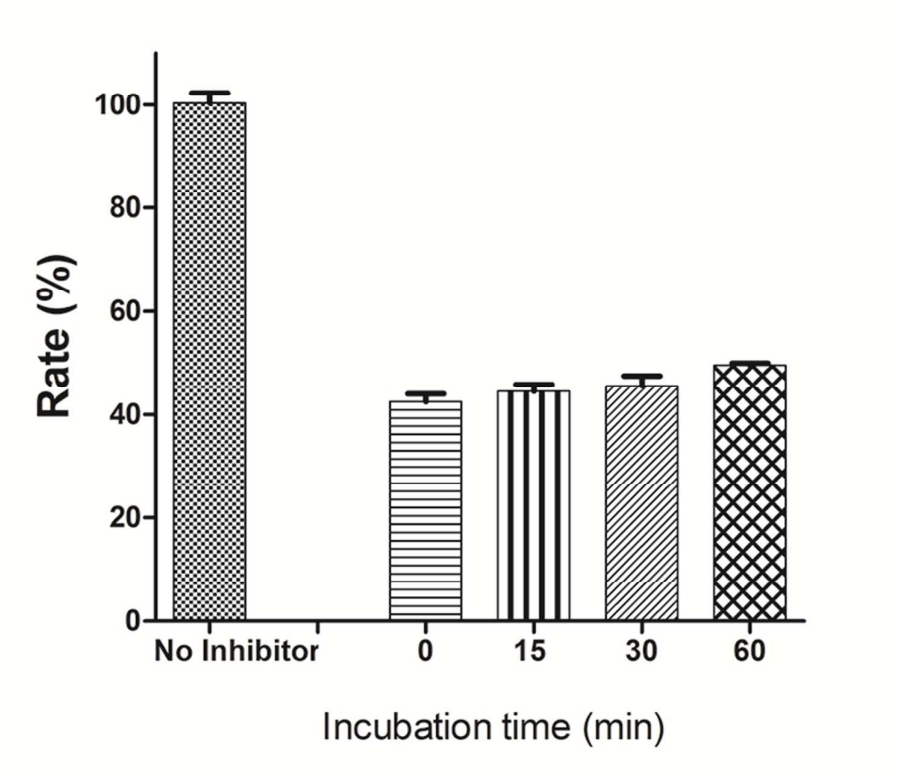

Figure 2. The time-dependent inhibition of *h*MAO-B by compound **4f**. Compound **4f** was preincubated for various periods of time (0-60 min) with *h*MAO-B at concentrations equal to twofold the IC_{50} values for the inhibition of the enzyme. After dilution to concentrations equal to the IC_{50}, the inhibitory rates were recorded.

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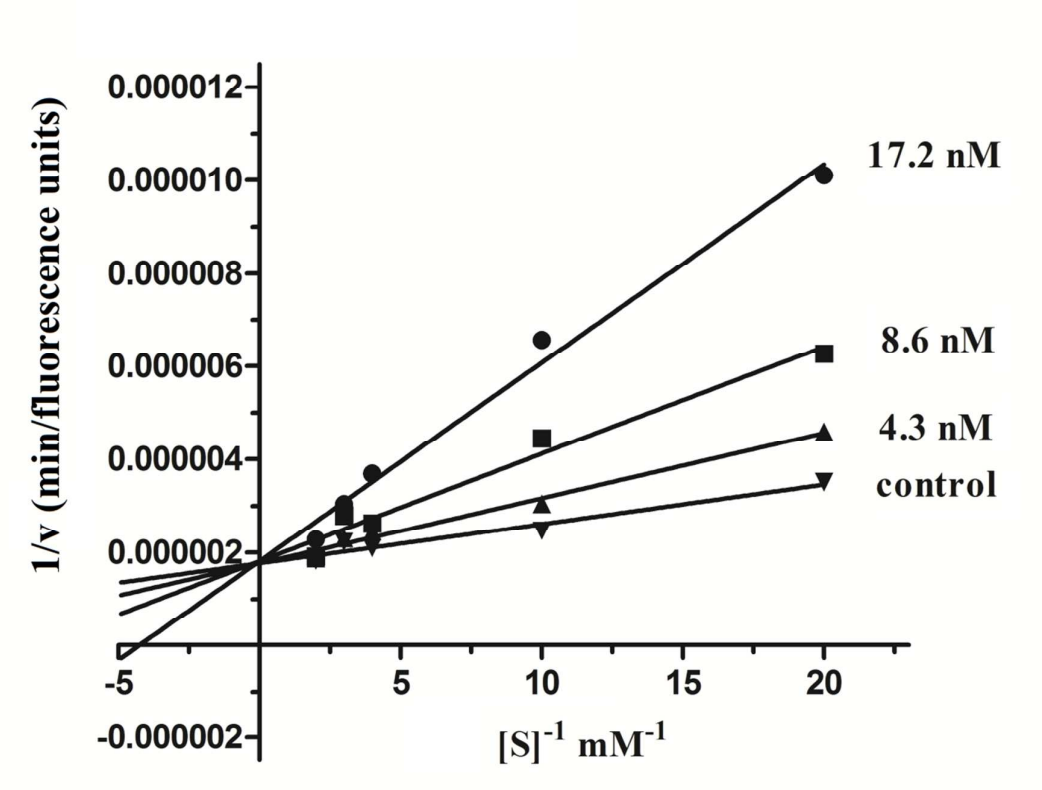

Figure 3. Kinetic study on the mechanism of *h*MAO-B inhibition by compound **4f**. Overlaid Lineweaver-Burk reciprocal plots of MAO-B initial velocity at increasing substrate concentration (50–500 μM) in the absence of inhibitor and in the presence of **4f** are shown. Lines were derived from a weighted least-squares analysis of the data points.

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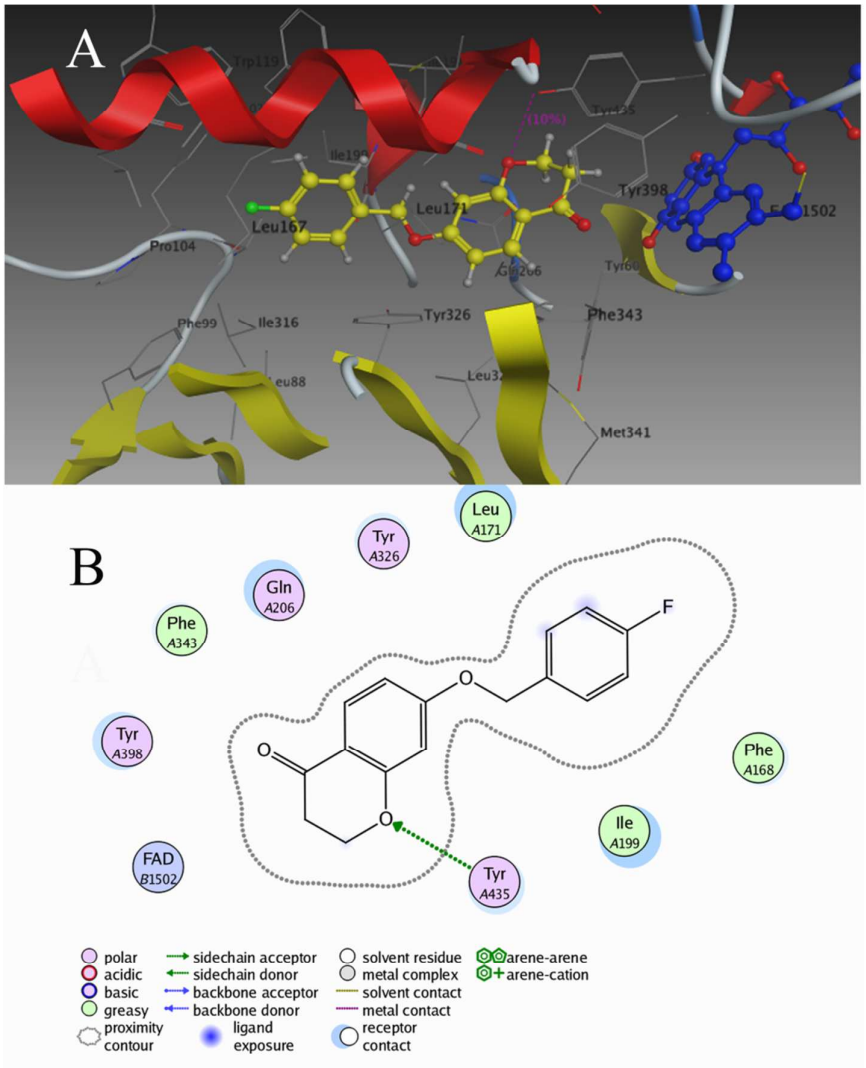

Figure 4. (A) 3D docking model of compound **4f** with *h*MAO-B. (B) 2D schematic diagram of docking model of compound **4f** with *h*MAO-B. The figure was prepared using the ligand interactions application in MOE. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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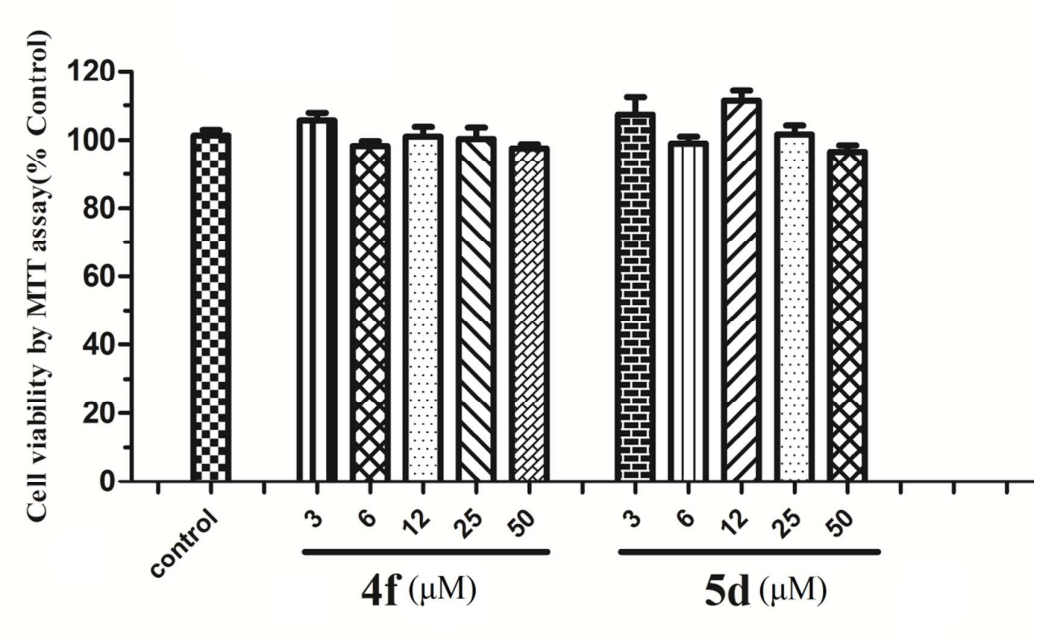

Figure 5. Effects of compounds on cell viability in SH-SY5Y cells. The cell viability was determined by the MTT assay after 48 h of incubation with various concentrations of **4f** and **5d**. The results were expressed as a percentage of control cells. Values are reported as the mean ± SD of three independent experiments.