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Potent and Selective Monoamine Oxidase-B Inhibitory Activity: Fluoro- *vs.* Trifluoromethyl-4-hydroxylated Chalcones Derivatives

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For various neurodegenerative disorders like *Alzheimer*'s and Parkinsonism diseases, selective and reversible MAO B inhibitors have a great therapeutic value. In our previous study, we have shown that a series of methoxylated chalcones with F functional group exhibited high binding affinity towards for human monoamine oxidase-B (hMAO-B). In continuation with our earlier study and to extend the structure–activity relationships, a series of new five chalcones were studied for their inhibition of hMAO. The results demonstrated that these compounds are reversible and selective hMAO-B inhibitors with a competitive mode of inhibition. The most active compound, (2*E*)-1-(4-hydroxyphenyl)-3-[4-(trifluoromethyl)phenyl]prop-2-en-1-one exhibited K_i value 0.33 ± 0.01 μM towards hMAO-B with a selectivity index of 26.36. Molecular docking study revealed that the presence of a H-bond network in hydroxylated chalcone with the N5 atom of FAD is crucial for MAO-B selectivity and potency.

Keywords: Chalcone, MAO-A, MAO-B, Molecular docking.

1. Introduction

The Monoamine oxidase (MAO) enzyme consists of two isoforms, MAO-A and MAO-B, which are widely distributed in central and peripheral nervous systems. These isoforms are encoded by separate genes and share approximately 70% sequence identity at the amino acid level and play crucial roles in the oxidative catabolism of neurotransmitter

amines in central and peripheral tissues [1,2]. Selective inhibition of MAO-A results in the elevated level of serotonin and noradrenaline and can be used for improving the symptoms of depression [3]. While selective MAO-B inhibitors are used with L-DOPA and/or dopamine agonists in the symptomatic treatment of *Parkinson*'s disease (PD) [4]. Side effects of most current monoamine oxidase inhibitors (MAOi) are due to lack of affinity and selectivity toward one of the isoforms. So, there is an urgent need to design new more potent, selective and reversible inhibitors of MAO-A and MAO-B have great therapeutic value.

Considering the pharmacological importance of MAO inhibitors, the design of new selective MAO inhibitors is pursued by several research groups. Chalcones are open-chain flavonoids in which the two phenyl rings are linked by a three-carbon consists of α,β-unsaturated carbonyl system. In this, aromatic ring *A* is generally nearer to the C=O group and the *B* ring to the β C-atom of three-carbon unit. Chalcones are reported for their potential MAO inhibition by different groups [5-11]. The most active chalcones were characterized by the presence of OH/MeO substituents either *ortho* positions with Cl-atom at *para* position of ring *B*. These compounds exhibited inhibitory activity in the nanomolar range with good selectivity [12].

Recently, our group has reported some potent, selective and reversible inhibitors of hMAO-B having methoxylated and thiophene based chalcones with F and CF_3 substitution. Amongst them, (2*E*)-1-(4-methoxyphenyl)-3-[4-(trifluoromethyl)phenyl]prop-2-en-1-one and (*2E*)-1-(thiophen-2-yl)-3-[4-(trifluoromethyl)phenyl]prop-2-en-1-one exhibited K_i value 0.22

± 0.01 and 0.90 ± 0.05 μM, respectively, towards hMAO-B. The study mainly highlighted the effect of orientation of F and CF_3 groups at the ring *B* of chalcone on the inhibition of hMAO [13, 14]. The ability of chalcone scaffolds to act on the central nervous system (CNS) mainly associated with its low polar surface area that can help to cross the blood brain barrier easily. This property, principally earned by the hydrophobic nature of two aromatic ring of *A* and *B* at the terminals of open chain of *α*,*β*-unsaturated system.

The present study aimed at expanding on the structure–activity relationships (SARs) of hMAO inhibition of chalcone derivatives by synthesizing a series of fluorinated chalcones with OH substituted phenyl system. The information regarding the orientation of F and CF_3 groups in the *B* ring system of hydroxylated chalcone and their effect on MAO inhibition is not explored so far. Earlier we reported fluorochalcones from 2-acetylthiophene and 4-methoxyacetophenone and observed that position of F (*ortho*, *meta*, *para*) on ring *B* had a negligible impact on inhibition of both hMAO-A and hMAO-B. A similar pattern is observed for CF_3 group, but with slight improvement in potency for 4-CF_3 derivative. This clearly ■showcasing■ the effect of electronegativity on biological activity. When thiophene is replaced with *para*-methoxyphenyl ring, the position of F and CF_3 has shown a great difference in potency in inhibiting both hMAO-A and hMAO-B [13, 14]. Chalcones having F substitution at *ortho*- and *para*-positions were found to inhibit hMAB-B potently in comparison with their *meta*-counterpart, whereas the reverse has been observed for hMAO-A. Similar trend was observed for CF_3 derivatives, but here they are found to have potency

equivalent to their F counterpart. From these past two experiences, we learnt that change in biological activity due to nature and position of F groups on ring *B* has also been greatly influenced by the nature and position of substituents on ring *A*. With this background, in the presented research we evaluated effect of *para*-hydroxyphenyl as ring *A* that is a better H-bond acceptor/donor when compared with *para*-methoxyphenyl. *para*-Hydroxyacetophenone moiety was selected as starting reagent for the synthesis of various hydroxylated chalcones. For this purpose, chalcones were obtained by a *Claisen–Schmidt* condensation of *p*-hydroxyacetophenone with various F and CF_3 substituted benzaldehydes under basic conditions as outlined in the *Scheme*.

2. Results and Discussion

2.1. Chemistry

The synthesis of the fluorinated hydroxylated chalcones was achieved by following *Claisen–Schmidt* condensation of 4-hydroxyacetophenone with various fluorinated aromatic aldehydes under 40% KOH/EtOH medium (*Scheme*). ^{1}H-NMR spectrum showed the peaks of eleven H-atoms compromising of signals of the *α*,*β*-unsaturated unit, the substituted two phenyl system and OH group. It has been noted that, the up-field H-atom of *α* C-atom and down-field H-atom of *β* C-atom coupled with CH H-atom with coupling constants of (15.6 – 16.0 Hz). This large coupling constant shows the presence of (*E*)-configuration in the fluorinated hydroxylated chalcones. In ^{13}C-NMR spectra, the peaks for C=O C-atom are in

the range of δ(C) 188.92 – 188.43. Mass spectra of the compounds postulated their corresponding molecular weights.

2.2. MAO inhibitory potencies of fluorinated hydroxylated chalcones

The potential inhibitory activities of the fluorinated hydroxylated chalcones on hMAO isozymes were determined by measuring their effects on the production of H_2O_2 from the substrate (*p*-tyramine), using the *Amplex Red* MAO assay kit and enzymes (recombinant MAO isoforms). All the newly synthesized chalcones were screened for their potential hMAO inhibitory activity using recombinant hMAO-A and hMAO-B. The results of the inhibitory potencies and selectivities of the chalcones under study towards MAO isoforms, and reference compounds, are depicted in *Table 1*. The presented study documents that all the fluorinated chalcones inhibited hMAO-B selectively and reversibly in a competitive mode. The most potent and selective hMAO-B inhibitor in this series was compound **B5** that inhibited hMAO-B isoform with a K_i value of 0.30 ± 0.05 μM and selective index of 26.36. This was having a CF_3 substitution at the *para*-position on the *B* ring of chalcone. The following order of hMAO inhibitory activity was observed for the fluorinated hydroxylated chalcones:

hMAO-A: 2-CF_3 > 3-F > 2-F > 4-F > 4-CF_3

hMAO-B: 4-CF_3 > 2-F > 4-F > 2-CF_3 > 3-F

The study clearly indicated that all the chalcones under the present study exhibited good activity in the submicromolar range towards the inhibition of hMAO-B. Chalcones having F substitution at *ortho*- and *para*-position were found to be better than their *meta*-counterpart in inhibiting hMAO-B, while a reverse trend has been observed for hMAO-A. Chalcone having CF_3 at *ortho*-position was found to inhibit hMAO-B at a concentration equal to the one shown by the chalcones having F at *ortho*- and *para*-position. Chalcone having CF_3 at *para*-position was found to be better than their F counterparts (*ortho*, *meta*, *para*) in inhibiting hMAO-B. The trend was quite similar to the one observed with the fluorinated chalcones having thiophene as ring *A*, reported by our group [14], these hydroxylated chalcones were found to be slightly better than their thienyl chalcones. But the hydroxylated chalcones were not better than their methoxylated counterparts reported by our group [13].

From the analysis of the inhibition data, it is clear that the presence of electron withdrawing lipophilic group on the *para*-position of ring *B* of chalcone resulted the enhance MAO-B inhibition, while the same substitution is associated with the decreased MAO-A inhibition activity. Shifting the CF_3 group from *para*- to *ortho*-position exhibiting three fold less inhibition activity of MAO-B. Based on high inhibition potency and hMAO-B selectivity as selection criteria, **B5** was considered a promising lead compound for the development of reversible hMAO-B inhibitors.

The presence of CF_3 substituent at *para*-position on ring *B* play a pivotal role in determining a productive binding of chalcone-based hMAO-B inhibitors. The enhancement of MAO-B inhibition potencies by CF_3 substitution has been previously described [15-17]. Compounds **B1**, **B3**, and **B4** were also found to be a potent MAO-B inhibitor with a K_i value of 0.74, 0.83, and 0.90 μM, respectively.

2.3. Kinetic analysis

Kinetic analyses were carried out for most potent hMAO-B inhibitor **B5** from this series. A set of *Lineweaver–Burk* plots were constructed in the absence and presence of various concentrations of compound **B5**. The observation that the lines were linear and intersects on the *y*-axis suggests that **B5** interacts with the catalytic site of hMAO-B, with a competitive mode of inhibition (*Fig. 1*). The replots of the slopes of the *Lineweaver–Burk* plots *vs.* inhibitor concentration is shown in *Fig. 2* and the K_i was estimated as 0.33 μM for **B5** competitively. This compound also showed the highest selectivity for hMAO-B (*SI* value is calculated as 26.36).

2.4. Reversibility studies

The reversibility of MAO inhibition with the newly synthesized compounds were investigated, since it has been previously shown that reversibility of MAO inhibition is highly important for drug safety over the irreversible inhibitors for the management of *Parkinson*'s

disease. Reversibility of the inhibition of hMAO-B with the new compounds is shown in *Table 2*. The results indicated that both MAO-A and MAO-B activities are recovered following dialysis. The reversiblity of compound **B5** (1.5 μM) was calculated as 24.77 ± 1.66 and 97.11 ± 2.33 before and after dialysis, respectively, whereas the percentage inhibition of hMAO-B inhibition (%) of lazabemide (0.15 μM) was calculated as 7.85 ± 0.23 and 89.00 ± 5.20 before and after dialysis, respectively.

2.5. Molecular docking

This preliminary molecular docking study points out that the presence of a H-bond network in hydroxylated chalcone with the N5 atom of Flavin Adenine Dinucleotide (FAD) is crucial for hMAO-B selectivity for compound **B5**. This stabilizing interaction makes the OH group of ring *A* of fluorinated chalcones showed good proximity towards the isoalloxazine of FAD unit (1.932 Å). This shorter distance between the phenolic OH and N5 atom of FAD argues its enhanced binding affinities to the inhibitor binding cavity of MAO-B (*Fig. 3*). Accordingly, it can be also anticipated that the *para*-position of the CF_3 substituent on the aromatic nucleus of *B* ring of chalcone can influence the strength of the H-bond and π-π stacking interaction and consequently affecting the potency of this type of hMAO-B inhibitor. Another possible explanation of MAO-B selectivity of **B5** may be that CF_3 group, by virtue of its higher *Hammett* constant, forms more productive interactions with the hMAO-B active site.

Depending on the bound substrate/inhibitor, MAO-B active site can exhibit a bipartite cavity so that the ligand has to negotiate a smaller entrance room before entering the substrate cavity where FAD is accommodated [18]. The presence of CF_3 group on the *para*-position of ring *B* can contribute significant lipophilic nature to the molecule. Since the entrance cavity is reported to be a highly hydrophobic environment, the CF_3 substituent is stabilized principally by *Van der Waals* interactions within the entrance cavity. The strong electron withdrawing CF_3 group in the *para*-position of the *B* ring of the **B5** significantly reduces the electron density and is capable of forming more π-π stacking interaction with FAD unit of the inhibitor binding cavity of MAO-B [19].

3. Conclusion

In the present work, evidence was acquired to demonstrate that hydroxylated chalcone is a privileged scaffold for the design of potent, selective, and reversible MAO-B inhibitors. It may thus be concluded that **B5** is a promising lead for the development of reversible and selective MAO-B inhibitors, which may be used in the treatment of *Parkinson*'s disease. It is also mentioned that these lead chalcones are the versatile intermediates for the preparation of 2-pyrzolines compounds for the development of remarkable MAO inhibition properties.

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4. Experimental Part

4.1. Chemistry

4.2. General procedure for the synthesis of chalcones

A mixture of 4-hydroxyacetophenone (0.01 mol, 1 equiv.), fluorinated aldehyde (0.01 mol, 1 equiv.) and 40% aq. KOH (15 ml) in EtOH (30 ml) was stirred at r.t. for about 2 – 6 h. The resulting product was kept overnight in refrigerator. The separated solid was filtered, washed with H_2O and recrystallized from EtOH. If the solid was not formed, dil. HCl is slightly added to the mixture was to afford products.

(2*E*)-3-(2-Fluorophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (**B1**). Yield: 53%. Brown powder. M.p. 138 – 140°. ^{1}H-NMR: 5.910 (*s*, 1 H); 7.696 – 7.657 (*dd*, *J* = 15.6, 1 H); 7.933 – 7.894 (*dd*, *J* = 15.6, 1 H); 6.955 – 8.043 (*m*, 8 H). ^{13}C-NMR: 188.92; 163.01; 160.17; 136.99; 131.69; 131.60; 131.26; 131.05; 129.88; 124.50; 116.40; 116.18; 115.52. ESI-MS: 242.256 ($[M + H]^+$).

(2*E*)-3-(3-Fluorophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (**B2**). Yield: 50%. Cream powder. M.p: 114 – 116°. ^{1}H-NMR: 5.932 (*s*, 1 H); 7.698 – 7.659 (*dd*, *J* = 15.6, 1 H);

7.931 – 7.896 (*dd*, *J* = 15.6, 1 H); 6.952 – 8.034 (*m*, 8 H). ^{13}C-NMR: 188.43; 163.35; 161.14; 137.19; 131.33; 131.43; 131.25; 129.62; 124.48; 116.12; 115.87. ESI-MS: 242.243 ([*M* + H]$^{+}$).

(2*E*)-3-(4-Fluorophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (**B3**). Yield: 56%. Pale-yellow powder. M.p: 158 – 160°. ^{1}H-NMR: 6.10 (*s*, 1 H); 7.508 – 7.469 (*dd*, *J* = 15.6, 1 H); 7.814 – 7.775 (*dd*, *J* = 15.6, 1 H); 6.955 – 8.043 (*m*, 8 H). ^{13}C-NMR: 188.67; 162.34; 160.67; 136.64; 131.19; 131.87; 131.75; 128.68; 124.92; 116.72; 115.12. ESI-MS: 242.244 ([*M* + H]$^{+}$).

(2*E*)-1-(4-Hydroxyphenyl)-3-[2-(trifluoromethyl)phenyl]prop-2-en-1-one (**B4**). Yield: 44%. Brown powder. M.p: 173 – 175°. ^{1}H-NMR: 5.693 (*s*, 1 H); 8.154 – 8.114 (*dd*, *J* = 16, 1 H); 7.834 – 7.795 (*dd*, *J* = 15.6, 1 H); 6.952 – 8.028 (*m*, 8 H). ^{13}C-NMR: 188.90; 163.01; 160.17; 136.99; 131.69; 131.26; 131.05; 129.88; 124.50; 116.40; 115.52. ESI-MS: 292.252 ([*M* + H]$^{+}$).

(2*E*)-1-(4-Hydroxyphenyl)-3-[4-(trifluoromethyl)phenyl]prop-2-en-1-one (**B5**). Yield: 62%. Pale-yellow powder. M.p: 161 – 163°. ^{1}H-NMR: 5.827 (*s*, 1 H); 7.847 – 7.808 (*dd*, *J* = 15.6, 1 H); 7.641 – 7.602 (*dd*, *J* = 15.6, 1 H); 6.868 – 8.259 (*m*, 8 H). ^{13}C-NMR: 188.92; 163.71; 161.37; 136.87; 131.39; 131.12; 131.01; 129.18; 124.58; 116.67; 115.63. ESI-MS: 292.252 ([*M* + H]$^{+}$).

4.3. Biochemistry

4.3.1. Determination of inhibitory activities of the chalcones on human MAO-A and B

hMAO isoform activities were determined by a fluorimetric method described and modified previously by using recombinant hMAO-A and hMAO-B (expressed in baculovirus-infected BTI insect cells, *Sigma*, Germany) as enzyme sources and *p*-tyramine (0.05 – 1.00 mM) as common substrate. Specific MAO activities were calculated as 168.22 ± 9.55 pmol mg^{-1} min^{-1} (n = 3) for hMAO-A and 148.25 ± 8.60 pmol mg^{-1} min^{-1} (n = 3) for hMAO-B [20 – 22].

Study medium contained 0.1 ml of sodium phosphate buffer (0.05M, pH 7.4), various concentrations of the synthesized compounds or known inhibitors (moclobemide, selegiline and lazabemide), and recombinant hMAO-A or hMAO-B. This mixture was incubated for 15 min at 37° in microplates, placed in the dark fluorimeter chamber. Reaction was started by adding 200 µM *Amplex Red* reagent, 1 U/ml horseradish peroxidase (HRP), and *p*-tyramine. The production of H_2O_2 catalyzed by MAO isoforms was detected using *Amplex®-Red* reagent, in the presence of HRP to produce the fluorescent product resorufin. Resorufin was quantified at 37° in a multidetection microplate fluorescence reader with excitation at 545 nm, and emission at 590 nm, over a 15 min period, in which the fluorescence increased linearly. The specific fluorescence emission was calculated after subtraction of the background activity, which was determined from wells containing all components except the

hMAO isoforms, which were replaced by a sodium phosphate buffer solution. In our experimental conditions, this background activity was negligible.

Control experiments were carried out by replacing the compound and known inhibitors. The possible capacity of compounds to modify the fluorescence generated in the reaction mixture due to non-enzymatic inhibition was determined by adding these compounds to solutions containing only the *Amplex Red* reagent in a sodium phosphate buffer. The new compounds and reference inhibitors themselves did not react directly with *Amplex® Red* reagent. Newly synthesized compounds did not cause any inhibition on the activity of HRP in the test medium.

4.3.2. Kinetic experiments

Synthesized compounds were dissolved in DMSO, with a maximum concentration of 1% and used in a wide concentration range of 0.01 µM – 10.00 mM. The mode of MAO inhibition was examined using *Lineveaver–Burk* plotting. The slopes of the *Lineweaver–Burk* plots were plotted *vs.* the inhibitor concentration, and the K_i values were determined from the *x*-axis intercept as $-K_i$. Each K_i value is the representative of single determination where the correlation coefficient (R^2) of the replot of the slopes *vs.* the inhibitor concentrations was at least 0.98. *SI* was calculated as K_i(hMAO-A)/K_i(hMAO-B. The protein was determined according to the *Bradford* method [23].

4.3.3. Reversibility experiments

Reversibility of the MAO inhibition with the compounds was determined by dialysis method previously described [24]. Dialysis tubing 16 × 25 mm (*Sigma*, Germany) with a molecular weight cut-off of 12,000 and a sample capacity of 0.5 – 10 ml was used. Adequate amounts of the recombinant enzymes (hMAO-A or B) (0.05 mg/ml) were incubated with a concentration equal to fourfold of the IC_{50} values for the inhibition of hMAO-A and -B, respectively, in potassium phosphate buffer (0.05M, pH 7.4, 5% sucrose containing 1% DMSO) for 15 min at 37°. Another sets were prepared by preincubation of same amount of hMAO-A and B with the reference inhibitors (moclobemide, selegiline and lazabemide) considering their IC_{50} values reported for the inhibition of hMAO-A and -B previously reported. IC_{50} value for the inhibition of hMAO-A with moclobemide was taken as 4 µM and IC_{50} values for the inhibition of hMAO-B with selegiline and lazabemide were taken as 0.079 µM and 0.03 µM, respectively [25, 26]. Enzyme-inhibitor mixtures were subsequently dialyzed at 4° in 80 ml of dialysis buffer (100 mM potassium phosphate, pH 7.4, 5% sucrose). The dialysis buffer was replaced with fresh buffer at 3 h and 7 h after the start of dialysis. At 24 h after dialysis, residual MAO activities were measured. All reactions were carried out in triplicate and the residual enzyme catalytic rates were expressed as mean ± SEM. For comparison, undialyzed mixtures of the MAOs and the inhibitors were kept at 4° over the same time period [27].

4.4. Molecular docking methodology

In the current molecular simulation study, AUTODOCK4.2 software was used to establish a ligand-based computer modeling program for the prediction of binding energy of the selected compounds with hMAO isoforms [28]. Docking protocol done with X-ray crystal structure of hMAO-A (2BXR) and hMAO-B (2BYB) downloaded from Protein Data Bank (www.rcsb.org) [29]. Protein preparation was carried out using Protein Preparation Wizard of Maestro-8.5 (Schrodinger LLC) [30]. Ligands were prepared through PRODRG webserver (http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg) [31]. Grid preparation and the docking parameters are prepared on the basis of reported method [32].

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Table 1. Experimental K_i values corresponding to the inhibition of hMAO isoforms by the chalcones.

Compound	Experimental K_i values [μM][a])		*SI*[b])	Inhibition type	Reversibility	MAO Selectivity
	MAO-A	MAO-B				
B1	4.96 ± 0.23	0.74 ± 0.04	6.70	Competitive	Reversible	B
B2	4.22 ± 0.28	1.03 ± 0.09	4.10	Competitive	Reversible	B
B3	5.55 ± –0.36	0.83 ± 0.04	6.69	Competitive	Reversible	B
B4	3.61 ± 0.19	0.90 ± 0.05	4.01	Competitive	Reversible	B
B5	8.70 ± 0.45	0.33 ± 0.01	26.36	Competitive	Reversible	B
Moclobemide	0.61 ± 0.03	5.50 ± 0.13	0.11	Competitive	Reversible	A
Selegiline	11.20 ± 1.08	0.09 ± 0.04	124.44	Suicide inhibitor	Irreversible	B
Lazabemide	1320.00 ± 302.50	0.006 ± 0.001	220.00	Competitive	Reversible	B

[a]) Values represents the mean ± SEM of three independent experiments. [b]) Selectivity index. It was calculated as K_i(MAO-A)/K_i(MAO-B).

Table 2. Reversibility of hMAO inhibition by the chalcones[a])

Test compound	hMAO-A Activity before dialysis [%]	hMAO-A Activity after dialysis [%]	hMAO-B Activity before dialysis [%]	hMAO-B Activity after dialysis [%]	Reversibility
With no inhibitor	100 ± 0.00	99.74 ± 1.45	100 ± 0.00	100.59 ± 1.64	–
Moclobemide (16.00 μM)	15.00 ± 1.04	98.55 ± 2.88	68.24 ± 3.69	98.21 ± 4.00	Reversible
Selegiline (0.35 μM)	77.90 ± 4.09	96.44 ± 2.99	9.88 ± 4.12	11.77 ± 5.45	Irreversible
Lazabemide (0.15 μM)	80.23 ± 3.87	98.03 ± 4.31	7.85 ± 0.23	89.00 ± 5.20	Reversible
B1 (3.00 μM)	68.65 ± 2.55	90.34 ± 2.38	38.77 ± 1.98	90.26 ± 5.55	Reversible
B2 (4.00 μM)	76.22 ± 3.21	88.45 ± 3.00	57.90 ± 3.59	89.40 ± 4.67	Reversible
B3 (3.50 μM)	80.00 ± 4.02	85.25 ± 3.25	39.29 ± 2.35	87.16 ± 3.90	Reversible
B4 (4.00 μM)	75.90 ± 3.00	90.00 ± 2.21	52.88 ± 3.75	90.21 ± 5.03	Reversible
B5 (1.50 μM)	86.24 ± 3.55	97.00 ± 2.08	24.77 ± 1.16	97.11 ± 2.33	Reversible

[a]) hMAO Isoforms were preincubated with no inhibitor, with the newly synthesized compounds and also with the reference inhibitors for 15 min, and enzyme activities were measured. The mixtures were then dialyzed for 24 h and residual activities were determined. Each value represents the mean ± SEM of three independent.

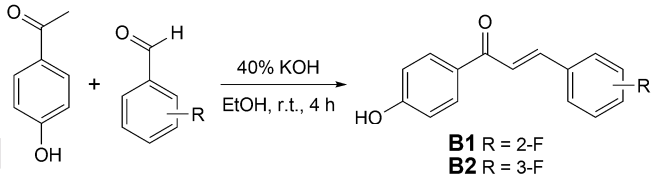
Captions:

Scheme 1. Synthesis of fluorinated hydroxylated chalcones.

Fig. 1. *Lineweaver–Burk* plots of the oxidation of *p*-tyramine by recombinant hMAO-B. The plots were constructed in the absence and presence of various concentration of compound **B5**.

Fig. 2. Replots of the slopes of the *Lineweaver–Burk* plots *vs.* inhibitor **B5** concentration.

Fig. 3. Docking pose of **B5** in the MAO-B active site. Yellow mesh indicates π-π stacking interaction.



B1 R = 2-F
B2 R = 3-F
B3 R = 4-F
B4 R = 2-CF_3
B5 R = 4-CF_3