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Full Paper

Synthesis and Screening of Human Monoamine Oxidase-A Inhibitor Effect of New 2-Pyrazoline and Hydrazone Derivatives

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A group of 3,5-diaryl-2-pyrazoline and hydrazone derivatives was prepared via the reaction of various chalcones with hydrazide compounds in ethanol. Twenty original compounds were synthesized. Ten of these original compounds have a pyrazoline structure, nine of these original compounds have a hydrazone structure, and one of these original compounds has a chalcone structure. Structural elucidation of the compounds was performed by IR, ^{1}H NMR, ^{13}C NMR, mass spectral data, and elemental analyses. These compounds were tested for their inhibitory activities toward the A and B isoforms of human monoamine oxidase (MAO). Except for **3k** and **6c**, all compounds were found to be competitive, reversible, and selective inhibitors for either one of the isoforms (hMAO-A or MAO-B). Compounds **3k** and **6c** were found to be competitive, reversible, but non-selective MAO inhibitors. Compound **6h** showed hMAO-B inhibitory activity whereas the others potently inhibited hMAO-A. Compound **5c** showed higher selectivity than the standard drug moclobemide. According to the experimental K_i values, compounds **6i**, **6d**, and **6a** exhibited the highest inhibitory activity toward hMAO-A. The AutoDock 4.2 program was employed to perform automated molecular docking. The calculated results obtained computationally were in good agreement with the experimental values.

Keywords: 2-Pyrazoline / Hydrazone / MAO inhibitors / Molecular docking

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Introduction

Monoamine oxidase (MAO) is an enzyme present in the outer mitochondrial membrane of neuronal and non-neuronal cells. Two isoforms of MAO exist as MAO-A and MAO-B. MAO enzymes are responsible for the oxidative deamination of endogenous and xenobiotic amines and have a different substrate preference, inhibitor specificity, and tissue distribution [1]. MAO-A preferably metabolizes serotonin, adrenaline, and noradrenaline [2] whereas β-phenylethylamine and benzylamine are predominantly metabolized by MAO-B [3]. Tyramine, dopamine, and some other important amines are common substrates for both the isoenzymes [4].

Although MAOs are widely distributed in various organs, most of the studies concerning their functional properties and involvement in pathological processes have been mainly

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focused on the central nervous system. In the periphery, MAO-A is predominant in the heart, adipose tissue, and skin fibroblasts; MAO-B is the major form found in platelets and lymphocytes, while both isoenzymes are expressed in the kidney and liver [5]. MAO inhibition allows endogenous and exogenous substrates to accumulate and may thereby alter the dynamics of regular monoamine transmitters, such as noradrenaline, serotonin, or dopamine. MAO inhibitors (MAOIs) are mainly used in psychiatry for the treatment of depressive disorders and in neurology for the treatment of Parkinson's and Alzheimer's diseases. Although the classical non-selective and irreversible MAOIs, such as phenelzine and tranylcypromine, are characterized by the risk of inducing a hypertensive crisis when dietary tyramine is ingested, selegiline (L-deprenyl), the selective and irreversible MAO-B inhibitor and moclobemide, the selective and reversible inhibitor of MAO-A, are free from this potential interaction [1].

Chalcones are an important class of natural compounds. Chalcones, or 1,3-diaryl-2-propen-1-ones, belong to the flavonoid family. They are precursors for flavonoid biosynthesis, which plays an ecological role in relation to plant color. Chemically they consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α,β-unsaturated carbonyl system [6, 7]. They are commonly synthesized via the Claisen–Schmidt condensation between acetophenone and benzaldehyde. Chalcones are important intermediates in the synthesis of many pharmaceuticals including 2-pyrazolines and hydrazones [8], and they have important biological activities such as anti-inflammatory [9], antibacterial [10], antiviral [11], antioxidant [12], antineoplastic [13], and MAO inhibitor activities [14, 15].

Of chalcones, 2-pyrazoline derivatives have been previously reported to exhibit various pharmacological activities such as antihypertensive [16], antimicrobial [17–19], anti-inflammatory [20–22], antidepressant [23], and MAO inhibitory activities [24]. Some hydrazone compounds also have MAO inhibitory activities [25, 26]. The necessity of discovering new, selective, and reversible hMAO inhibitors directed us to synthesize novel compounds with more selective and potent MAO inhibitory activity. Thus, in the current study, we synthesized new compounds containing 2-pyrazoline or hydrazone and investigated their hMAO inhibitory potencies.

Results and discussion

Chemistry

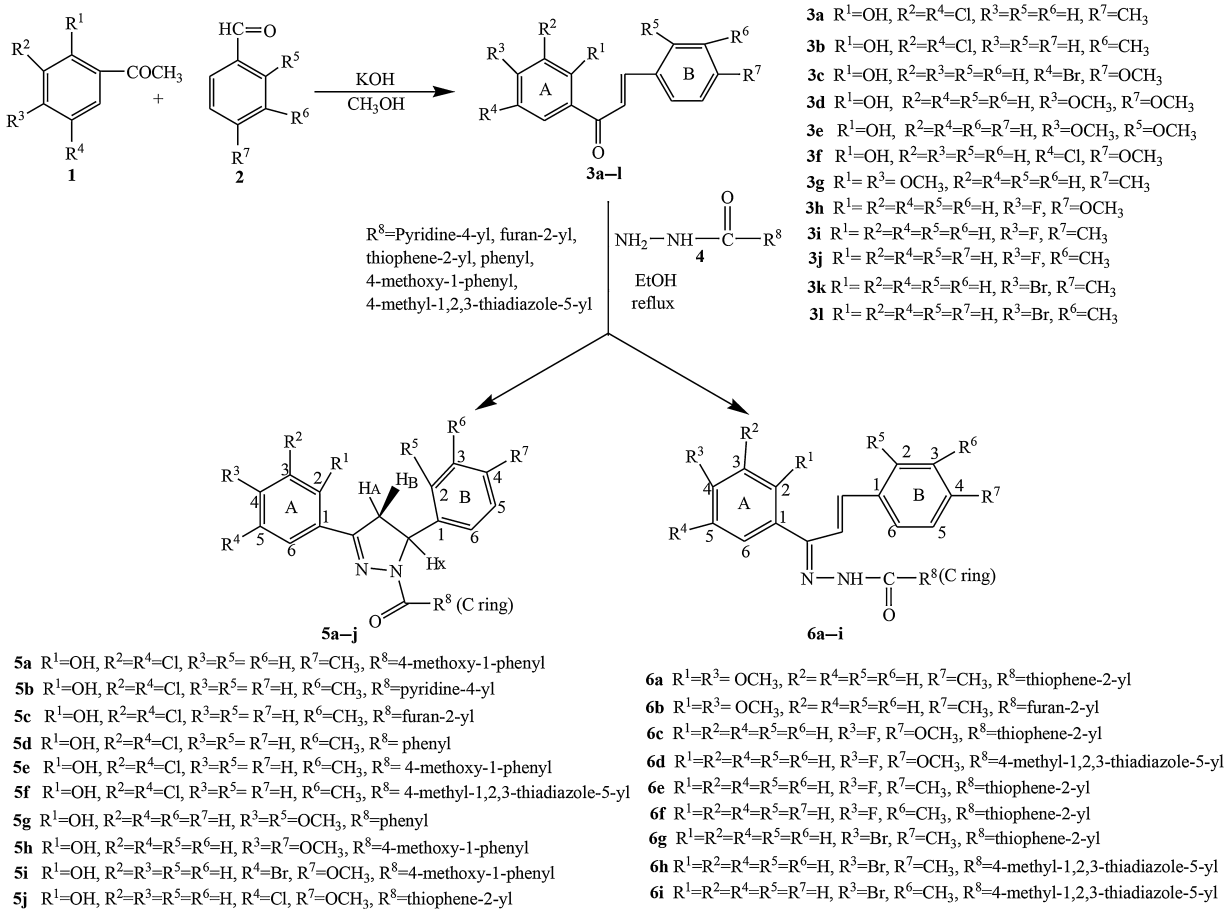
Chalcone derivatives were prepared with the reaction of some acetophenone and benzaldehyde derivatives in KOH/MeOH. The ensuing chalcone derivatives were then reacted with hydrazide compounds to furnish hydrazone and 2-pyrazoline derivatives (Scheme 1). Structures, physicochemical, and spectral characterization of the synthesized compounds are given in the Experimental section.

The structure of synthesized hydrazone and 2-pyrazoline derivatives was elucidated by IR, ^{1}H NMR, ^{13}C NMR, mass spectral data, and elementary analysis findings. The IR data provided evidence for the formation of the expected structures. OH, C=O, and C=N functions absorbed strongly in the expected regions: OH at 3450–3410 cm^{-1}, NH at 3420–3348 cm^{-1}, C=O at 1694–1617 cm^{-1}, and C=N at 1607–1505 cm^{-1}, respectively. In ^{1}H NMR spectrum of compounds **5a–j**, the CH_2 protons of the pyrazoline ring resonated as a pair of doublets of doublets at 2.83–3.02 and 3.32–3.62 ppm. The CH proton appeared as a doublet of doublets at δ 5.18–5.43 ppm. In ^{1}H NMR spectrum of compounds **6a–i**, ethylenic protons were observed at 6.34–7.80 ppm. The protons belonging to the aromatic ring and the other aliphatic groups were observed with the expected chemical shift and integral values. ^{13}C NMR spectra of compounds **3b**, **3l**, **5a**, **5e**, **5i**, **6b**, **6d**, **6g**, and **6i** are given in the Experimental section. Mass analysis of compounds was performed by using ESI (+) and ESI (−) method. The characteristic peaks were observed in the mass spectra of the compounds. Molecular ion peaks ($[M]^+$) provided the molecular formula of all synthesized compounds **5a–j/6a–i**. Characteristic [M+2] isotope peaks were observed in the mass spectra of the compounds having a Cl or a Br atom; [M+4] isotope peaks were observed in the mass spectra of the compounds which have two Cl atoms. All compounds provided satisfactory elemental analysis.

Biological activity

All the newly synthesized compounds were screened for their potential hMAO inhibitory activity using recombinant hMAO-A and hMAO-B. The results of hMAO-A and hMAO-B inhibition studies with title compounds are reported in Table 1 together with their selectivity indexes. It has been previously reported that the natural and synthetic chalcone derivatives exhibit hMAO inhibitory activity [27, 28]. Although chalcones have been shown to inhibit hMAO-B more potently than hMAO-A [14], there are some exceptions such as isoliquiritigenin which inhibits MAO-A more potently than MAO-B [28]. Kinetic studies indicate that chalcones are competitive inhibitors of MAOs [27]. In general, reversibility of the inhibition of MAO by chalcones is controversial. In a study, chalcones have been shown to bind irreversibly to hMAO-B, by a binding mode similar to those of rasagiline and (*R*)-deprenyl, two known potent MAO-B inhibitors [14]. Since the side effects of known MAO inhibitors are mostly associated with the irreversible inhibition particularly of MAO-A, design of potent, selective, and reversible new MAO-A inhibitors is of value.

In our study, newly synthesized chalcones (compounds **3a–l**) inhibited hMAO-A selectively and reversibly in a competitive mode except for compound **3k**. Compound **3k** inhibited both hMAO-A and hMAO-B potently, but with similar K_i values (Table 1) suggesting that this compound is a non-selective hMAO inhibitor. The most potent hMAO-A inhibitor in this series was found to be compound **3b** which carries two



Scheme 1. General synthesis of **5a–j** and **6a–i**.

chloride atoms at the third and fifth positions of A ring and is substituted with a methyl group at the third position of B ring. Compound **3b** inhibited the hMAO-A isoform reversibly and selectively with a K_i value of $0.056 \pm 0.004\,\mu M$. Since chalcones **3a** and **3b**, which have 3,5-dichloro substitution on the A ring inhibited hMAO-A potently, chloride substitution at the third and fifth positions of A ring is suggested to improve the MAO-A inhibitory activity of the chalcone. The additional hydrophobic interaction between the chloride at the substitution third position and PHE352, ILE180, ILE335, and LEU337 residues and the chloride at the substitution fifth position and PHE208, ILE335, and LEU337 residues causes high potency. According to the selectivity index, compound **3f** which has a chloride substitution at the fifth position of A ring and carries a methoxy group at the fourth position of the B ring appeared to be the most selective hMAO-A inhibitor ($SI_{Ki\,hMAO\text{-}A/Ki\,hMAO\text{-}B} = 0.060$) among the synthesized chalcones. Methoxy substitution on B ring seems to enhance the selectivity of the compound toward the hMAO-A isoform. These enhancements can be rationalized based on the observation of molecular docking results such that the chloride substitution at the fifth position of A ring makes two important hydrophobic interactions with TYR197 and TYR407. Also a strong hydrogen bond occurs between the oxygen of the methoxy substitution on B ring and the side chain hydrogen and LYS305. Experimental data concerning the hMAO inhibitory activities of chalcones were found to be in good agreement with the calculated data obtained from the molecular docking studies (Table 2).

Newly synthesized pyrazolines (compounds **5a–j**) were found to inhibit human MAO potently and reversibly in a competitive mode (Table 1). All compounds inhibited hMAO-A selectively with K_i values ranging between 0.005 ± 0.001 and $14.445 \pm 1.087\,\mu M$. Data showed that compounds **5a, 5e**, and **5g** exhibited high activity toward hMAO-A with K_i values of 0.005 ± 0.001, 0.010 ± 0.001, and $0.013 \pm 0.001\,\mu M$, respectively. Among them, compound **5a** which carries two chloride atoms at the third and fifth positions of A ring and bears a 4-methoxy-1-phenyl as C ring showed the best activity for hMAO-A. It is striking that compound **5a** inhibited hMAO-A more potently than moclobemide, a known potent MAO-A

Table 1. Experimentally determined K_i values of the newly synthesized hydrazone and 2-pyrazoline derivatives for the inhibition of hMAO isoforms A and B[a].

Compounds	K_i(MAO-A) [μM][b]	K_i(MAO-B) [μM][b]	SI[c]	Inhibition type	Reversibility	Selectivity
3a	0.060 ± 0.004	0.320 ± 0.013	0.188	Competitive	Reversible	MAO-A
3b	0.056 ± 0.004	0.328 ± 0.019	0.233	Competetive	Reversible	MAO-A
3c	0.070 ± 0.005	0.560 ± 0.033	0.125	Competetive	Reversible	MAO-A
3d	0.390 ± 0.025	1.960 ± 0.134	0.199	Competetive	Reversible	MAO-A
3e	0.175 ± 0.010	1.115 ± 0.090	0.157	Competetive	Reversible	MAO-A
3f	0.078 ± 0.008	1.280 ± 0.099	0.060	Competitive	Reversible	MAO-A
3g	0.488 ± 0.027	0.621 ± 0.038	0.785	Competetive	Reversible	MAO-A
3h	0.755 ± 0.058	2.154 ± 0.194	0.351	Competetive	Reversible	MAO-A
3i	0.748 ± 0.055	2.005 ± 0.152	0.373	Competetive	Reversible	MAO-A
3j	0.628 ± 0.042	1.302 ± 0.105	0.482	Competetive	Reversible	MAO-A
3k	0.387 ± 0.030	0.389 ± 0.035	0.995	Competetive	Reversible	Non-selective
3l	0.201 ± 0.018	0.278 ± 0.020	0.723	Competetive	Reversible	MAO-A
5a	0.005 ± 0.001	0.100 ± 0.009	0.050	Competetive	Reversible	MAO-A
5b	0.088 ± 0.005	1.300 ± 0.097	0.068	Competetive	Reversible	MAO-A
5c	0.123 ± 0.012	2200.00 ± 90.365	5.591×10^{-5}	Competetive	Reversible	MAO-A
5d	0.120 ± 0.009	0.290 ± 0.016	0.414	Competetive	Reversible	MAO-A
5e	0.010 ± 0.001	0.097 ± 0.002	0.103	Competetive	Reversible	MAO-A
5f	0.655 ± 0.039	1.020 ± 0.098	0.642	Competetive	Reversible	MAO-A
5g	0.013 ± 0.001	0.102 ± 0.009	0.127	Competetive	Reversible	MAO-A
5h	12.250 ± 1.060	36.880 ± 2.450	0.332	Competetive	Reversible	MAO-A
5i	14.445 ± 1.087	857.000 ± 29.700	0.016	Competetive	Reversible	MAO-A
5j	0.377 ± 0.020	1.752 ± 0.110	0.215	Competetive	Reversible	MAO-A
6a	0.010 ± 0.001	0.103 ± 0.009	0.097	Competetive	Reversible	MAO-A
6b	0.078 ± 0.005	0.102 ± 0.009	0.764	Competetive	Reversible	MAO-A
6c	0.032 ± 0.002	0.349 ± 0.021	0.082	Competetive	Reversible	Non-selective
6d	0.008 ± 0.001	0.103 ± 0.010	0.077	Competetive	Reversible	MAO-A
6e	0.016 ± 0.002	0.159 ± 0.011	0.100	Competetive	Reversible	MAO-A
6f	0.035 ± 0.002	0.099 ± 0.006	0.354	Competetive	Reversible	MAO-A
6g	0.075 ± 0.004	0.108 ± 0.009	0.416	Competetive	Reversible	MAO-A
6h	0.874 ± 0.051	0.501 ± 0.033	1.745	Competetive	Reversible	MAO-B
6i	$0.008 \pm 0.10 \times 10^{-3}$	0.500 ± 0.026	0.016	Competetive	Reversible	MAO-A
Selegiline	17.112 ± 1.334	0.301 ± 0.020	56.850	Competetive	Irreversible	MAO-B
Moclobemide	0.012 ± 0.001	1.466 ± 0.07	0.008	Competetive	Reversible	MAO-A

[a] Racemic compounds were used for the experiments. Compounds **3a–l** are chalcones, compounds **5a–j** are pyrazoline derivatives, and compounds **6a–i** are hydrazone derivatives.
[b] Each value represents the mean ± SEM of three independent experiments.
[c] The selectivity index (SI) was calculated as K_i(MAO-A)/K_i(MAO-B).

inhibitor (K_i values are 0.005 ± 0.001 and 0.012 ± 0.001 μM, respectively). From an inspection of the chemical structures of these two compounds, it can be concluded that chloride substitution at the third and fifth positions of A ring and a methoxy substitution at the fourth position of C ring improve the MAO-A inhibitory activity of the pyrazolines. These results are also supported by the computational modeling studies such that a methoxy group at the fourth position of C ring with the side chain of TYR197, and a chloride atom at the fifth position of A ring and TYR69 make hydrophobic interactions. Compound **5c** which carries two chloride atoms at the third and fifth positions of A ring and bears a furane ring as a C ring appeared to be the most selective hMAO-A inhibitor of this series with a SI of 5.60 ± 10^{-5} (Table 1). Since the replacement of C ring with furane in the pyrazoline nucleus enhanced the selectivity of pyrazolines for hMAO-A (Tables 1 and 2) and docking studies also endorse this finding (Fig. 1), it is suggested that the furane ring improves the selectivity of pyrazolines toward hMAO-A. Experimental data concerning the hMAO inhibitory activities of novel pyrazolines were found to be in good agreement with the calculated data obtained from the molecular docking studies (Table 2).

Newly synthesized hydrazones (compounds **6a–i**) inhibited hMAO-A or -B selectively and reversibly in a competitive mode except for compound **6c**. Compound **6h** inhibited hMAO-B selectively, but not as potently as selegiline. Compound **6c** inhibited both hMAO-A and -B potently, but with similar K_i values (Table 1) suggesting that this compound is a non-selective hMAO inhibitor. Among hydrazones, compound **6i** which carries a bromide at the fourth position of A ring, and

Table 2. Calculated K_i values of the newly synthesized hydrazone and 2-pyrazoline derivatives for the inhibition of hMAO isoforms A and B.

Compounds[a)]	Calculated ΔG for MAO-A (kcal/mol)	K_i(MAO-A) [μM]	Calculated ΔG for MAO-B (kcal/mol)	K_i(MAO-B) [μM]	SI[b)]	Selectivity
3a	−9.99	0.048	−8.98	0.261	0.184	MAO-A
3b	−10.36	0.026	−9.03	0.241	0.108	MAO-A
3c	−9.81	0.064	−8.58	0.508	0.126	MAO-A
3d	−9.02	0.245	−7.84	1.800	0.136	MAO-A
3e	−9.27	0.161	−8.17	1.030	0.156	MAO-A
3f	−9.55	0.100	−8.15	1.070	0.093	MAO-A
3g	−8.82	0.345	−8.54	0.549	0.628	MAO-A
3h	−8.41	0.686	−7.75	2.070	0.341	MAO-A
3i	−8.48	0.609	−7.97	1.430	0.426	MAO-A
3j	−8.58	0.511	−8.05	1.260	0.406	MAO-A
3k	−8.97	0.268	−8.88	0.307	0.873	MAO-A
3l	−9.24	0.169	−9.00	0.251	0.673	MAO-A
5a (R)	−11.72	0.003	−9.28	0.157	0.019	MAO-A
5a (S)	11.20	0.006	−6.50	0.033	0.182	MAO-A
5b (R)	−9.76	0.070	−8.05	1.260	0.055	MAO-A
5b (S)	−8.57	0.520	−6.50	17.180	0.030	MAO-A
5c (R)	−9.47	0.115	−9.40	0.130	0.885	MAO-A
5c (S)	−8.16	1.050	−2.00	34300.000	3.060×10^{-5}	MAO-A
5d (R)	−10.09	0.040	−8.99	0.257	0.155	MAO-A
5d (S)	−8.78	0.364	−5.33	0.124	2.940	MAO-B
5e (R)	−12.22	0.001	−10.58	0.017	0.059	MAO-A
5e (S)	10.94	0.010	−9.76	0.070	0.143	MAO-A
5f (R)	−9.68	0.080	−8.06	1.230	0.065	MAO-A
5f (S)	−8.31	0.812	−8.20	0.968	0.839	MAO-A
5g (R)	−11.94	0.002	−10.20	0.033	0.060	MAO-A
5g (S)	−11.17	0.006	−9.99	0.048	0.125	MAO-A
5h (R)	−7.75	2.080	−7.03	7.000	0.298	MAO-A
5h (S)	−6.52	16.61	−1.83	45390.000	3.66×10^{-4}	MAO-A
5i (R)	−6.54	16.15	−7.46	3.41	4.740	MAO-B
5i (S)	−6.48	17.70	−3.13	5050.000	0.004	MAO-A
5j (R)	−8.83	0.337	−7.77	2.030	0.166	MAO-A
5j (S)	−8.80	0.353	−4.79	308.140	0.001	MAO-A
6a	−11.10	0.007	−9.58	0.095	0.074	MAO-A
6b	−9.93	0.053	−9.57	0.096	0.552	MAO-A
6c	−10.41	0.023	−9.12	0.207	0.111	MAO-A
6d	−11.19	0.006	−9.65	0.084	0.071	MAO-A
6e	−10.77	0.013	−9.33	0.145	0.090	MAO-A
6f	−10.80	0.012	−9.88	0.057	0.211	MAO-A
6g	−10.02	0.045	−9.60	0.092	0.489	MAO-A
6h	−10.10	0.040	−10.34	0.026	1.540	MAO-B
6i	−11.20	0.006	−10.72	0.014	0.043	MAO-A

[a)] Compounds **3a–l** are chalcones, compounds **5a–j** are pyrazoline derivatives, and compounds **6a–i** are hydrazone derivatives.
[b)] The selectivity index (SI) was calculated as K_i(MAO-A)/K_i(MAO-B).

bears a 4-methyl-1,2,3-thiadiazole-5-yl ring as C ring was found to be the most potent and selective hMAO-A inhibitor. Compound **6i** inhibited hMAO-A more potently than moclobemide, a known potent MAO-A inhibitor (K_i values are $0.008 \pm 0.10 \times 10^{-3}$ and 0.012 ± 0.001 μM, respectively). It is suggested that 4-methyl-1,2,3-thiadiazole-5-yl ring increases the inhibitory potency and selectivity of hydrazones toward hMAO-A since docking studies showed that this moiety interacts with the FAD co-factor located in the active site cavity of hMAO-A (Fig. 2). Experimental data concerning the hMAO inhibitory activities of novel hydrazones were found to be in good agreement with the calculated data obtained from the molecular docking studies (Table 2).

Palaska et al. [29] synthesized a series of 1-thiocarbamoyl-3,5-diphenyl-4,5-dihydro-1*H*-pyrazole derivatives, screened their MAO inhibitory activities and suggested that chloride

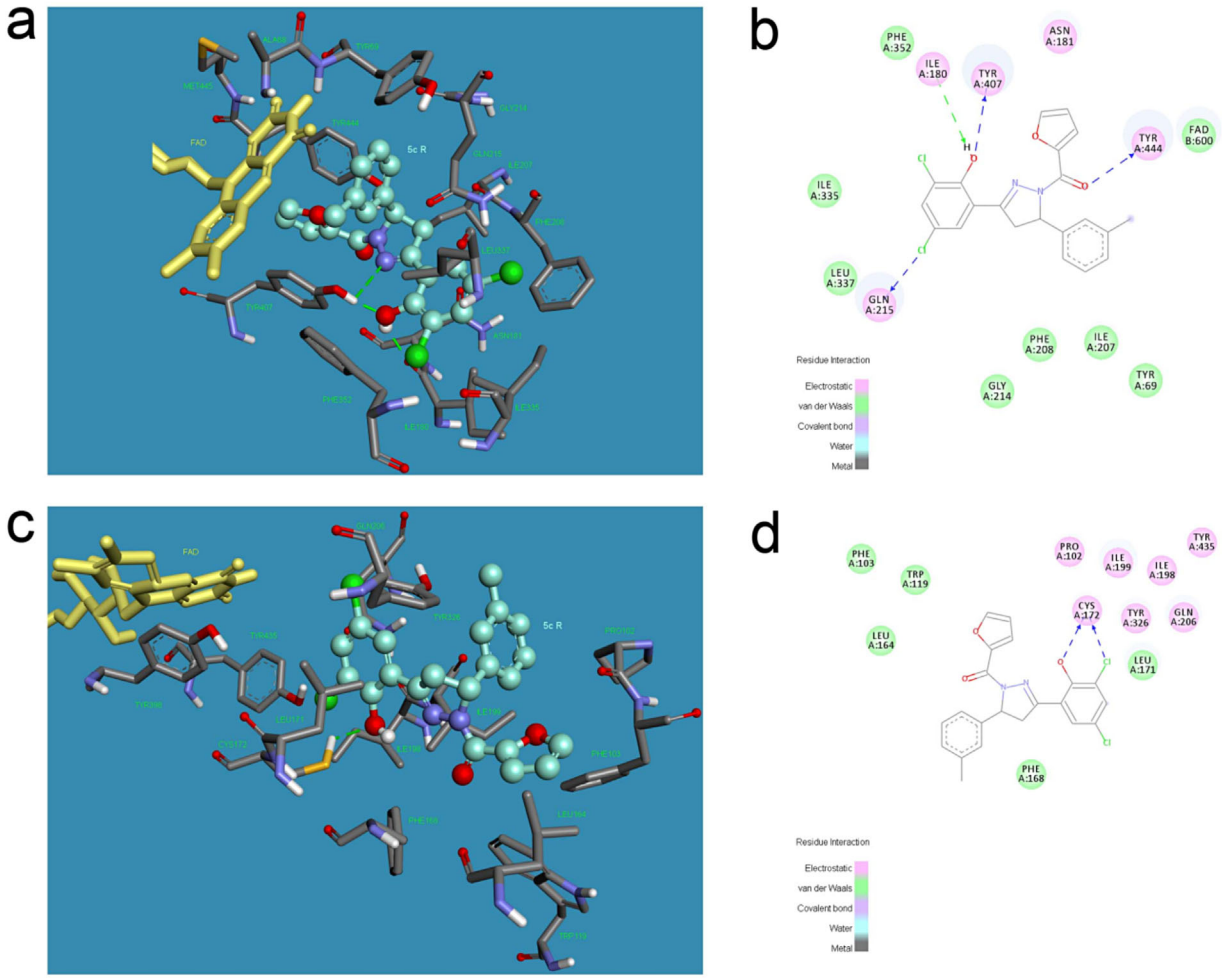


Figure 1. Three-dimensional and two-dimensional orientations of **5c** (*R*) in the active site of hMAO-A (a and b) and hMAO-B (c and d). Amino acid side chains are shown as sticks, the inhibitor is shown as a ball and stick (green), and the co-factor FAD is depicted as a yellow stick. In 2D pictures, purple color shows electrostatic and green color shows van der Waals attractions.

substitution on the phenyl ring located at the third position of 2-pyrazoline increased the inhibitory potency toward MAO-A. They also indicated that replacement of chloride with bromide significantly decreased the inhibitory potency. Our data showing that the MAO-A inhibitory potency of compound **5i** in our series which is having a bromide atom at R4 position significantly reduced when compared with those of having a chloride atom at the same position of the molecule is in good agreement with that of Palaska group. Sahoo et al. [30] suggested that substitution on the nitrogen atom at the first position of pyrazoline ring increases the MAO-A inhibitory potency. Likewise, almost all of our new compounds having a substitution on the same position of pyrazoline moiety inhibited hMAO-A selectively and potently.

All tested compounds inhibited the hMAO reversibly. Table 3 presents the reversibility of hMAO-A inhibition with the compounds **3f, 5c**, and **6i** which possessed the most selective and potent inhibitory activities toward the hMAO-A isoform. Reversibility of hMAO-A inhibition with the compounds was compared with that of moclobemide, and the reversibility of hMAO-A inhibition with compound **5c** was found to be better than that of moclobemide.

Modeling studies

In order to see the binding pose of these types of compounds in detail, two representative ligands **5c** and **6i** were chosen.

To render visible the detailed interactions of the docked poses of the inhibitor **5c** and **6i** were chosen. In order to generate pictures, Accelrys Visualization 4.5 program was used. Analysis of the optimal binding mode for **5c** (Fig. 1a and b) in the hMAO-A active site cavity revealed that the furane ring of the compound is inserted between TYR444 and TRY407 from the *re* face of the FAD co-factor. **5c**

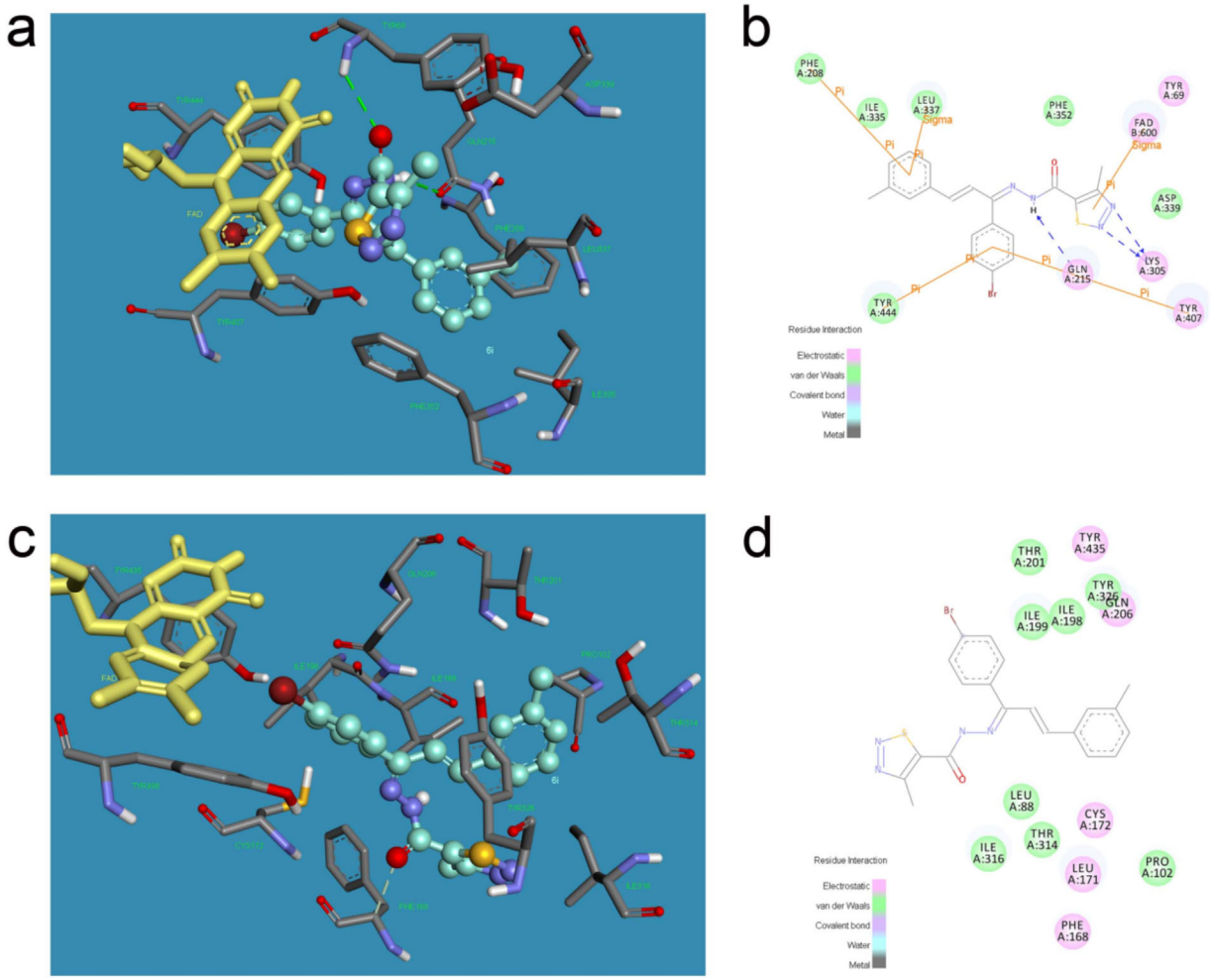


Figure 2. Three-dimensional and two-dimensional orientations of **6i** in the active site of hMAO-A (a and b, respectively) and hMAO-B (c and d, respectively). Amino acid side chains are shown as sticks, the inhibitor is shown as a ball and stick (green), and the co-factor FAD is depicted as a yellow stick. In 2D pictures, purple color shows electrostatic and green color shows van der Waals attractions.

makes various electrostatic and van der Waals interactions with active site residues lining the cavity. A hydrogen bond occurs between the carbonyl group of the inhibitor and the hydroxy moiety of TYR444. The second hydrogen bond forms between the hydroxyl moiety of the **5c** and TYR407 side chain hydroxyl group. The other strong electrostatic interactions occur between GLN215 and the chloride atom on the phenyl ring of **5c** and the hydroxyl group of the **5c** and backbone carbonyl of ILE180. The binding mode adopted by **5c** fits snugly within a cavity lined with hydrophobic amino acid residues. This hydrophobic pocket includes PHE352, ILE180, ILE335, LEU337, GLY214, PHE208, and ILE207 amino acids. Figure 1c and d shows the binding pose (3D and 2D) of **5c** in the active site of the MAO-B isoform. **5c** occupies a space far from the hydrophobic cage, surrounded by TYR398, TYR435, and FAD co-factor, near the entrance cavity. The major interaction is the hydrogen bond that forms between the hydroxyl group of **5c** and the side chain residue of CYS172. The other hydrophobic amino acid residues are PHE168, LEU171, TYR326, ILE198, PHE103, GLN206, ILE198, ILE199, PRO102, TRP119, and LEU164 amino acids.

Table 3. Reversibility of hMAO inhibition with the compounds 3f, 5c, 6i, and moclobemide.

Compounds	hMAO-A inhibition (%)	
	Before washing	After washing
Moclobemide (0.30 μM)	90.00 ± 5.02	8.22 ± 0.50
Compound **3f** (0.30 μM)	81.80 ± 0.45	12.00 ± 1.10
Compound **5c** (0.30 μM)	89.95 ± 4.55	8.14 ± 0.61
Compound **6i** (0.30 μM)	83.26 ± 4.11	10.96 ± 0.95

Each value represents the mean ± SEM of three experiments.

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Figure 2(a and b) shows the 3D and 2D pictures of **6i** in the active site of the hMAO-A isoform. The bromophenyl ring was inserted between the TYR444 and TYR407 residues, which constitute the hydrophobic cage of the active site, making one π–π interaction with TYR407 and TYR444. The methylphenyl ring of **6i** makes two more π–π interactions, one with PHE208 and the other with LEU337. The 4-methyl-1,2,3-thiadiazole-5-yl ring also makes one π–σ with FAD and two electrostatic interactions with LYS305. Tyr69, Phe352, Ile335, Gln215, and ASP339 are the other active site residues involved in hydrophobic and polar interactions with the inhibitor. Figure 2c and d shows the binding pattern of **6i** to hMAO-B. The inhibitor is located near the hydrophobic cage surrounded by Tyr398, Tyr435, and FAD. Ile171, ILE316, LEU88, THR314, CYS172, PRO102, THR201, ILE198, ILE199, GLN201, TYR326, and THR201 are the other active site residues interacting with the inhibitor. The potency of the both **5c** and **6i** molecules on hMAO-A compared to hMAO-B can be evaluated from the above data, indicating that **5c** is stabilized by various π–π interactions in hMAO-A compared to hMAO-B. All of these data may suggest why the hMAO-A inhibitory potency of **5c** ($K_i = 0.115\ \mu M$) is better than that for hMAO-B (0.13 μM). Inhibitor **6i** also more effectively binds to the hMAO-A isoform than to the MAO-B isoform [Selectivity Index (SI) $= K_i$(hMAO-B)/K_i(hMAO-A), **5c** $= 1.13$ and **6i** $= 2.33$].

Compounds **6h** and **6i** are positional isomers in which a methyl group is at different positions (**6h** has it on R7 while **6i** has it on R6). This single variation could be able to make the molecules behave differently (Table 1); **6h** is selective toward MAO-B while **6i** is selective toward MAO-A. Compound **6h** has a methyl group at the *p*-position of the phenyl ring, which makes favorable hydrophobic interactions with Lys305, Phe353 residues as well as three more interactions with the FAD of MAO-A. These interactions force the phenyl ring to go into the hydrophobic pocket exactly between the Phe352 and Tyr69 residues. In the same manner, in MAO-B, the methyl group interacts with Phe343, Tyr60 residues, and co-factor FAD. On the other hand, when the methyl group is in *m*-position of compound **6i**, a completely different binding pose is observed. In the MAO-A binding site, **6i** is flipped, forcing the methyl group to interact with Phe208, Ile335, and Leu337. In MAO-B *m*-methyl group makes another interaction with Leu88 and Tyr326 located in the entrance cavity hindering interaction with the FAD co-factor in the hydrophobic pocket. That might be the reason for **6h** to be selective MAO-B and **6i** selective MAO-A inhibitors. Details of the interactions of the compounds **6h** and **6i** are given in the Supporting Information.

Conclusion

Chalcones are intermediates for synthesizing various heterocyclic compounds which have been reported to possess various biological activities [6–15]. 2-Pyrazoline and hydrazone derivatives which have been synthesized by starting from chalcone derivatives have been previously reported to exhibit various pharmacological activities including MAO inhibitory activities [16–26]. The necessity of discovering new, selective, and reversible hMAO inhibitors directed us to synthesize novel chalcone derivatives as more selective, potent, and reversible MAO inhibitors. Thus, in the present study, we synthesized new compounds containing 2-pyrazoline or hydrazone and investigated their hMAO inhibitory potencies.

Conventional methods were used for the synthesis of chalcones, pyrazolines, and hydrazones in our study. These methods have been previously reported by different researches and shown to give satisfactory results [31, 32]. MAO activities were determined by a fluorimetric method previously reported using recombinant hMAO-A and -B as enzyme sources [33–35]. We used *p*-tyramine as a substrate in the activity measurements as it is a common substrate for both MAO-A and -B which caused no interference in our experimental set. The possible capacity of the new compounds to quench the fluorescence generated in the reaction mixture was determined by adding these compounds to solutions containing only the Amplex Red reagent or resorufin in a sodium phosphate buffer [36]. The new compounds and reference inhibitors themselves did not react directly with Amplex® Red reagent or did not effect the fluorescence generated in the reaction mixture. Newly synthesized compounds did not cause any inhibition on the activity of HRP in the test medium.

AutoDock 4.2 docking program which was the most used program among the community was used to investigate the molecular mechanisms of the interaction of newly synthesized compounds with hMAO. AutoDock 4.2 has semiempirical scoring function, which was calibrated against experimentally dependable inhibition values and it was shown to produce the least error and artifacts comparing the other programs [37].

The newly synthesized compounds were found to be mostly competitive, reversible, and selective inhibitors of hMAO-A. 2-Pyrazoline compounds showed higher selectivity than hydrazones. The data indicated that 2-hydroxy-3,5-dichloro phenyl and 4-methoxy-1-phenyl rings designed as A and C rings, respectively, increased the inhibitory potency for hMAO-A in the series of **5a–j**. Compounds **5a** and **5e** in this series exhibited higher inhibition potency for hMAO-A than moclobemide. hMAO-A inhibitory potency of compound **5a** was found to be higher than that of compound **5e** suggesting that methyl substitution on the R^6 position of B ring decreases the inhibition potency and selectivity for hMAO-A.

Replacement of A ring by 2-hydroxy-3,5-dichlorophenyl ring and addition of pyridine, phenyl, or 4-methyl-1,2,3-thiadiazole-5-yl rings to the scaffold as a C ring decreased the potency of inhibition. Addition of 2-furyl to the scaffold as the C ring decreased the potency, but increased the selectivity. Among the hydrazones, compounds **6a**, **6d**, and **6i** appeared to be the most potent compounds. These derivatives showed higher inhibitory potency but lower selectivity for hMAO-A than moclobemide. Data indicated that a 4-methyl-1,2,3-thiadiazole-5-yl ring as the C ring is essential

for inhibitory potency and selectivity for hMAO-A in the hydrazone series. Replacement of the thiophene-2-yl or furan-2-yl ring by 4-methyl-1,2,3-thiadiazole-5-yl ring increased the potency. When A ring has a 2,4-dimethoxy scaffold, replacement of 2-furyl by the thiophene-2-yl increased the potency and selectivity. Replacement of the fluorine by a bromine atom on the third position of A ring decreased the potency and the selectivity of inhibition.

Biological experiments were carried out using only racemic mixtures of compounds **5a–j**. The calculated K_i values (Table 2), at least for one of the enantiomers, agreed with the experimentally determined values (Table 1). Overall, the docking studies provided valuable data for the rationalization of the experimental results, and allowed us to estimate the binding mode, the inhibition constant, and the free energy of binding, suggesting that molecular docking is one of the promising tools for use in the discovery of novel, potent, and selective MAO inhibitors potentially useful as pharmacological agents.

Experimental

Chemistry

Melting points were determined with an Electrothermal 9100 type melting point apparatus (Engineering Ltd., Essex, UK), and are uncorrected. All instrumental analyses were performed at the Central Laboratory of the Faculty of Pharmaceuticals of Ankara University, Ankara, Turkey. IR spectra were recorded on a Jasco FT/IR-420 spectrometer (Jasco, Easton, MD, USA). ^{1}H NMR and ^{13}C NMR spectra were measured with a Varian Mercury 400 MHz FT-NMR spectrometer (Varian Inc., Palo Alto, CA, USA) in DMSO-d_6. All chemical shifts were reported as δ (in ppm) values. Mass spectra were recorded on a Waters Micromass ZQ instrument (Waters Corporation, Milford, MA, USA). Elementary analyses were performed on a Leco CHNS 932 analyzer (Leco, St. Joseph, MI, USA), and satisfactory results ±0.4% of calculated values (C, H, N) were obtained. Reactions were followed and purity of compounds was assessed by thin-layer chromatography (TLC) on silica gel-protected aluminum sheets (Type 60 GF254; Merck, Darmstadt, Germany), and spots were detected by exposure to a UV lamp at 254 nm for a few seconds. The chemical reagents used in syntheses were purchased from Merck and Aldrich (Milwaukee, MI, USA).

*General procedure for the preparation of the chalcone derivatives **3a–l***

To a stirred solution of KOH (50%) in water (5 mL) cooled to 0°C in an ice bath was added dropwise a solution of acetophenone (4.99 mmol) and aldehyde (6.018 mmol) in ethanol (20 mL). The reaction mixture was stirred at room temperature through night. Then the mixture was poured into ice, adjusted to pH 3–4 with 1N HCl, and then filtered. The resultant was crystallized from ethanol [38].

*(E)-1-(3,5-Dichloro-2-hydroxyphenyl)-3-p-tolylprop-2-en-1-one (**3a**)*

Orange powder (ethyl alcohol). Yield: 57.5%, m.p. 138°C [lit. 115°C] [39]. $C_{16}H_{12}Cl_2O_2$.

*(E)-1-(3,5-Dichloro-2-hydroxyphenyl)-3-m-tolylprop-2-en-1-one (**3b**)*

Yellow-orange powder (ethyl alcohol). Yield: 73.80%, m.p. 103°C. IR (KBr): $\nu = 3430$ (OH), 1711 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 2.42$ (s, 3H, –CH_3), 7.26–7.50 (4H, aromatic-H), 7.52 (d, 1H, $J = 15.2$ Hz, α-ethylenic-H), 7.58 (d, 1H, $J = 2$ Hz, 4′-H), 7.82 (d, 1H, $J = 2$ Hz, 6′-H), 7.96 (d, 1H, $J = 15.2$ Hz, β-ethylenic-H), 13.4 (s, 1H, –OH). ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 20.80$ (–CH_3), 120.57, 122.03, 122.54, 122.83, 127.09, 128.82, 128.85, 129.83, 132.19, 134.09, 135.17, 138.26, 147.09, 156.63, 192.86 (C=O). MS, ESI: $m/z = 307$ $[M+H]^+$ (100%), 309 $[M+H^++2]^+$ (66%), 311 $[M+H^++4]^+$ (12%). $C_{16}H_{12}Cl_2O_2$: C 62.20, H 3.93; calcd. C 62.56, H 3.94.

*(E)-1-(5-Bromo-2-hydroxyphenyl)-3-(4-methoxyphenyl)-prop-2-en-1-one (**3c**)*

Orange powder (ethyl alcohol). Yield: 90.75%, m.p. 113°C [lit. 104°C] [40]. $C_{16}H_{13}BrO_3$.

*(E)-1-(2-Hydroxy-4-methoxyphenyl)-3-(4-methoxyphenyl)-prop-2-en-1-one (**3d**)*

Yellow powder (ethyl alcohol). Yield: 76%, m.p. 90.1°C [lit. 106–108°C] [41]. $C_{17}H_{16}O_4$.

*(E)-1-(2-Hydroxy-4-methoxyphenyl)-3-(2-methoxyphenyl)-prop-2-en-1-one (**3e**)*

Yellow powder (ethyl alcohol). Yield: 94.63%, m.p. 95.40°C [lit. 92°C] [42]. $C_{17}H_{16}O_4$.

*(E)-1-(5-Chloro-2-hydroxyphenyl)-3-(4-methoxyphenyl)-prop-2-en-1-one (**3f**)*

Yellow powder (ethyl alcohol). Yield: 84.20%, m.p. 106.50°C [lit.104°C] [43]. $C_{16}H_{13}ClO_3$.

*(E)-1-(2,4-Dimethoxyphenyl)-3-p-tolylprop-2-en-1-one (**3g**)*

Yellow powder (ethyl alcohol). Yield: 55.60%, m.p. 118°C [lit. 117°C] [44]. $C_{18}H_{18}O_3$.

*(E)-1-(4-Fluorophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**3h**)*

White crystals (ethyl alcohol). Yield: 97.70%, m.p. 107.2°C [lit. 98°C] [45]. $C_{16}H_{13}FO_2$.

*(E)-1-(4-Fluorophenyl)-3-p-tolylprop-2-en-1-one (**3i**)*

White crystals (ethyl alcohol). Yield: 98.00%, m.p. 142.4°C [lit. 135–137°C] [46]. $C_{16}H_{13}FO$.

*(E)-1-(4-Fluorophenyl)-3-m-tolylprop-2-en-1-one (**3j**)*

White crystals (ethyl alcohol). Yield: 88.10%, m.p. 86°C. ^{1}H NMR δ ppm ($CDCl_3$, 400 MHz): $\delta = 2.41$ (s, 3H, –CH_3),

7.16–7.34 (m, 4H, 2,4,5,6-H), 7.44–7.51 (3H, 3′,5′-H; $J = 15.6$ Hz, α-ethylenic-H), 7.80 (d, 1H, $J = 16$ Hz, β-ethylenic-H), 8.06 (t, 2H, 2′,6′-H). MS (ESI): $m/z = 241$ $[M+H]^+$ (100%). $C_{16}H_{13}FO$.

*(E)-1-(4-Bromophenyl)-3-p-tolylprop-2-en-1-one (**3k**)*
Yellow powder (ethyl alcohol). Yield: 94.70%, m.p. 167.6°C [lit. 163–164] [47]. $C_{16}H_{13}FO$.

*(E)-1-(4-Bromophenyl)-3-m-tolylprop-2-en-1-one (**3l**)*
Cream powder (ethyl alcohol). Yield: 97.90%, m.p. 129.5°C. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 2.40$ (s, 3H, $-CH_3$), 7.23–7.48 (5H, 2,4,5,6-H; $J = 15.6$ Hz, α-ethylenic-H), 7.64 (d, 2H, A_2X_2, 3′,5′-H), 7.79 (d, 1H, $J = 16$ Hz, β-ethylenic-H), 7.90 (d, 2H, A_2X_2, 2′,6′-H). ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 20.84$ ($-CH_3$), 121.46, 126.44, 127.28, 128.80, 129.21, 130.54, 131.49, 131.83, 134.46, 136.53, 138.17, 144.68, 188.28 (C=O). MS (ESI): $m/z = 301$ $[M+H]^+$ (97%), 303 $[M+H+2]^+$ (100%). $C_{16}H_{13}BrO$.

*General procedure for the preparation of compounds **5a–j** and **6a–i***
A mixture of chalcone (1.42 mmol) and hydrazide derivatives (1.42 mmol) was refluxed in ethanol (20 mL) for 36–80 h. The precipitate was filtered and recrystallized from ethanol.

*(3-(3,5-Dichloro-2-hydroxyphenyl)-5-p-tolyl-4,5-dihydropyrazole-1-yl)(4-methoxyphenyl)methanone (**5a**)*
Yellow powder (ethyl alcohol). Yield: 67%, m.p. 238.9°C. IR (KBr): $\nu = 3440$ (OH), 1636 (amide C=O), 1600 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 2.34$ (s, 3H, $-CH_3$), 2.98 (dd, 1H, $J_1 = 11.6$ Hz, $J_2 = 17.2$ Hz, H_A), 3.55 (dd, 1H, $J_1 = 2.8$ Hz, $J_2 = 16.8$ Hz, H_B), 3.82(s, 3H, $-OCH_3$), 5.37 (dd, 1H, $J_1 = 2.8$ Hz, $J_2 = 11.6$ Hz, H_X), 7.03 (d, 2H, $J = 8.8$ Hz, A_2X_2, C ring H^3, H^5), 7.27 (d, 2H, $J = 7.6$ Hz, A_2X_2, B ring H^2, H^6), 7.45 (d, 2H, B ring H^3, H^5), 7.69 (s, 1H, A ring H^4), 7.87 (d, 2H, $J = 8.4$ Hz, A_2X_2, C ring H^2, H^6), 7.93 (s, 1H, A ring H^6), 10.92 (s, 1H, –OH). ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 20.68$ ($-CH_3$), 31.24, 55.36, 77.16, 113.41, 122.45, 122.78, 123.28, 125.07, 125.58, 126.36, 129.01, 130.13, 135.88, 137.85, 151.13, 161.95. MS (ESI): $m/z = 455$ $[M+H]^+$ (20%), 477 $[M+Na]^+$ (100%), 479 $[M+Na+2]^+$ (66%), 481 $[M+Na+4]^+$ (12%). $C_{24}H_{20}Cl_2N_2O_3 \cdot 0.25\ H_2O$: C 62.79, H 4.25, N 6.33; calcd. C 62.81, H 4.47.

*(3-(3,5-Dichloro-2-hydroxyphenyl)-5-m-tolyl-4,5-dihydropyrazole-1-yl)(pyridine-4-yl)methanone (**5b**)*
White powder (ethyl alcohol). Yield: 38.6%, m.p. 219.1°C. IR (KBr): $\nu = 3446$ (OH), 1652 (amide C=O), 1558 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 2.35$ (s, 3H, $-CH_3$), 2.96 (dd, 1H, $J_1 = 12.4$ Hz, $J_2 = 16.8$ Hz, H_A), 3.57 (dd, 1H, $J_1 = 2.8$ Hz, $J_2 = 17.2$ Hz, H_B), 5.37 (dd, 1H, $J_1 = 2.8$ Hz, $J_2 = 12$ Hz, H_X), 7.22–7.96 (8H, aromatic-H), 8.75 (d, 2H, pyridine H^2, H^6), 11.24 (s, 1H, –OH). MS (ESI): $m/z = 426$ $[M+H]^+$ (100%), 428 $[M+H+2]^+$ (66%), 430 $[M+H+4]^+$ (12%). $C_{22}H_{17}Cl_2N_3O_2 \cdot 0.25\ H_2O$: C 61.53,H 4.22, N 9.90; calcd. C 61.47, H 4.07, N 9.78.

*(3-(3,5-Dichloro-2-hydroxyphenyl)-5-m-tolyl-4,5-dihydropyrazole-1-yl)(furane-2-yl)methanone (**5c**)*
White powder (ethyl alcohol). Yield: 36.74%, m.p. 235.2°C. IR (KBr): $\nu = 3420$ (OH), 1646 (amide C=O), 1558 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 2.36$ (s, 3H, $-CH_3$), 2.95 (dd, 1H, $J_1 = 12.8$ Hz, $J_2 = 16.8$ Hz, H_A), 3.53 (dd, 1H, H_B), 5.37 (dd, 1H, $J_1 = 2.8$ Hz, $J_2 = 12$ Hz, H_X), 6.71–7.95 (9H, aromatic-H), 10.97 (s, 1H, –OH). MS (ESI): $m/z = 415$ $[M+H]^+$ (100%), 417 $[M+H+2]^+$ (66%), 419 $[M+H+4]^+$ (12%). $C_{21}H_{16}Cl_2N_2O_3$: C 60.70, H 3.63, N 6.98; calcd. C 60.74, H 3.88, N 6.75.

*(3-(3,5-Dichloro-2-hydroxyphenyl)-5-m-tolyl-4,5-dihydropyrazole-1-yl)(phenyl)methanone (**5d**)*
Yellow powder (ethyl alcohol). Yield: 26%. M.p. 250.2°C. IR (KBr): $\nu = 3424$ (OH), 1642 (amide C=O), 1574 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 2.33$ (s, 3H, $-CH_3$), 2.93 (dd, 1H, $J_1 = 11.6$ Hz, $J_2 = 16.8$ Hz, H_A), 3.55 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 17.2$ Hz, H_B), 5.34 (dd, 1H, $J_1 = 2.8$ Hz, $J_2 = 12.4$ Hz, H_X), 7.19–7.94 (11H, aromatic-H), 11.00 (s, 1H, OH). MS (ESI): $m/z = 447$ $[M+Na]^+$ (100%), 449 $[M+Na+2]$ (66%), 451 $[M+Na+4]$ (12%). $C_{23}H_{18}Cl_2N_2O_2 \cdot 0.25\ H_2O$: C 64.34, H 4.37, N 6.78; calcd. C 64.41, H 4.32, N 6.53.

*(3-(3,5-Dichloro-2-hydroxyphenyl)-5-m-tolyl-4,5-dihydropyrazole-1-yl)(4-methoxyphenyl)methanone (**5e**)*
Yellow powder (ethyl alcohol). Yield: 37.95%, m.p. 220°C. IR (KBr): $\nu = 3420$ (OH), 1637 (amide C=O), 1567 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 2.36$ (s, 3H, $-CH_3$), 2.95 (dd, 1H, $J_1 = 12$ Hz, $J_2 = 16.8$ Hz, H_A), 3.57 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 17.2$ Hz, H_B), 3.82 (s, 3H, $-OCH_3$), 5.36 (dd, 1H, $J_1 = 2.8$ Hz, $J_2 = 12.4$ Hz, H_X), 7.03 (d, 2H, $J = 8.8$ Hz, A_2X_2, C ring H^3, H^5), 7.23 (s, 1H, A ring H^4) 7.34–7.39 (3H, B ring-H), 7.70 (s, 1H, $J = 2.4$ Hz, A ring H^6), 7.87 (d, 2H, $J = 8.8$ Hz, A_2X_2, C ring H^2, H^6), 7.90 (s, 1H, B ring H), 10.91 (s, 1H, –OH). ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 20.99$ ($-CH_3$), 31.48, 55.35, 77.37, 113.39, 122.46, 122.78, 123.26, 123.52, 125.09, 125.57, 126.91, 128.41, 129.07, 130.13, 137.68, 138.83, 151.16, 161.94. MS (ESI): $m/z = 455$ $[M+H]^+$ (30%), 477 $[M+Na]^+$ (100%), 479 $[M+Na+2]^+$ (66%), 481 $[M+Na+4]^+$ (12%). $C_{24}H_{20}Cl_2N_2O_3$: C, 63.20, H 4.47, N 6.41; calcd. C 63.31, H4.43, N 6.15.

*(3-(3,5-Dichloro-2-hydroxyphenyl)-5-m-tolyl-4,5-dihydropyrazole-1-yl)(4-methyl-1,2,3-thiadiazole-5-yl)-methanone (**5f**)*
Yellow powder (ethyl alcohol). Yield: 45.10%, m.p. 250°C. IR (KBr): $\nu = 3410$ (OH), 1652 (amide C=O), 1576 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 2.36$ (s, 3H, $-CH_3$), 2.95–3.02 (4H, C ring CH_3 and H_A), 3.62 (dd, 1H, $J_1 =$ 3.2 Hz, $J_2 = 17.2$ Hz, H_B), 5.42 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 11.6$ Hz, H_X), 7.22–7.36 (4H, B ring-H), 7.77 (d, 2H, A ring-H), 11.78 (s, 1H, –OH). MS (ESI): $m/z = 445$ $[M-H]^+$ (99%), 447 $[M-H+2]^+$ (66%), 449 $[M-H+4]$ (12%). $C_{20}H_{16}Cl_2N_4O_2S$: C 53.51, H 3.38, N 12.63, S 7.21; calcd. C 53.70, H 3.61, N 12.52, S 7.17.

*(3-(2-Hydroxy-4-methoxyphenyl)-5-(2-methoxyphenyl)-4,5-dihydropyrazole-1-yl)(phenyl)methanone (**5g**)*
White powder (ethyl alcohol). Yield: 18%, m.p. 220°C. IR (KBr): $\nu = 3450$ (OH), 1663 (amide C=O), 1599 (C=N) cm^{-1}. ^{1}H NMR

(DMSO-d_6, 400 MH): $\delta = 2.83$ (dd, 1H, H_A), 3.32 (dd, 1H, H_B), 3.78 (d, 3H, –OCH_3), 3.82 (s, 3H, –OCH_3), 5.43 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 12.8$ Hz, H_X), 6.56–7.98 (12H, aromatic-H), 10.81 (s, 1H, –OH). MS (ESI): $m/z = 403$ [M+H]$^+$ (100%). $C_{24}H_{22}N_2O_4$: C 71.28, H 5.31, N 7.14; calcd. C 71.63, H 5.51, N 6.96.

*(3-(2-Hydroxy-4-methoxyphenyl)-5-(4-methoxyphenyl)-4,5-dihydropyrazole-1-yl)(4-methoxyphenyl)methanone (**5h**)*
White powder (ethyl alcohol). Yield: 21%, m.p. 207.6°C. IR (KBr): $\nu = 3439$ (OH), 1630 (amide C=O), 1604 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 2.85$ (dd, 1H, $J_1 = 12$ Hz, $J_2 = 16.8$ Hz, H_A), 3.32 (dd, 1H, H_B), 3.77 (s, 6H, A and B ring –OCH_3), 3.81 (s, 3H, C ring –OCH_3), 5.18 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 12.4$ Hz, H_X), 6.55–7.93 (11H, aromatic-H), 10.66 (s, 1H, –OH). MS (ESI): $m/z = 433$ [M+H]$^+$ (100%), 455 [M+Na]$^+$ (100%). $C_{25}H_{24}N_2O_5 \cdot 0.25\ H_2O$: C 68.41, H 5.76, N 6.38; calcd. C 68.73, H 5.61, N 6.41.

*(3-(5-Bromo-2-hydroxyphenyl)-5-(4-methoxyphenyl)-4,5-dihydropyrazole-1-yl)(4-methoxyphenyl)methanone (**5i**)*
White powder (ethyl alcohol). Yield: 24.19%, m.p. 258.6°C. IR (KBr): $\nu = 3410$ (OH), 1626 (amide C=O), 1515 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 2.92$ (dd, 1H, H_A), 3.46 (dd, 1H, $J_1 = 2.8$ Hz, $J_2 = 17.2$ Hz, H_B), 3.78 (s, 3H, B ring –OCH_3), 3.82 (s, 3H, C ring –OCH_3), 5.22 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 12$ Hz, H_X), 6.97–7.03 (m, 5H, A ring H^3, B ring H^3 and H^5, C ring H^3 and H^5), 7.49 (d, 2H, B ring H^2 and H^6, s, 1H, A ring H^4), 7.86 (d, 2H, C ring H^2 and H^6), 8.11 (s, 1H, A ring H^6), 10.83 (s, 1H, –OH). ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 31.75$, 37.28, 55.12, 55.32, 76.44, 112.96, 113.36, 113.75, 120.05, 122.29, 125.65, 126.48, 128.00, 129.99, 131.28, 133.48, 155.99, 159.28, 161.85. MS (ESI): $m/z = 481$ [M+H]$^+$ (20%), 483 [M+H+2]$^+$ (30%), 503 [M+Na]$^+$ (95%), 505 [M+Na+2]$^+$ (100%). $C_{24}H_{21}BrN_2O_4 \cdot 0.5\ H_2O$: C 58.71, H 4.18, N 5.96; calcd. C 58.89, H 4.49, N 5.72.

*(3-(5-Chloro-2-hydroxyphenyl)-5-(4-methoxyphenyl)-4,5-dihydropyrazole-1-yl)(thiophene-2-yl)methanone (**5j**)*
Yellow powder (ethyl alcohol). Yield: 74.74%, m.p. 279.8°C. IR (KBr): $\nu = 3445$ (OH), 1617 (amide C=O), 1558 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 2.92$ (dd, 1H, $J_1 = 12.4$ Hz, $J_2 = 17.2$ Hz, H_A), 3.62 (dd, 1H, H_B), 3.80 (s, 3H, –OCH_3), 5.26 (dd, 1H, H_X), 7.00 (d, 1H, A ring H^3), 7.06 (d, 2H, B ring H^3 and H^5), 7.24 (s, 1H, thiophene H^4), 7.40 (dd, 1H, $J_1 = 2.8$ Hz, $J_2 = 8.4$ Hz, A ring H^4), 7.47 (d, 2H, B ring H^2 and H^6), 8.04 (s, 3H, A ring H^6, thiophene H^{3}and H^5), 11.20 (s, 1H, –OH). MS (ESI): $m/z = 413$ [M+H]$^+$ (20%), 435 [M+Na]$^+$ (100%), 437 [M+Na+2] (33%). $C_{21}H_{17}ClN_2O_3S$: C 61.10, H 4.12, N 6.86, S 7.74; calcd. C 61.09, H 4.15, N 6.78, S 7.76.

*N′-(1-(2,4-Dimethoxyphenyl)-3-p-tolylallylidene)-thiophene-2-carbohydrazide (**6a**)*
Yellow powder (ethyl alcohol). Yield: 23.00%, m.p. 178.2. IR (KBr): $\nu = 3420$ (NH), 1631 (amide C=O), 1505 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 2.30$ (s, 3H, –CH_3), 3.77 (s, 3H, –OCH_3), 3.87 (s, 3H, –OCH_3), 6.36 (d, 1H, $J = 16$ Hz, α-ethylenic-H), 6.73 (d, 1H, A ring H^5), 6.78 (s, 1H, A ring H^3), 7.10–7.43 (7H, B ring H^3 and H^5, B ring H^2 and H^6, A ring H^6, β-ethylenic-H, C ring-H), 7.93 (s, 2H, C ring –H), 9.51 (s, 1H, –NH). MS (ESI): $m/z = 407$ [M+H]$^+$ (100%). $C_{23}H_{22}N_2O_3S \cdot 0.25\ H_2O$: C 67.12, H 5.38, N 7.03, S 7.74; calcd. C 67.24, H 5.48, N 6.82, S 7.79.

*N′-(1-(2,4-Dimethoxyphenyl)-3-p-tolylallylidene)furane-2-carbohydrazide (**6b**)*
White powder (ethyl alcohol). Yield: 51.85%, m.p. 188.5. IR (KBr): $\nu = 3348$ (NH), 1694 (amide C=O), 1607 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 2.28$ (s, 3H, –CH_3), 3.77 (s, 3H, –OCH_3), 3.87 (s, 3H, –OCH_3), 6.34 (d, 1H, $J = 16$ Hz, α-ethylenic-H), 6.65 (s, 1H, A ring H^3), 6.76 (d, 1H, A ring H^5), 6.82 (1H, C ring –H), 7.14–7.22 (5H, B ring H^3 and H^5, β-ethylenic-H, A ring H^6, C ring –H), 7.41 (d, 2H, B ring H^2 and H^6), 7.83 (1H, C ring –H), 9.25 (s, 1H, –NH). ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 20.83$, 55.43, 55.80, 99.33, 106.37, 109.51, 110.52, 112.33, 127.02, 127.85, 130.71, 133.05, 136.27, 138.29, 145.99, 157.30, 162.07. MS (ESI): $m/z = 391$ [M+H]$^+$ (30%), 413 [M+Na]$^+$ (100%). $C_{23}H_{22}N_2O_4$: C 70.74, H 5.27, N 7.21; calcd. C 70.75, H 5.68, N 7.17.

*N′-(1-(4-Fluorophenyl)-3-(4-methoxyphenyl)allylidene)-thiophene-2-carbohydrazide (**6c**)*
Yellow powder (ethyl alcohol). Yield: 21%, m.p. 197.2. IR (KBr): $\nu = 1633$ (amide C=O), 1602 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 3.80$ (s, 3H, –OCH_3), 6.76 (d, 1H, $J = 16$ Hz, α-ethylenic-H), 6.98 (d, 2H, B ring H^3 and H^5), 7.18 (broad t, 1H, C ring –H), 7.34 (t, 2H, A ring H^3 and H^5), 7.69 (t, 5H, A ring H^2 and H^6, B ring H^2 and H^6, β-ethylenic-H), 7.88 (d, 1H, C ring –H), 8.04 (d, 1H, C ring –H), 11.60 (s, 1H, –NH). MS (ESI): $m/z = 381$ [M+H]$^+$ (100%). $C_{21}H_{17}FN_2O_2S$: C 66.42, H 4.43, N 7.59, S 8.28; calcd. C 66.30, H 4.50, N 7.36, S 8.43.

*N′-(1-(4-Fluorophenyl)-3-(4-methoxyphenyl)allylidene)-4-methyl-1,2,3-thiadiazole-5-carbohydrazide (**6d**)*
Yellow powder (ethyl alcohol). Yield: 52.31%, m.p. 206.4. IR (KBr): $\nu = 1677$ (amide C=O), 1597 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 2.99$ (s, 3H, –CH_3), 3.81 (s, 3H, –OCH_3), 6.81 (d, 1H, $J = 16$ Hz, α-ethylenic-H), 7.00 (d, 2H, B ring H^3 and H^5), 7.41 (t, 2H, A ring H^3 and H^5), 7.62–7.72 (m, 5H, B ring H^2 and H^6, A ring H^2 and H^6, β-ethylenic-H), 12.25 (s, 1H, –NH). ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 15.13$, 55.24, 114.14, 115.31, 115.53, 115.71, 128.07, 129.79, 131.67, 131.76, 132.19, 132.23, 135.14, 140.97, 150.64, 160.60, 161.13, 161.51, 163.06, 163.95. MS (ESI): $m/z = 395$ [M−H]$^+$ (100%). $C_{20}H_{17}FN_4O_2S \cdot 0.25\ H_2O$: C 60.09, H 4.31, N 14.16, S 8.14; calcd. C 59.93, H 4.37, N 13.98,S 7.99.

*N′-(1-(4-Fluorophenyl)-3-p-tolylallylidene)thiophene-2-carbohydrazide (**6e**)*
Yellow powder (ethyl alcohol). Yield: 28.81%, m.p. 198.3. IR (KBr): $\nu = 1652$ (amide C=O), 1558 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 3.32$ (s, 3H, –CH_3), 6.78 (d, 1H, $J = 16$ Hz, α-ethylenic-H), 7.18 (t, 1H, C ring –H), 7.23 (d, 2H,

B ring H^3 and H^5), 7.34 (t, 2H, A ring H^3 and H^5), 7.62–7.69 (m, 5H, B ring H^2 and H^6, β-ethylenic-H, A ring H^2 and H^6), 7.89 (d, 1H, C ring –H), 8.04 (d, 1H, C ring –H), 11.6 (s, 1H, –NH). MS (ESI): $m/z = 365$ $[M+H]^+$ (100%). $C_{21}H_{17}FN_2OS$: C 68.97, H 4.74, N 7.80, S 8.73; calcd. C 69.21, H 4.70, N 7.69.

*N′-(1-(4-Fluorophenyl)-3-m-tolylallylidene)thiophene-2-carbohydrazide (**6f**)*
Yellow powder (ethyl alcohol). Yield: 33.17%, m.p. 176.2. IR (KBr): $\nu = 1630$ (amide C=O), 1558 (C=N) cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): 2.34 (s, 3H, $–CH_3$), 6.78 (d, 1H, $J = 16$ Hz, α-ethylenic-H), 7.19–7.8 (m, 10H, aromatic and β-ethylenic-H), 7.90 (dd, 1H, C ring –H), 8.05 (dd, 1H, C ring –H), 11.69 (s, 1H, –NH). MS (ESI): $m/z = 364$ $[M+H]^+$ (50%), 387 $[M+Na]^+$ (100%). $C_{21}H_{17}FN_2OS$: C 69.12, H 4.58, N 7.76, S 8.80; calcd. C 69.21, H 4.70, N 7.69, S 8.80.

*N′-(1-(4-Bromophenyl)-3-p-tolylallylidene)thiophene-2-carbohydrazide (**6g**)*
Yellow powder (ethyl alcohol). Yield: 32.75%, m.p. 207.6. IR (KBr): $\nu = 1628$ (amide C=O), 1575 (C=N) cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): 2.33 (s, 3H, $–CH_3$), 6.79 (d, 1H, $J = 16$ Hz, α-ethylenic-H), 7.18 (t, 1H, C ring –H), 7.23 (d, 2H, B ring H), 7.56–7.71 (7H, B ring –H, β-ethylenic-H, A ring –H), 7.88 (d, 1H, C ring –H), 8.03 (d, 1H, C ring –H), 11.70 (s, 1H, –NH). ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 20.92$, 116.77, 122.48, 127.95, 129.23, 130.11, 131.32, 132.96, 135.99, 139.22, 139.77. MS (ESI): $m/z = 447$ $[M+Na]^+$ (100%), 449 $[M+Na+2]^+$ (97%). $C_{21}H_{17}BrN_2OS$: C 59.11, H 3.70, N 6.73, S 7.59; calcd. C 59.30, H 4.03, N 6.59, S 7.54.

*N′-(1-(4-Bromophenyl)-3-p-tolylallylidene)-4-methyl-1,2,3-thiadiazole-5-carbohydrazide (**6h**)*
Yellow powder (ethyl alcohol). Yield: 19.90%, m.p. 209.4. IR (KBr): $\nu = 1675$ (amide C=O), 1575 (C=N) cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): 2.33(s, 3H, B ring $–CH_3$), 2.99 (s, 3H, C ring $–CH_3$), 6.84 (d, 1H, $J = 16$ Hz, α-ethylenic-H), 7.25 (d, 2H, $J = 8.4$ Hz, B ring H^3 and H^5, A_2X_2), 7.57–7.79 (m, 7H, $J = 8.4$ Hz, B ring H^2 and H^6, A_2X_2, $J = 20$ Hz, β-ethylenic-H, A ring –H), 12.25 (s, 1H, –NH). MS (ESI): $m/z = 441$ $[M+H]^+$ (50%), 443 $[M+H+2]$ (45%), 463 $[M+Na]^+$ (90%), 465 $[M+Na+2]^+$ (100%). $C_{20}H_{17}BrN_4OS$: C 54.83, H 3.96, N 12.43, S 7.19; calcd. C 54.43, H 3.88, N 12.69, S 7.26.

*N′-(1-(4-Bromophenyl)-3-m-tolylallylidene)-4-methyl-1,2,3-thiadiazole-5-carbohydrazide (**6i**)*
Yellow powder (ethyl alcohol). Yield: 21.30%, m.p. 177.6. IR (KBr): $\nu = 1640$ (amide C=O), 1558 (C=N) cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): 2.34 (s, 3H, B ring $–CH_3$), 2.99 (s, 3H, C ring $–CH_3$), 6.83 (d, 1H, $J = 16$ Hz, α-ethylenic-H), 7.21–7.79 (9H, aromatic-H, β-ethylenic-H), 12.33 (s, 1H, –NH). ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 15.21$, 20.92, 117.69, 123.09, 125.54, 128.54, 128.61, 130.50, 131.57, 131.63, 134.98, 135.13, 135.38, 137.91, 141.39, 150.26, 161.34, 163.23. MS (ESI): $m/z = 439$ $[M-H]^+$ (100%), 441 $[M–H+2]^+$ (97%). $C_{20}H_{17}BrN_4OS$: C 54.22, H 3.88, N 12.65, S 7.27; calcd. C 54.43, H 3.88, N 12.69, S 7.26.

Biochemistry

Chemicals

hMAO-A and hMAO-B (both recombinant, expressed in baculovirus-infected BTI insect cells), *R*-(−)-deprenyl hydrochloride (selegiline), moclobemide, resorufin, dimethyl sulfoxide (DMSO), and other chemicals were purchased from Sigma–Aldrich (Munich, Germany). The Amplex®-Red MAO assay kit (Cell Technology Inc., Mountain View, CA, USA) contained benzylamine, *p*-tyramine, clorgyline (MAO-A inhibitor), pargyline (MAO-B inhibitor), and horseradish peroxidase.

Determination of hMAO-A and -B activities

The activities of hMAO-A and hMAO-B were determined using *p*-tyramine as common substrate and calculated as 161.50 ± 8.33 pmol/mg/min ($n = 3$) and 140.00 ± 7.55 pmol/mg/min ($n = 3$), respectively. The interactions of the synthesized compounds with hMAO isoforms were determined by a fluorimetric method described and modified previously [33–35]. The production of H_2O_2 catalyzed by MAO isoforms was detected using Amplex®-Red reagent, a non-fluorescent probe that reacts with H_2O_2 in the presence of horseradish peroxidase to produce the fluorescent product resorufin. The reaction was started by adding 200 μM Amplex Red reagent, 1 U/mL horseradish peroxidase, and *p*-tyramine (concentration range 0.1–1mM). Control experiments were carried out by replacing the compound and reference 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.

Kinetic experiments

Synthesized compounds were dissolved in dimethyl sulfoxide, with a maximum concentration of 1% and used in the concentration range of 1–100 μM. Kinetic data for interaction of the enzyme isoforms with the compounds were determined using the Microsoft Excel package program. The slopes of the Lineweaver–Burk plots were plotted versus 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 versus the inhibitor concentrations was at least 0.98. SI (K_i(MAO-A)/K_i(MAO-B)) was also calculated. The protein was determined according to the Bradford method [48].

Reversibility experiments

Reversibility of the MAO inhibition with synthesized compounds was evaluated by a centrifugation–ultrafiltration method [49]. In brief, adequate amounts of the recombinant hMAO-A or -B were incubated together with a single

concentration of the synthesized compounds or the reference inhibitors in a sodium phosphate buffer (0.05 M, pH 7.4) for 1 h at 37°C. After this incubation period, an aliquot was stored at 4°C and used for the measurement of MAO-A and -B activity. The remaining incubated sample was placed in an Ultrafree-0.5 centrifugal tube with a 30 kDa Biomax membrane and centrifuged at 9000*g* for 20 min at 4°C. The enzyme retained in the 30 kDa membrane was resuspended in a sodium phosphate buffer at 4°C and centrifuged again twice. After the third centrifugation, the enzyme retained in the membrane was resuspended in sodium phosphate buffer (300 mL) and an aliquot of this suspension was used for MAO-A and -B activity determination. Control experiments were performed simultaneously (to determine the 100% MAO activity) by replacing the test compounds with appropriate dilutions of the vehicles. The corresponding values of percent (%) MAO isoform inhibition were separately calculated for samples with and without repeated washing.

Molecular docking

Molecular models of the inhibitors were built and optimized using SPARTAN 10.0 at PM3 level on iMac 3.4 GHz Intel Core i7 processor. The crystal structure of hMAO-A complexed with the reversible inhibitor harmine (coded 2Z5X, with a resolution of 2.20 Å) [50] and the crystal structure of hMAO-B co-crystallized with the reversible inhibitor safinamide (coded 2V5Z, with a resolution of 1.60 Å) were extracted from the Protein Data Bank (PDB) (http://www.rcsb.org) [51] for protein setup. Studies were carried out on only one subunit of the enzymes. Each structure was cleaned of all water molecules and inhibitors as well as all non-interacting ions were removed before being used in the docking studies. For each protein, all hydrogens were added and minimized using "Clean Geometry" toolkit, then for more complete optimization final geometry was submitted to "Prepare Macromolecule" protocol of Discovery Studio (Accelrys) assigning CHARMM force field. Missing hydrogen atoms were added based on the protonation state of the titratable residues at a pH of 7.4. Ionic strength was set to 0.145 and the dielectric constant was set to 10. Autodock Tool (ADT) interface was used for the preparation of input files of macromolecules and ligands for docking calculations. Docking was achieved using AutoDock 4.2.6 coding scripts [52] using HPxw8600_Work_Station. The detailed docking procedures are given elsewhere [53, 54]. Accelrys Visualization 4.0 program was used for rendering the 2D and 3D pictures.

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References

[1] M. Yamada, H. Yasuhara, *Neurotoxicology* **2004**, *25*, 215–221.
[2] J. P. Johston, *Biochem. Pharmacol.* **1968**, *17*, 1285–1297.
[3] J. Knoll, K. Magyar, *Adv. Biochem. Psychopharmacol.* **1972**, *5*, 393–408.
[4] A. M. O'Carroll, C. J. Fowler, J. P. Phillips, I. Tobbia, K. F. Tipton, *Naunyn Schmiedebergs Arch. Pharmacol.* **1983**, *322*, 198–202.
[5] J. C. Shih, K. Chen, M. J. Ridd, *Annu. Rev. Neurosci.* **1999**, *22*, 197–217.
[6] B. A. Bohm, in *The Flavonoids* (Eds.: J. B. Harborne, T. J. Mabry, H. Mabry), Chapman and Hall, London **1975**, pp. 442–504.
[7] Z. Nowakowska, *Eur. J. Med. Chem.* **2007**, *42*, 125–137.
[8] E. Perozo-Rondón, R. M. Martín-Aranda, B. Casal, C. J. Durán-Valle, W. N. Lau, X. F. Zhang, K. L. Yeung, *Catal. Today* **2006**, *114*, 183–187.
[9] Y. Xia, Z. Y. Yang, P. Xia, K. F. Bastow, Y. Nakanishi, K. H. Lee, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 699–701.
[10] S. F. Nielsen, A. Kharazmi, S. B. Christensen, *Bioorg. Med. Chem.* **1998**, *6*, 937–945.
[11] V. Opletalova, J. Hartl, K. Palat, Jr., A. Patel, *J. Pharm. Biomed. Anal.* **2000**, *23*, 55–59.
[12] L. Mathıesen, K. E. Malterud, R. B. Sund, *Free Radical. Biol. Med.* **1997**, *22*, 307–311.
[13] S. Ducki, R. Forrest, J. A. Hadfield, A. Kendall, N. J. Lawrence, A. T. Mcgown, D. Rennison, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1051–1056.
[14] F. Chimenti, R. Fioravanti, A. Bolasco, P. Chimenti, D. Secci, F. Rossi, M. Yanez, F. Orallo, F. Ortuso, S. Alcaro, *J. Med. Chem.* **2009**, *52*, 2818–2824.
[15] B. Mathew, J. Suresh, S. Anbazghagan, J. Paulra, G. K. Krishnan, *Biomed. Prev. Nutr.* **2014**, *4*, 451–458.
[16] G. T. Zitouni, P. Chevallet, F. S. Kiliç, K. Erol, *Eur. J. Med. Chem.* **2000**, *35*, 635–641.
[17] M. S. Karthikeyan, B. S. Holla, N. S. Kumari, *Eur. J. Med. Chem.* **2007**, *42*, 30–36.
[18] G. T. Zitouni, A. Özdemir, K. Güven, *Arch. Pharm.* **2005**, *338*, 96–104.
[19] G. T. Zitouni, A. Özdemir, Z. A. Kaplancikli, P. Chevallet, Y. Tunali, *Phosphorus Sulfur Silicon Relat. Elem.* **2005**, *180*, 2717–2724.
[20] F. F. Barsoum, H. M. Hosnib, A. S. Girgis, *Bioorg. Med. Chem.* **2006**, *14*, 3929–3937.
[21] P. Rani, V. K. Srivastava, A. Kumar, *Eur. J. Med. Chem.* **2004**, *39*, 449–452.
[22] N. Gökhan-Kelekçi, S. Yabanoglu, E. Küpeli, U. Salgın, Ö. Özgen, G. Uçar, E. Yeşilada, E. Kendi, A. Yeşilada, A. Bilgin, *Bioorg. Med. Chem.* **2007**, *15*, 5775.
[23] E. Palaska, D. Erol, R. Demirdamar, *Eur. J. Med. Chem.* **1996**, *31*, 43–47.
[24] N. Soni, K. Pande, R. Kalsi, T. K. Gupta, S. S. Parmer, J. P. Barthwal, *Res. Commun. Chem. Pathol. Pharmacol.* **1987**, *56*, 129–132.

[25] F. Chimenti, D. Secci, A. Bolasco, P. Chimenti, A. Granese, S. Carradori, E. Maccioni, M. C. Cardia, M. Yáñez, F. Orallo, S. Alcaro, F. Ortuso, R. Cirilli, R. Ferretti, S. Distinto, J. Kirchmair, T. Langer, *Bioorg. Med Chem*, **2010**, *18*, 5063–5070.
[26] S. Distinto, M. Yáñez, S. Alcaro, M. C. Cardia, M. Gaspari, M. Sanna Luisa, R. Meleddu, F. Ortuso, J. Kirchmair, P. Markt, A. Bolasco, G. Wolber, D. Secci, E. Maccioni, *Eur. J. Med. Chem.* **2012**, *48*, 284–295.
[27] H. Haraguchi, Y. Tanaka, A. Kabbash, T. Fujioka, T. Ishizu, A. Yagi, *Phytochemistry* **2004**, *65*, 2255–2260.
[28] X. Pan, L. D. Kong, Y. Zhang, C. H. Cheng, R. X. Tan, *Acta Pharmacol. Sinica* **2000**, *21*, 949–953.
[29] E. Palaska, F. Aydın, G. Ucar, D. Erol, *Arch Pharm.* **2008**, *341*, 209–215.
[30] A. Sahoo, S. Yabanoglu, B. N. Sinha, G. Ucar, A. Basu, V. Jayaprakash, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 132–136.
[31] A. A. Pimenov, N. V. Makarova, I. K. Moiseev, M. N. Zemtsova, *Chem. Heterocycl. Comp.* **2004**, *40*, 575–581.
[32] Y. Li, Y. Luo, W. Huang, J. Wang, W. Lu, *Tetrahedron Lett.* **2006**, *47*, 4153–4155.
[33] F. Chimenti, E. Maccioni, D. Secci, A. Bolasco, P. Chimenti, A. Granese, S. Carradori, S. Alcaro, F. Ortuso, M. Yanez, F. Orallo, R. Cirilli, R. Ferretti, F. La Torre, *J. Med. Chem.* **2008**, *51*, 4874–4880.
[34] M. C. Anderson, F. Hasan, J. M. Mc Crodden, K. F. Tipton, *Neurochem. Res.* **1993**, *18*, 1145–1149.
[35] M. Yáñez, N. Fraiz, E. Cano, F. Orallo, *Biochem. Biophys. Res. Commun.* **2006**, *344*, 688–695.
[36] B. V. Nayak, S. Ciftci-Yabanoglu, S. Bhakat, A. K. Timiri, B. N. Sinha, G. Ucar, M. E. S. Soliman, V. Jayaprakash, *Bioorg. Chem.* **2015**, *58*, 72–80.
[37] E. Yuriev, P. A. Ramsland, *J. Mol. Recognit.* **2013**, *26*, 215–239.
[38] N. Jun, G. Hong, K. Jun, *Bioorg. Med. Chem.* **2007**, *15*, 2396–2402.
[39] S. S. Tiwari, A. Sing, *J. Ind. Chem. Soc.* **1961**, *38*, 931–932.
[40] P. Mahanthy, S. P. Panda, B. K. Sabata, M. K. Rout, *Ind. J. Chem.* **1965**, *3*, 121–123.
[41] A. Maiti, M. Cuendet, V. L. Croy, D. C. Endringer, J. M. Pezzuto, M. J. Cushman, *Med. Chem.* **2007**, *50*, 2799–2806.
[42] T. H. Simpson, W. B. Whalley, *J. Chem. Soc.* **1955**, 166–170.
[43] N. N. Pawar, A. G. Doshi, *Orient. J. Chem.* **2005**, *21*, 369–371.
[44] D. N. Dhar, *J. Ind. Chem. Soc.* **1960**, *37*, 363–364.
[45] W. T. A. Harrison, H. S. Yathirajan, H. G. Anilkumar, B. K. Sarojinic, B. Narayana, *Acta Cryst.* **2006**, *E62*, o3251–o3253.
[46] J. Montes-Avila, S. P. Díaz-Camacho, J. Sicairos-Félix, F. Delgado-Vargas, I. A. Rivero, *Bioorg. Med. Chem.* **2009**, *17*, 6780–6785.
[47] D. R. Palleros, *J. Chem. Educ.* **2004**, *81*, 1345–1347.
[48] M. M. Bradford, *Anal. Biochem.* **1976**, *72*, 248–254.
[49] F. Chimenti, S. Carradori, D. Secci, A. Bolasco, B. Bizzarri, P. Chimenti, A. Granese, M. Yanez, F. Orallo, *Eur. J. Med. Chem.* **2010**, *45*, 800–804.
[50] S. Y. Son, J. Ma, Y. Kondou, M. Yoshimura, E. Yamashita, T. Tsukihara, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 5739–5744.
[51] C. Binda, J. Wang, L. Pisani, C. Caccia, A. Carotti, P. Salvati, D. E. Edmondson, A. Mattevi, *J. Med. Chem.* **2007**, *50*, 5848–5852.
[52] G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell, A. J. Olson, *J. Comput. Chem.* **2009**, *30*, 2785–2791.
[53] K. Yelekci, B. Buyukturk, N. Kayrak, *J. Neural Transm.* **2013**, *120*, 853–858.
[54] N. Gökhan-Kelekçi, O. Simsek, A. Ercan, K. Yelekci, Z. S. Sahin, S. Isık, G. Uçar, A. A. Bilgin, *Bioorg. Med. Chem.* **2009**, *17*, 6761–6772.