Bioorganic & Medicinal Chemistry Letters 24 (2014) 3278–3284

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of some novel hydrazone and 2-pyrazoline derivatives: Monoamine oxidase inhibitory activities and docking studies

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ARTICLE INFO

Article history:
Received 14 April 2014
Revised 4 June 2014
Accepted 5 June 2014
Available online 17 June 2014

Keywords:
2-Pyrazoline
Hydrazone
MAO inhibitors
Molecular docking

ABSTRACT

A novel series of 2-pyrazoline and hydrazone derivatives were synthesized and investigated for their human monoamine oxidase (hMAO) inhibitory activity. All compounds inhibited the hMAO isoforms (MAO-A or MAO-B) competitively and reversibly. With the exception of **5i**, which was a selective MAO-B inhibitor, all derivatives inhibited hMAO-A potently and selectively. According to the experimental K_i values, compounds **6e** and **6h** exhibited the highest inhibitory activity towards the hMAO-A, whereas compound **5j**, which carries a bromine atom at R^4 of the A ring of the pyrazoline, appeared to be the most selective MAO-A inhibitor. Tested compounds were docked computationally into the active site of the hMAO-A and hMAO-B isozymes. The computationally obtained results were in good agreement with the corresponding experimental values.

Monoamine oxidases (MAOs) are flavoenzymes which play an important role in the oxidative catabolism of amine neurotransmitters and dietary amines.[1,2] MAO contains flavin adenine dinucleotide (FAD) as a cofactor and exist in two isoforms in mammals, namely MAO-A and MAO-B.[3] MAO-A preferentially deaminates serotonin and norepinephrine and is selectively inhibited by clorgyline, whereas MAO-B preferentially deaminates -phenylethylamine and benzylamine and is selectively inhibited by l-deprenil.[4,5]

Inhibitors of MAO-A are clinically used as antidepressants and anxiolytics,[6,7] while MAO-B inhibitors are used in the treatment of Parkinson's disease and in the management of symptoms associated with Alzheimer's disease.[8] The availability of the crystal structures of the two isoforms of human MAO facilitates the understanding of the selective interactions between these proteins and their ligands, making it possible to investigate the catalytic mechanism and recognize the pharmacophoric requirements necessary for the rational design of new inhibitors.[8–12]

Substrates and inhibitors of MAO usually carry an amino or imino group, which seems to play an essential role in the orientation and complex formation at the active site of the enzyme. Numerous substituted hydrazines and hydrazides have been studied as MAO inhibitors.[13,14] 2-Pyrazolines can be considered a cyclic hydrazine moiety,[15] and it has been found that they confer MAO inhibitory and antidepressant activity.[1,15–23] Acetyl substitution of the 2-pyrazoline ring on N1 has been found to favor inhibitory activity on MAO isoforms. This substitution increases the positive charge of N1 of the heterocycle which strengthens the charge-transfer bond with the isoalloxazine nucleus of FAD and reduces the steric hindrance of the molecules.[24–26]

Most currently used MAO inhibitors produce side effects due to a lack of affinity and selectivity towards one of the isoforms. For this reason, it is necessary to design more potent, reversible and selective inhibitors of MAO-A and MAO-B. A series of chalcones have been found to exhibit MAO inhibitory activity.[27] It is also known that pyrazoline and hydrazone derivatives inhibit MAO.[28,29] In this study, we have synthesized new hydrazone and 2-pyrazoline derivatives and evaluated their MAO inhibitory activities.

Chalcone derivatives were prepared by the reaction of acetophenone and benzaldehyde derivatives, **1** and **2**, in KOH/MeOH. The ensuing chalcone derivatives **3a**–**3h** were then reacted with hydrazide compounds to furnish hydrazone and 2-pyrazoline derivatives, **5a**–**5j** and **6a**–**6i** (Scheme 1). Structures, physicochemical and spectral characterization of the synthesized compounds are given in Supplementary data.

Hydrazone formation is dependent on the Schiff base reaction, and thus the optimization of the pH value affects the product yield. In these reactions, 2-pyrazoline and hydrazone derivatives are

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http://dx.doi.org/10.1016/j.bmcl.2014.06.015
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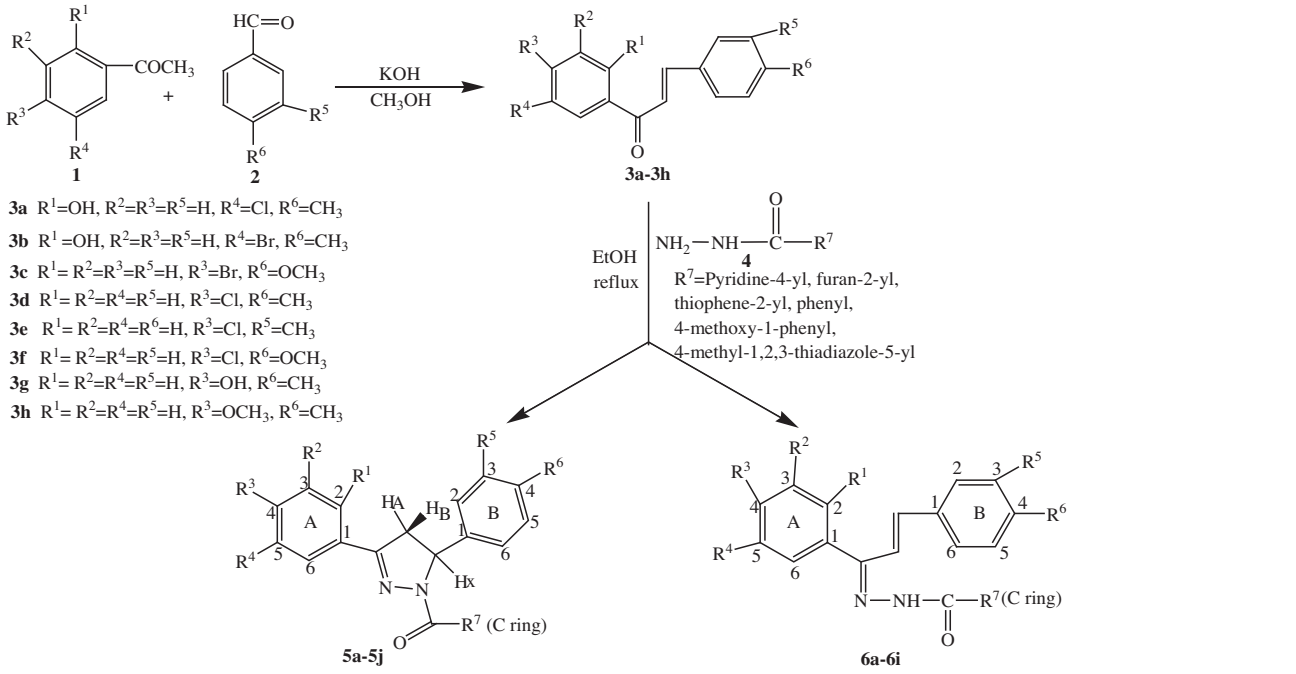

3a R^1=OH, R^2=R^3=R^5=H, R^4=Cl, R^6=CH_3
3b R^1 =OH, R^2=R^3=R^5=H, R^4=Br, R^6=CH_3
3c R^1= R^2=R^3=R^5=H, R^3=Br, R^6=OCH_3
3d R^1= R^2=R^4=R^5=H, R^3=Cl, R^6=CH_3
3e R^1= R^2=R^4=R^6=H, R^3=Cl, R^5=CH_3
3f R^1= R^2=R^4=R^5=H, R^3=Cl, R^6=OCH_3
3g R^1= R^2=R^4=R^5=H, R^3=OH, R^6=CH_3
3h R^1= R^2=R^4=R^5=H, R^3=OCH_3, R^6=CH_3

5a R^1=OH, R^2=R^3=R^5=H, R^4=Cl, R^6=CH_3, R^7=pyridine-4-yl
5b R^1=OH, R^2=R^3=R^5=H, R^4=Cl, R^6=CH_3, R^7=furan-2-yl
5c R^1=OH, R^2=R^3=R^5=H, R^4=Cl, R^6=CH_3, R^7=phenyl
5d R^1=OH, R^2=R^3=R^5=H, R^4=Cl, R^6=CH_3, R^7=4-methoxy-1-phenyl
5e R^1=OH, R^2=R^3=R_5=H, R^4=Cl, R^6=CH_3, R^7=4-methyl-1,2,3-thiadiazole-5-yl
5f R^1=OH, R^2=R^3=R^5=H, R^4=Br, R^6=CH_3, R^7=pyridine-4-yl
5g R^1=OH, R^2=R^3=R^5=H, R^4=Br, R^6=CH_3, R^7=furan-2-yl
5h R^1=OH, R^2=R^3=R^5=H, R^4=Br, R^6=CH_3, R^7=phenyl
5i R^1=OH, R^2=R^3=R^5=H, R^4=Br, R^6=CH_3, R^7=4-methoxy-1-phenyl
5j R^1=OH, R^2=R^3=R^5=H, R^4=Br, R^6=CH_3, R^7=thiophene-2-yl

6a R^1=R^2=R^4=R^5=H, R^3=Br, R^6=OCH_3, R^7=4-methoxy-1-phenyl
6b R^1=R^2=R^4=R^5=H, R^3=Br, R^6=OCH_3, R^7=furan-2-yl
6c R^1=R^2=R^4=R^5=H, R^3=Cl, R^6=CH_3, R^7=thiophene-2-yl
6d R^1=R^2=R^4=R^5=H, R^3=Cl, R^6=CH_3, R^7=4-methyl-1,2,3-thiadiazole-5-yl
6e R^1=R^2=R^4=R^6=H, R^3=Cl, R^5=CH_3, R^7=4-methyl-1,2,3-thiadiazole-5-yl
6f R^1=R^2=R^4=R^5=H, R^3=Cl, R^6=OCH_3, R^7=4-methyl-1,2,3-thiadiazole-5-yl
6g R^1=R^2=R^4=R^5=H, R^3=Cl, R^6=OCH_3, R^7=4-methoxy-1-phenyl
6h R^1=R^2=R^4=R^5=H, R^3=OH, R^6=CH_3, R^7=4-methyl-1,2,3-thiadiazole-5-yl
6i R^1=R^2=R^4=R^5=H, R^3=OCH_3, R^6=CH_3, R^7=4-methyl-1,2,3-thiadiazole-5-yl

Scheme 1. General synthesis of compounds **3a**–**3h**, **5a**–**5j**, and **6a**–**6i**.

formed together. At the end of the reaction, only one of the products—either the hydrazone or the 2-pyrazoline derivative—can be isolated. For this Letter, hydrazones were obtained (15–25.7% yield) in an ethanol solution with the reaction of chalcone and acylhydrazines at 78 °C during a period of 40–50 h. When we used chalcones having a 2′-OH group as the starting compound, only 2-pyrazolines were produced, but with chalcones not having a hydroxy group at position 2′, only hydrazones were generated. Generally, 2-pyrazoline derivatives were obtained with a higher yield than hydrazones. The highest yield was achieved with 2′-hydroxy-5′-chloro chalcone derivatives (27.33–74.48%).

Structures of the synthesized hydrazone and 2-pyrazoline derivatives were elucidated by IR, ^{1}H NMR, ^{13}C NMR, mass spectral data, and elemental analyses. The IR spectra of the compounds showed OH bonds at 3178–3446 cm^{-1}, C=O stretching bonds at 1665–1626 cm^{-1}, and C=N stretching bonds at 1605–1546 cm^{-1}. In the ^{1}H NMR spectrum of compounds **5a**–**5j**, the CH_2 protons of the pyrazoline ring resonated as a pair of doublets of doublets at δ_H 2.88–2.92 ppm and δ_H 3.43–3.52 ppm. The CH proton appeared as a doublet of doublets at δ_H 5.24–5.30 ppm. In the ^{1}H NMR spectrum of compounds **6a**–**6i**, ethylenic protons were observed at δ_H 6.51–7.90 ppm. The protons belonging to the aromatic ring and the other aliphatic groups were observed with the expected chemical shifts and integral values. ^{13}C NMR spectrum of compounds **3b**, **5a**, **5d**, **5g**, **5i**, **6d**, **6e**, **6i** were given in Supplementary data. Mass spectral analysis of the compounds was performed using the ESI (+) or ESI (−) method, and the characteristic peaks were observed in the mass spectra. Molecular ion peaks ([M]+) provided the molecular formula of all synthesized compounds **5a**–**5j**/**6a**–**6i**. Characteristic [M+2] isotope peaks were observed in the mass spectra of the compounds having a halogen atom. All compounds provided satisfactory elemental analyses.

The MAO-A and MAO-B inhibitory activities of the newly synthesized 2-pyrazoline and hydrazone derivatives were determined using the respective hMAO isoforms. Except compound **5i**, all tested compounds were found to inhibit MAO-A selectively and competitively (Table 1). These novel compounds were reversible inhibitors of hMAO-A, since the enzyme activity was restored after the centrifugation-ultrafiltration steps. Compound **5i** showed selectivity towards the MAO-B isoform.

Among compounds **5a**–**5j**, which are 2-pyrazoline derivatives carrying a chloride substitution on the A ring at position 5, compound **5c**, which carries a unsubstituted phenyl ring (C ring), was found to be the most potent MAO-A inhibitor according to its lowest K_i value for hMAO-A (Table 1). However, compound **5j**, which carries a bromide atom at R^4 of the A ring of pyrazoline, appeared as the most selective MAO-A inhibitor in the pyrazoline series according to its highest selectivity index (*SI*) value. *SI* was calculated as K_i (MAO-B)/K_i (MAO-A); the experimental SI value calculated for a compound increases as the selectivity to MAO-A isoform also increases whereas the experimental SI value calculated for a compound decreases, the selectivity to MAO-B increases. For this group of compounds, chloride substitution at R^4 of the phenyl ring was identified as favorable in terms of MAO-A inhibitory potency, whereas bromide substitution at R^4 of the phenyl ring increased the selectivity towards hMAO-A. Compound **5i**, which has a

Table 1
Calculated and experimental values (K_i values, SI, ΔG_b, free energy binding) of the newly synthesized 2-pyrazoline and hydrazone derivatives for hMAO isoforms A and B

Compound		Calculated					Experimental				
		ΔG_b (MAO-A) [kcal/mol]	K_i (MAO-A) [μM]	Calculated energy value for MAO-B	K_i (MAO-B) [μM]	SI[a]	Selectivity	K_i (MAO-A) [μM][b]	K_i (MAO-B) [μM][b]	*SI*[a]	Selectivity
3a		−9.20	0.18	−6.94	8.14	45.22	MAO-A	0.14 ± 0.01	7.22 ± 0.24	51.57	MAO-A
3b		−9.51	0.11	−7.18	5.44	49.45	MAO-A	0.13 ± 0.009	4.22 ± 0.20	32.46	MAO-A
3c		−9.31	0.15	6.99	7.50	50.00	MAO-A	0.10 ± 0.009	5.55 ± 0.33	55.50	MAO-A
3d		−8.91	0.30	6.93	8.38	27.93	MAO-A	0.24 ± 0.01	7.70 ± 0.40	32.08	MAO-A
3e		−9.18	0.19	6.98	7.68	40.42	MAO-A	0.16 ± 0.01	7.00 ± 0.34	29.17	MAO-A
3f		−9.0	0.25	6.62	14.00	56.00	MAO-A	0.24 ± 0.01	13.90 ± 1.05	57.91	MAO-A
3g		−8.57	0.52	6.21	28.17	54.17	MAO-A	0.60 ± 0.23	27.00 ± 1.97	45.00	MAO-A
3h		−8.62	0.48	6.57	15.41	32.10	MAO-A	0.56 ± 0.03	14.90 ± 1.19	26.61	MAO-A
5a	R	−10.57	0.018	−7.95	1.49	82.78	MAO-A	0.60 ± 0.04	5.23 ± 0.27	8.72	MAO-A
	S	−7.85	1.77	−6.67	12.84	7.25	MAO-A				
5b	R	−10.17	0.035	−9.31	0.15	4.29	MAO-A	0.11 ± 0.002	0.99 ± 0.04	9.00	MAO-A
	S	−7.08	6.48	−6.27	25.47	3.93	MAO-A				
5c	R	−10.95	0.009	−8.46	0.63	70.00	MAO-A	0.012 ± 0.002	0.75 ± 0.03	62.50	MAO-A
	S	−6.55	15.80	−5.43	105.39	6.67	MAO-A				
5d	R	−9.02	0.25	−7.19	5.34	21.36	MAO-A	4.67 ± 0.03	20.00 ± 1.23	4.28	MAO-A
	S	−6.38	21.07	-4.58	441.84	20.97	MAO-A				
5e	R	−10.81	0.012	−9.64	0.09	7.50	MAO-A	0.24 ± 0.011	72.00 ± 4.55	300.00	MAO-A
	S	−8.47	0.62	−5.42	107.00	172.58	MAO-A				
5f	R	−11.42	0.004	−9.05	0.23	57.50	MAO-A	0.18 ± 0.008	2.00 ± 0.13	11.11	MAO-A
	S	−8.57	0.52	−7.28	4.63	8.90	MAO-A				
5g	R	−8.97	0.27	−8.98	0.26	0.96	Non selective	0.45 ± 0.02	14.00 ± 1.07	31.11	MAO-A
	S	−8.23	0.93	−6.15	30.89	33.22	MAO-A				
5h	R	−9.13	0.20	−6.92	8.46	42.30	MAO-A	0.040 ± 0.002	60.00 ± 3.24	1500.00	MAO-A
	S	−10.76	0.013	−5.56	84.37	6490	MAO-A				
5i	R	−9.53	0.10	−7.64	2.53	25.30	MAO-A	79.22 ± 5.03	29.66 ± 1.90	0.3744	MAO-B
	S	−5.19	157.80	−5.70	66.63	0.4222	MAO-B				
5j	R	−9.21	0.18	−7.74	2.11	11.72	MAO-A	0.13 ± 0.007	1640.00 ± 65.00	12615.38	MAO-A
	S	−10.26	0.030	−3.67	2050.00	68333	MAO-A				
6a		−10.19	0.034	−8.25	0.89	26.18	MAO-A	0.045 ± 0.002	0.98 ± 0.05	21.78	MAO-A
6b		−9.92	0.054	−8.78	0.36	6.67	MAO-A	0.091 ± 0.005	0.70 ± 0.04	7.69	MAO-A
6c		−10.29	0.029	−8.72	0.41	14.14	MAO-A	0.034 ± 0.001	0.52 ± 0.03	15.29	MAO-A
6d		−11.16	0.007	−8.52	0.57	81.42	MAO-A	0.012 ± 0.001	0.76 ± 0.04	63.33	MAO-A
6e		−11.32	0.005	−8.44	0.65	130.00	MAO-A	0.010 ± 0.001	0.99 ± 0.008	99.00	MAO-A
6f		−10.86	0.010	−8.22	0.95	95.00	MAO-A	0.012 ± 0.001	1.05 ± 0.009	87.50	MAO-A
6g		−10.33	0.027	−8.33	0.78	28.89	MAO-A	0.034 ± 0.001	0.87 ± 0.04	25.59	MAO-A
6h		−11.76	0.002	−8.25	0.89	445.00	MAO-A	0.010 ± 0.001	1.48 ± 0.10	148.00	MAO-A
6i		−10.32	0.027	−8.54	0.55	20.37	MAO-A	0.041 ± 0.002	0.90 ± 0.05	21.95	MAO-A
Selegiline		−6.55	15.93	−5.33	122.93	7.72	MAO-B	13.55 ± 1.08	0.22 ± 0.01	0.016	MAO-B
Moclobemide		−8.00	1.37	−6.04	37.38	27.28	MAO-A	0.014 ± 0.007	1.34 ± 0.08	95.71	MAO-A

[a] The selectivity index (*SI*) was calculated as K_i(MAO-B)/K_i(MAO-A).
[b] Each value represents the mean ± SEM of three independent experiments. Racemic compounds were used for the experiments. All compounds inhibited hMAO isoforms competitively and reversibly.

bromide substitution at R^4 and 4-methoxy-1-phenyl at R^7, was found to be a weak MAO-B inhibitor.

The hydrazone derivatives **6a**–**6i** were also found to be potent MAO-A inhibitors. Among this series, compounds **6e** and **6h**, having a 4-methyl-1,2,3-thiadiazole-5-yl substitution at the R^7 position of the C ring, were the most potent MAO-A inhibitors. Thus, this substitution is suggested to play a favorable role in terms of MAO-A inhibition for the novel hydrazone derivatives. However, the selectivities of the newly synthesized hydrazones for hMAO-A were found weaker than those of the 2-pyrazolines (Table 1).

In the present Letter, we successfully identified new compounds which are reversible and selective inhibitors of hMAO-A. The combination of chalcones (compounds **3a**–**3h**) with the hydrazide moiety caused a remarkable increase in selectivity to MAO-A in novel series (Table 1). For compounds **6a**–**6i**, the 4-methyl-1,2,3-thiadiazole-5-yl substitution on the C ring favors MAO-A inhibition. For compounds **5a**–**5j**, the phenyl substitution on the C ring favors MAO-A inhibition, whereas the 4-methoxy-1-phenyl substitution at the R^7 position (compound **5i**) favors MAO-B inhibition. The results of this study provide useful information for the design of a new series of potent, selective, and reversible MAO-A inhibitors in the future.

In order to gain more insight on the binding mode of the compounds with MAO-A and MAO-B, docking studies were employed (Supplementary data). Molecular modeling approaches were performed for compounds **5a**–**5j** and **6a**–**6i** for which K_i values had been experimentally obtained (Table 1). The calculated inhibition constants and free energies of the binding of these inhibitors to MAO-A and MAO-B are presented in Table 1. Inhibitors **5a**–**5j** were tested experimentally only as their racemates, due to the difficulty of separating the enantiomers, whereas calculations were done separately for each enantiomer of these inhibitors for both isozymes. As to be expected, for the isomeric compounds **5a**–**5j** different inhibition patterns were calculated for the respective enantiomers for both MAO-A and MAO-B. According to the molecular docking data, 2-pyrazolines (compounds **5a**–**5j**) appeared as MAO-A inhibitors, and their (*R*)-isomers, with the exception of **5h** and **5j** are predicted to inhibit MAO-A more effectively than the (*S*)-isomers. The (*R*)-isomer of compound **5g** was computed to be non-selective, whereas the (*S*)-isomer of the same compound

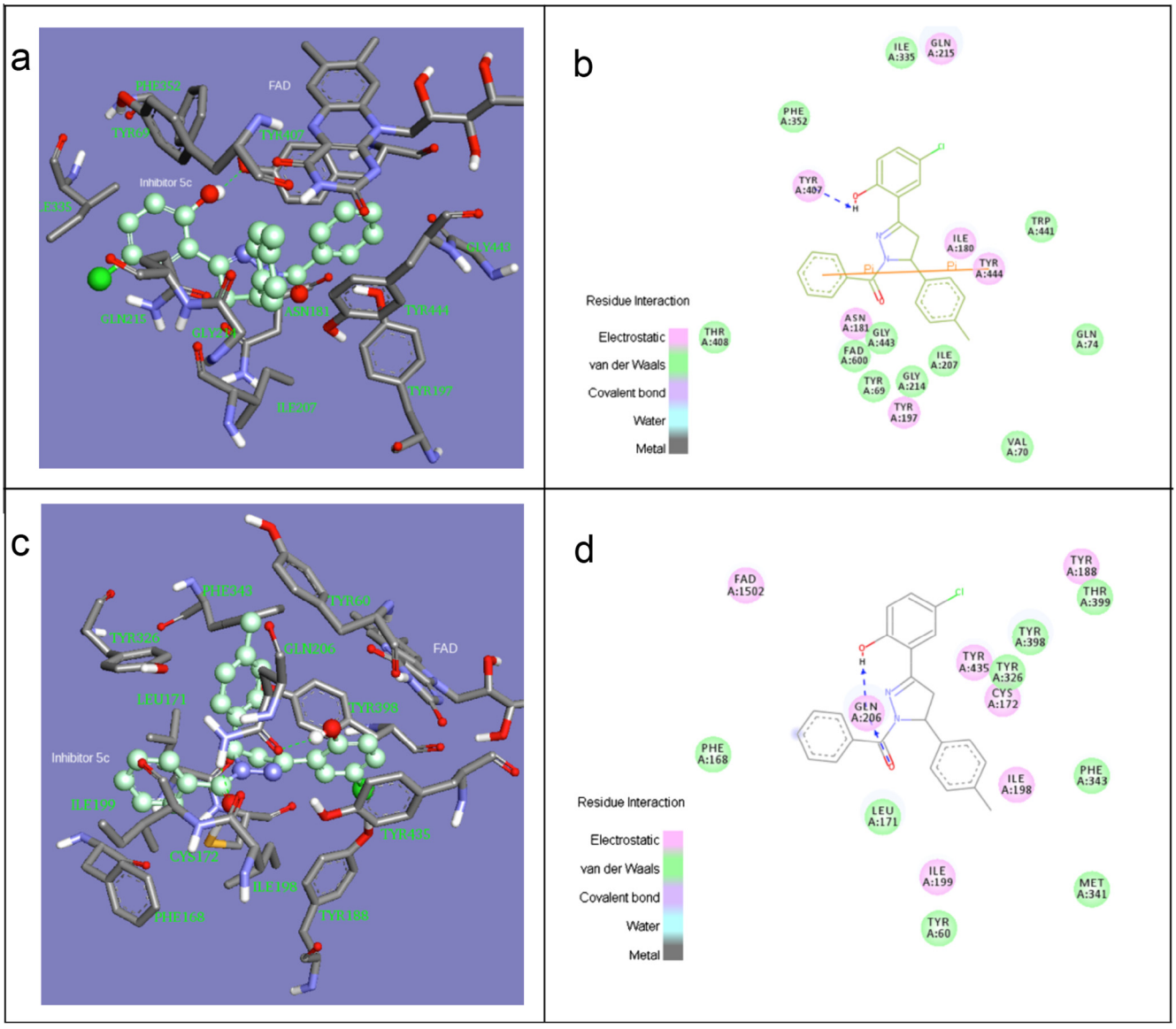

Figure 1. (a) Three-dimensional orientation of **5c** (*R*) in the active site of MAO-A. (b) Two-dimensional picture of **5c** (*R*) in the active site of MAO-A. (c)Three-dimensional orientation of **5c** (*R*) in the active site of MAO-B. (d) Two-dimensional picture of **5c** (*R*) in the active site of MAO-B.

is MAO-A selective. The (*R*)-isomer of **5i** is predicted to be a better MAO-A inhibitor than the (*S*)-isomer, and conversely, the (*S*)-isomer a better MAO-B inhibitor. The calculated K_i values of compounds **6a**–**6i** for MAO-A were in good agreement with the experimental values. The reference compound, selegiline, is a well-known irreversible MAO-B inhibitor[30] and the experimentally determined inhibition constants were 13.55 μM and 0.22 μM for MAO-A and MAO-B, respectively (Table 1). However, the calculated values were 15.93 μM (MAO-A) and 122.93 μM (MAO-B). In the molecular docking calculations, we only simulated the best docking conformation and the maximum interactions between the ligand and active site residues of the enzyme. If the ligand binds irreversibly or exhibits suicide-type inhibition, then it is hard to calculate the resulting interaction with docking simulations. In other words, for docking simulation, we only took the initial enzyme-inhibitor complex formation into consideration. Moclobemide is a well-known reversible MAO-A inhibitor[30] and the calculated values are in agreement with this fact, even though the experimental and calculated values for moclobemide differ more than those for our new inhibitors.

For visualization of the enzyme inhibitor complexes, the pyrazoline **5c** and the hydrazone **6h** were chosen, based on their high inhibitory potency and selectivity for one or the other of the two MOA isoenzymes.

According to Edmondson et al.[31] the human MAO-B enzyme has two cavities connected by the amino acid ILE199 that serves as a 'gate'. The entrance cavity has a volume of 290 Å^3 and is very hydrophobic in nature. The second cavity, with a volume of 390 Å^3, harbors the substrate binding site. At the far end of the substrate cavity, the coenzyme FAD is located. Computer analysis of this cavity indicated that the amino acid side chains lining the cavity are very hydrophobic and favorable for amine binding. The FAD and nearly two parallel tyrosyl residues (398 and 435) form an 'aromatic cage'. On the other hand, human MAO-A has only a single cavity of 550 Å^3, in which FAD and two nearly parallel tyrosyl residues (407 and 444) also form an 'aromatic cage'. The substrate binding sites of both MAO-A and MAO-B are quite hydrophobic in nature.

The (*R*)-enantiomer of **5c** (calculated K_i = 0.009 μM) was docked in the active site of MAO-A as shown in Figure 1a and b. The phenyl

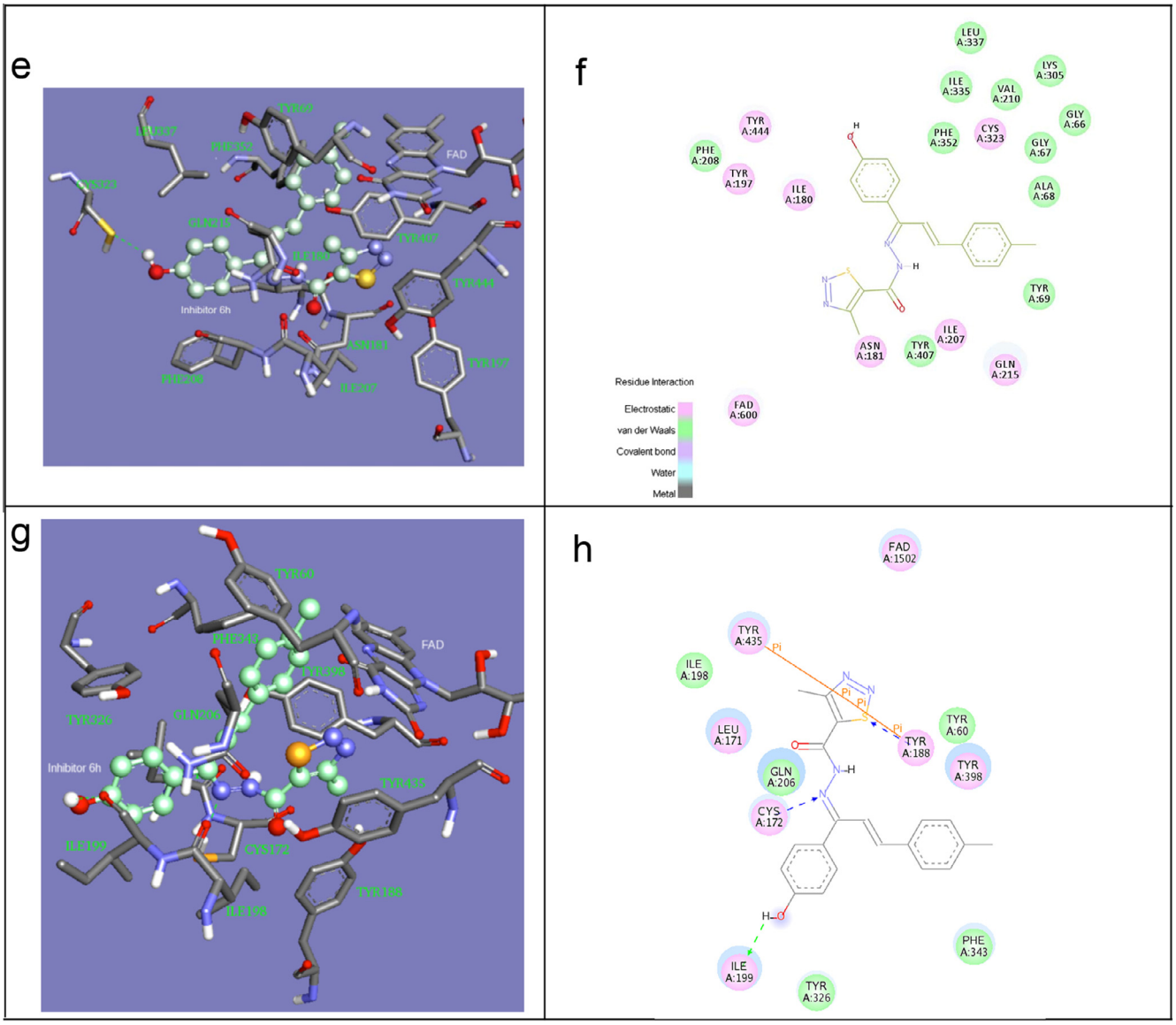

Figure 2. (e) Three-dimensional orientation of **6h** in the active site of MAO-A. (f) Two-dimensional picture of **6h** in the active site of MAO-A. (g) Three-dimensional orientation of **6h** in the active site of MAO-B. (h) Two-dimensional picture of **6h** in the active site of MAO-B.

ring is oriented horizontally between the phenolic side chains of Tyr444 and Tyr407 residues, and it approached from the *re* face of FAD making one π–π interaction. The hydrogen atom of the hydroxy group of the 2-hydroxy phenyl ring of the inhibitor forms a hydrogen bond with the hydroxy group of Tyr407. Ile207, Gly214, Tyr197, Tyr69, Asn181, Phe352, Ile335, Gln215, Ile180, Trp441, and Gln74 are the other active site residues interacting with the inhibitor. The same compound exhibits different binding patterns with MAO-B (K_i = 0.63 μM), as can be seen in Figure 1c and d. The inhibitor is placed distant from the hydrophobic cage by Tyr398, Tyr435, and FAD. In this case, the inhibitor is located close to the entrance cavity. The hydroxy group hydrogen of the 2-hydroxy phenyl ring of the inhibitor forms a hydrogen bond with the entrance site residue Gln206, while the carbonyl group of the inhibitor is hydrogen-bound to the side chain of Gln206. Ile171, Phe168, Tyr188, Tyr398, Tyr435, Tyr326, Cys172, Ile198, Phe343, and Met341 are the other active site residues interacting with the inhibitor.

Compound **6h** (calculated K_i = 0.002 μM) was docked in the active site of MAO-A as shown in Figure 2e and f. The 4-methyl-1,2,3-thiadiazole-5-yl ring is inserted into the hydrophobic cage which is comprised of Tyr444, Tyr407, and FAD. The inhibitor approached from the *re* face of FAD and makes a π–π interaction with Tyr444. The hydroxy group of the 4-hydroxy phenyl ring forms a hydrogen bond with the hydrogen atom of the Cys323 side chain. Tyr407, Ile207, Tyr197, Tyr69, Phe352, Ile335, Gln215, Gly67, Gly66, Lys305, Phe305, and Met350 are the other active site residues involved in hydrophobic and polar interactions with the inhibitor. Figure 2g and h show the binding pattern of **6h** to MAO-B (calculated K_i = 0.89 μM). The inhibitor is located in the hydrophobic cage surrounded by Tyr398, Tyr435, and FAD. The methyl-1,2,3-thiadiazole-5-yl ring is sandwiched between Tyr435 and Tyr398 and makes one pp interaction with Tyr435. The hydrogen atom of the hydroxy group of the 4-hydroxy phenyl ring is hydrogen-bound to Ile199. The azo group of the inhibitor makes another polar attraction with the sulfhydryl group of Cys172. The 4-hydroxy phenyl ring makes the second pp interaction with Tyr60. Ile171, Ile198, Tyr188, Tyr326, Phe343, and Gly57 are the other active site residues interacting with the inhibitor.

The inhibitor, **6h**, was optimized using SPARTAN 10 program at PM3 level. The docking simulation was started from these lowest energy conformations. The macromolecule (enzymes) was held stationary and the inhibitors are flexible. Depending on the inhibitors 6–7 single rotatable bonds are selected as flexible. The docking process generated the best conformer by rotating these single bonds which snuggly fits the active site of the enzyme. These final conformers may not be the same as started minimized structures in the active site. The structure of the compound **6h** was assigned and their stereoisomers were estimated based on QM calculations: Both isomers were optimized and their energies were calculated. According to B3LYP/6-31G* optimization E isomer (Fig. 4) is only 0.54 kcal/mol more stable than Z (Fig. 3) isomer.

The synthesized compounds were found to be mostly competitive, reversible, and selective inhibitors of hMAO-A. 2-Pyrazoline compounds showed higher selectivity towards hMAO-A than chalcones and hydrazones while the combination of 2-pyrazoline and hydrazone increased the inhibitory potency.

The data indicate that the 2-hydroxy-5-bromo phenyl ring (A ring) is essential both for selectivity and potency of hMAO-A inhibition by compounds **5a**–**5j**. Selectivity decreased, when the 2-hydroxy-5-bromo phenyl ring was replaced by the 2-hydroxy-5-chloro phenyl ring. Addition of 4-methoxyphenyl or 2-furyl to the scaffold as the C ring decreased the potency and selectivity of inhibition, while the addition of a phenyl ring as the C ring to the scaffold increased this effect. Addition of thiophene-2-yl as a C ring to 2-pyrazoline compounds increased their selectivity, as evidenced by the *SI* value of compound **5j**, which carries such a C ring, is almost 130-fold higher than that of the reference MOA-A inhibitor moclobemide. On the contrary, addition of thiophene-2-yl as a C ring to hydrazone compounds decreased their potency and selectivity. The addition of a pyridine moiety as the C ring to 2-pyrazoline compounds reduced their potency and selectivity towards hMAO-A. For hydrazone compounds, replacement of the chlorine atom at R^3 of the A ring by a hydroxy group increased the potency and selectivity towards hMAO-A, while a methoxy group in this position decreased both these parameters.

All biological experiments were carried out using only racemic mixtures of compounds **5a**–**5j**. The calculated K_i values, at least for one of the enantiomers, agreed with the experimentally determined values (Table 1).

Overall, the docking method provided us with invaluable data for the rationalization of the observed experimental results, and allowed us to estimate the binding mode, the inhibition constant

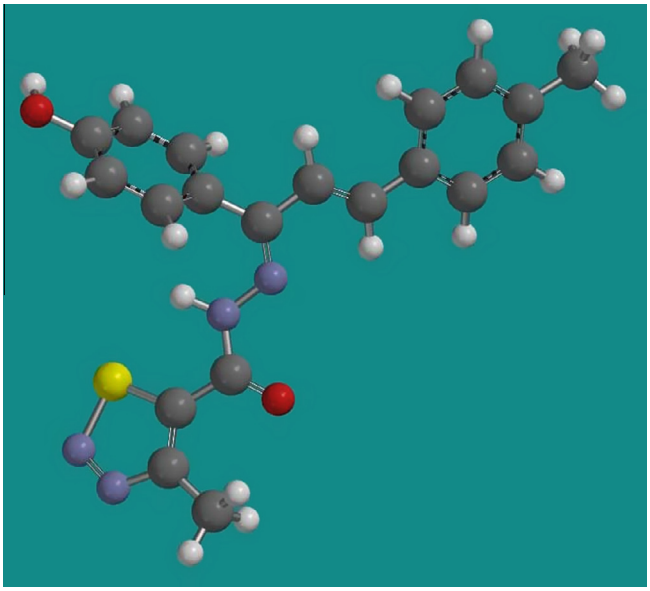

Figure 3. Z isomer of compound **6h**.

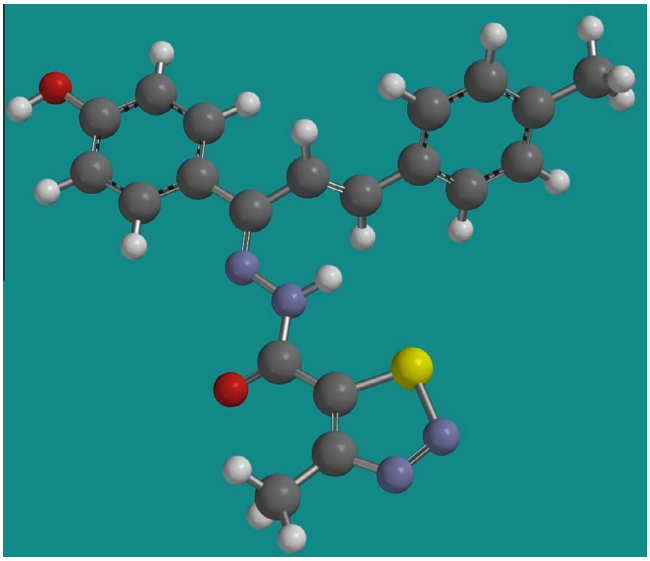

Figure 4. E isomer of compound **6h**.

and the free energy of binding, all of which are promising tools for the discovery of novel, potent, and selective MAO inhibitors potentially useful as pharmacological agents.

Acknowledgment

We would like to express our deep gratitude to Professor Hakan Göker for providing the spectroscopic data.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.06.015.

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