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Pyrazoline based MAO inhibitors: Synthesis, biological evaluation and SAR studies

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ABSTRACT

Twenty-two pyrazoline derivatives were synthesized and tested for their human MAO (hMAO) inhibitory activity. Twelve molecules with unsubstituted ring A and substituted ring C (**5–16**) were found to be potent inhibitors of hMAO-A isoform with SI_{MAO-A} in the order 10^3 and 10^4. Ten molecules with unsubstituted ring A and without ring C (**21–30**), in which eight molecules (**21**, **23–26**, and **28–30**) were selective for hMAO-A, one for hMAO-B (**22**) and the other one non-selective (**27**). Presence of ring C increases potency as well as SI towards hMAO-A; however its absence decreases both potency and SI towards hMAO-A and hMAO-B.

Selective MAO-B inhibitors gained greater attention due to their multiple roles that they can play in the therapy of neurodegenerative disorders.[1,2] Our group had reported earlier a few 3,5-diaryl carbothioamide pyrazolines with MAO inhibitory activity. Two molecules (**12** and **13**) without any substitution in the ring A and one molecule without ring C (**11**) were found to be selective against rat liver MAO-B (Fig. 1).[3] Recently Boppana et al. have proposed few molecules with potential MAO-B selectivity through pharmacophore modelling. They also proposed three molecules with unsubstituted ring A and without ring C in pyrazoline carboxamides (Fig. 1).[4] In order to explore the possibility of getting potential MAO-B selective compound we prepared 22 pyrazoline derivatives with unsubstituted ring A, with or without ring C.

Twelve molecules (**5–16**) with substituted ring C carrying unsubstituted ring A were prepared using the method reported earlier[3] by following the Scheme 1. Another 10 molecules (**21–30**) were prepared without ring C carrying unsubstituted ring A according to the method reported earlier[5] by following the Scheme 2. Chalcones **1–2** and **17–20** were prepared through Claisen–Schmidt condensation. To an equimolar quantity of acetophenone and appropriate benzaldehyde in ethanol was added an aqueous solution of sodium hydroxide (60%) dropwise with continuous stirring at 0 °C over a period of 30 min. The reaction mixture was kept at room temperature for about 48 h with occasional shaking. It was then poured into ice-cold water to obtain the **17–20**. Chalcones **1–2** were obtained by adjusting the pH to 2 using 6 N hydrochloric acid. The pyrazoline intermediate (**3–4**) were obtained by the condensation of **1–2** with excess hydrazine hydrate (99%) in ethanol. The reaction mixture was refluxed for about 3 h and upon concentration and cooling provided **3–4**. The final pyrazoline thiocarboxamide derivatives (**5–16**) were obtained by the reaction of **3–4** with appropriately substituted phenylisothiocyanates. The reaction mixture in ethanol was refluxed for a period of 30–45 min and upon cooling or concentration provided **5–16**. Pyrazoline derivatives (**21–30**) were prepared by the condensation of **17–20** with 2 Molar equiv of hydrazine derivatives (semicarbazide hydrochloride/thiosemicarbazide/aminoguanidine bicarbonate). The reaction mixture in ethanol was refluxed for a period of 12–18 h. The product (**21–30**) precipitated in hot solution or upon concentration and cooling. All the intermediates were characterised by IR spectroscopic and elemental analysis for CHNS. In the elemental analysis, the observed values were within ±0.4% of the calculated values. Final compounds were characterised by ^{1}H NMR and FAB-MS (Supplementary data).

Monoamine oxidase A human (recombinant, expressed in baculovirus infected BTI insect cells), monoamine oxidase B human (recombinant, expressed in baculovirus infected BTI insect cells), *R*-(−)-deprenyl hydrochloride, resorufin, dimethyl sulfoxide (DMSO) and some other chemicals were purchased from Sigma–Aldrich™ (Germany). Moclobemide was a gift (Roche

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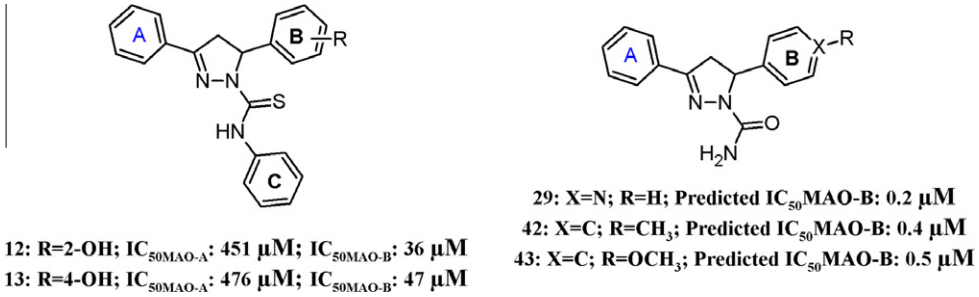
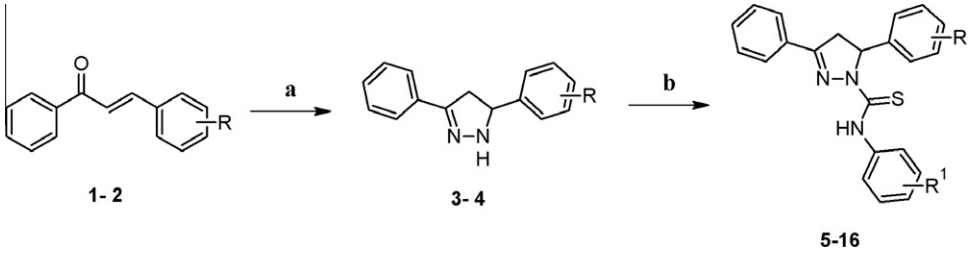
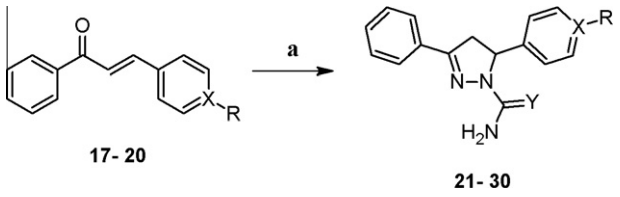


Figure 1. Selective MAO-B inhibitors reported[3] and predicted[4] earlier.



Scheme 1. Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$ (99%), C_2H_5OH, reflux, 3 h; (b) R^2-C_6H_4–NCS, C_2H_5OH, reflux, 30–45 min.



Scheme 2. Reagents and conditions: (a) NH_2–NH–C(=Y)–NH_2 (where X = O, S, NH), C_2H_5OH, reflux, 12–18 h.

Pharmaceuticals, Germany). Amplex®-Red MAO Assay Kit (Molecular Probes, USA) contained benzylamine, *p*-tyramine, Clorgyline (MAO-A inhibitor), Pargyline (MAO-B inhibitor) and horse radish peroxidase. The interactions of the synthesized compounds (**5–16** and **21–30**) with hMAO isoforms were determined by a fluorimetric method described and modified previously.[6,7] The production of H_2O_2 catalysed by MAO isoforms was detected using 10-acetyl-3,7-dihydroxyphenoxazine (Amplex®-Red reagent), a non-fluorescent, highly sensitive and stable probe that reacts with H_2O_2 in the presence of horseradish peroxidase to produce a fluorescent product, resorufin. The results are presented in Table 1(a and b).

All the compounds were found to inhibit MAO-A selectively and reversibly, except **22**, **23** and **27** those were non-selective towards either MAO isoforms. The compounds with ring C (**5–16**) were potent MAO-A inhibitors with K_i in nM range (except **15**) and SI_{MAO-A} is in the order of 10^3–10^4. Compounds **7** and **11** were found to be the most potent MAO-A inhibitors within this series with K_i values 99.55 ± 9.07 and 90.45 ± 4.73 nM, respectively. SAR within this series reveals (i) 2-hydroxy substitution in ring B is better than 4-hydroxy (except **11** and **14**), (ii) methoxy substitution in ring C is better than methyl substitution (except **15**) and (iii) *meta* substitution found to be favourable when above two factors exists. Ortho hydroxy substitution in ring B increases potency and selectivity index towards MAO-A when there is methoxy substitution in meta position of ring C. Whereas para hydroxy substitution in ring B increases potency and selectivity towards MAO-A, with both methoxy (favourable at *para* > *meta* position) and methyl (favourable at *meta* > *para* position) group at either para or meta positions.

The compounds without ring C (**21–30**) were found to be selective towards MAO-A (except **22**, **23** and **27**) but the potency and selectivity index are poor when compared with **5–16**. Compound **22** is slightly selective towards MAO-B with K_i of 4.79 ± 0.24 μM and SI_{MAO-B} of 1.46. The experimental K_i values of **21**, **22** and **23** were found to be 25-, 8- and 10-fold higher than the predicted values (Fig. 1).[4] This may be due to the fact that Boppanna et al.[4] has developed the pharmacophore model by including the ligands in training set that could able to interact covalently with the receptor. Moreover these three molecules were proposed by Boppana et al.[4] as potent MAO-B selective inhibitors. But, only **22** was found to be slightly selective towards MAO-B. They have predicted the database only with the model developed for MAO-B inhibitors. No model for MAO-A inhibitors was developed and molecules were predicted against the model by Boppana et al.[4] This may be the reason why these molecules were predicted and reported as potent MAO-B inhibitors. Possibly prediction of these molecules against model for MAO-A inhibitors and MAO-B inhibitors might have provided a true picture of selectivity towards isoforms.

Further compound **7**, **11**, **22** and **27** were selected for molecular docking simulations studies. Both *R* and *S* conformers were docked to understand the impact of configuration at C5 carbon of pyrazoline towards activity and selectivity. It has been reported already that enantiomer exhibited improved selectivity than racemates through chiral separation of racemates[8–10] and also through modelling studies.[11,12] Compounds **7** and **11** were studied against h MAO-A isoform to understand the factors contributing towards potency, while compounds **22** and **27** were studied against both hMAO-A and hMAO-B to understand the factors determining the selectivity. The Estimated Ki (EKi in μM) and Selectivity Index (SI) for *R*, *S* and average of *R* and *S* were presented in Table 2. Docking protocol reported already by our group has been followed.[3,5,13] Docking reports revealed that S-conformers are slightly better than R-conformer (except **11** for MAO-A and 27 for MAO-B).

Since racemates were screened for MAO inhibitory activity, the average Estimated K_i (EKi in μM) for *S* and *R* conformers was also calculated (EKi_{av}) for comparison. From the EKi_{av}, it is clear that **7** and **11** were selective towards MAO-A, **22** was slightly selective

Table 1
Experimental K_i values corresponding to the inhibition of human MAO isoforms by the newly synthesized pyrazoline derivatives

Code	K_i values for MAO-A (μM)	K_i values for MAO-B (μM)	SI[a]	Inhibition type	Reversibility	MAO inhibitory selectivity
(a)						
5	0.17 ± 0.01	60.22 ± 2.80	0.001	Competitive	Reversible	Selective for MAO-A
6	0.29 ± 0.02	69.90 ± 3.90	0.127	Competitive	Reversible	Selective for MAO-A
7	0.10 ± 0.01	979.00 ± 41.00	1.02×10^{-4}	Competitive	Reversible	Selective for MAO-A
8	0.30 ± 0.02	250.00 ± 11.77	0.001	Competitive	Reversible	Selective for MAO-A
9	0.31 ± 0.06	60.00 ± 3.79	0.005	Competitive	Reversible	Selective for MAO-A
10	0.25 ± 0.02	22.00 ± 1.50	0.001	Competitive	Reversible	Selective for MAO-A
11	0.09 ± 0.00	400.90 ± 21.00	2.26 ± 10^{-4}	Competitive	Reversible	Selective for MAO-A
12	0.36 ± 0.02	300.01 ± 20.00	0.001	Competitive	Reversible	Selective for MAO-A
13	0.35 ± 0.02	355.00 ± 16.08	9.88×10^{-4}	Competitive	Reversible	Selective for MAO-A
14	0.13 ± 0.01	255.60 ± 10.50	5.09×10^{-4}	Competitive	Reversible	Selective for MAO-A
15	2.15 ± 0.10	2130 ± 200	0.001	Competitive	Reversible	Selective for MAO-A
16	0.31 ± 0.02	309.22 ± 10.00	0.001	Competitive	Reversible	Selective for MAO-A
Selegiline	9.060 ± 440	0.091 ± 4.26	99.92	Competitive	Reversible	Selective for MAO-B
Moclobemide	0.005 ± 0.13	1.080 ± 300	0.005	Competitive	Reversible	Selective for MAO-A
(b)						
21	0.490 ± 0.03	3.20 ± 0.18	0.153	Competitive	Reversible	Selective for MAO-A
22	7.00 ± 0.53	4.79 ± 0.24	1.461	Competitive	Reversible	Selective for MAO-B
23	0.980 ± 0.04	1.88 ± 0.09	0.521	Competitive	Reversible	Selective for MAO-A
24	0.300 ± 0.02	1.66 ± 0.10	0.181	Competitive	Reversible	Selective for MAO-A
25	0.333 ± 0.02	1.80 ± 0.11	0.185	Competitive	Reversible	Selective for MAO-A
26	0.456 ± 0.02	4.00 ± 0.20	0.114	Competitive	Reversible	Selective for MAO-A
27	0.805 ± 0.04	0.805 ± 0.04	1.000	Competitive	Reversible	Non-selective
28	0.400 ± 0.02	4.11 ± 0.25	0.099	Competitive	Reversible	Selective for MAO-A
29	0.670 ± 0.03	4.80 ± 0.21	0.140	Competitive	Reversible	Selective for MAO-A
30	0.150 ± 0.01	4.22 ± 0.19	0.035	Competitive	Reversible	Selective for MAO-A
Selegiline	9.060 ± 440	0.091 ± 4.26	99.92	Competitive	Reversible	Selective for MAO-B
Moclobemide	0.005 ± 0.13	1.080 ± 300	0.005	Competitive	Reversible	Selective for MAO-A

K_i values were determined from the kinetic experiments in which *p*-tyramine (substrate) was used at 500 μM to measure MAO-A and 2.5 mM to measure MAO-B. Pargyline or clorgyline were added at 0.50 μM to determine the isoenzymes A and B. Newly synthesized compounds and the known inhibitors were preincubated with the homoganates for 60 min at 37 °C. Each value represents the mean ± SEM of three independent experiments.

[a] Selectivity index. It was calculated as K_i (MAO-A)/K_i (MAO-B).

Table 2
Molecular docking results of compound 7, 11, 22 and 27

Code	MAO-A (2BXR)					MAO-B (2BYB)					SI_{MAO-A}		
	R		*S*		Average	*R*		*S*		Average			
	EFEB	EKi	EFEB	EKi	EKi_{av}	EFEB	EKi	EFEB	EKi	EKi_{av}	rac/rac	*R*/*R*	*S*/*S*
7	−9.43	0.13	−9.77	0.07	0.10	−3.48	2830	−4.78	315.00	1572.50	15,725	21769.2	4500
11	−8.44	0.65	−7.87	1.70	1.18	−2.86	8020	−4.54	468.90	4244.45	3612.3	12338.5	275.8
22	−7.56	2.88	−7.67	2.39	2.64	−7.74	2.10	−7.78	1.99	2.05	0.8	0.7	0.8
27	−8.80	0.35	−8.81	0.35	0.35	−8.39	0.71	−7.77	2.00	1.36	3.9	2.0	5.7

EFEB—Estimated Free Energy of Binding in Kcal/mol; Eki—Estimated K_i in μM; SI—Selectivity Index.

towards MAO-B, very much in agreement with experimental result. The compound **27** has been found to have ~4-fold selectivity towards MAO-A, Experimental results suggest the molecule as non-selective.

The compounds **7** and **11**, Estimated K_i of *R*-conformers (EKi_R) were ~2-fold better than its *S*-counterpart, but exhibits ~17,000- and ~12,000-fold increased selectivity towards MAO-A, respectively. Compounds **22**, EKi_R was almost equal to EKi_S against MAO-A as well as for MAO-B. The selectivity towards MAO-B is marginal and the *S*-conformer exhibits only ~0.1-fold better selectivity compared with its *R*-counterpart/racemate. Compound **27**, EKi_R was almost equal to EKi_S against MAO-A, while against MAO-B EKi_R was ~3-fold better than EKi_S. Due to this the molecule displayed ~4-, ~2- and ~6-fold selectivity towards MAO-A for racemate, *R*-conformers and *S*-conformer, respectively. Simulation results suggested that for compounds **7** and **11** the *R*-isomer exhibited improved selectivity but compounds **22** and **27** displayed a marginal improvement.

In *R*-conformers of compound **7**, the ring A is accommodated in Pocket3 (P3, delimited by ILE180, ILE335, LEU337, MET350 and PHE352). *Ortho* substitution in ring B (**7**) keeps it at the entrance of the aromatic cage (P1, FAD, TYR407 and TYR444) with edge–edge interaction and accommodates ring C in Pocket 2 (P2, delimited by GLY71, GLN74, ARG206, ILE207, PHE208, GLU216 and TRP441). Both ring A (with PHE208 and TYR444) and ring C (TRP441) displayed π→π interaction. The *S*-conformer did not display any π→π interaction, but ring C is very well accommodated in P1, ring B in P2 and ring A in P3. The *o*-hydroxy group in ring B could able establishes an H-bond with SER209. In *R*-conformer of compound **11**, the ring A is accommodated in P3 exhibiting π→π interaction with PHE208 and TYR444 similar to **7**. But the *p*-substitution in ring B is not tolerated by P1, a rotation around the single bond between C3 of pyrazoline and ring A, moves ring B to P2 and kept ring C away from P1. This allows ring B to exhibit π→π interaction with TRP441. These factors contribute towards reduced potency of **11** compared with **7**. The *S*-conformer did not display any π→π interaction similar to **7**. Here too the ring C is kept away from P1, a rotation around the single bond between thiocarbamoyl C and N1 of pyrazoline puts ring A in P2 and ring B in P3. It clearly shows that *p*-substitution in either ring B or ring C reduces potency towards MAO-A as well as have a great impact on selectivity index also. The interaction of **7**(*R*) and **7**(*S*) are shown in Figure 2.

In MAO-A active site, the *R*-conformer of compound **22**, ring A interacts with P2 while ring B with P3. The carbamoyl group was

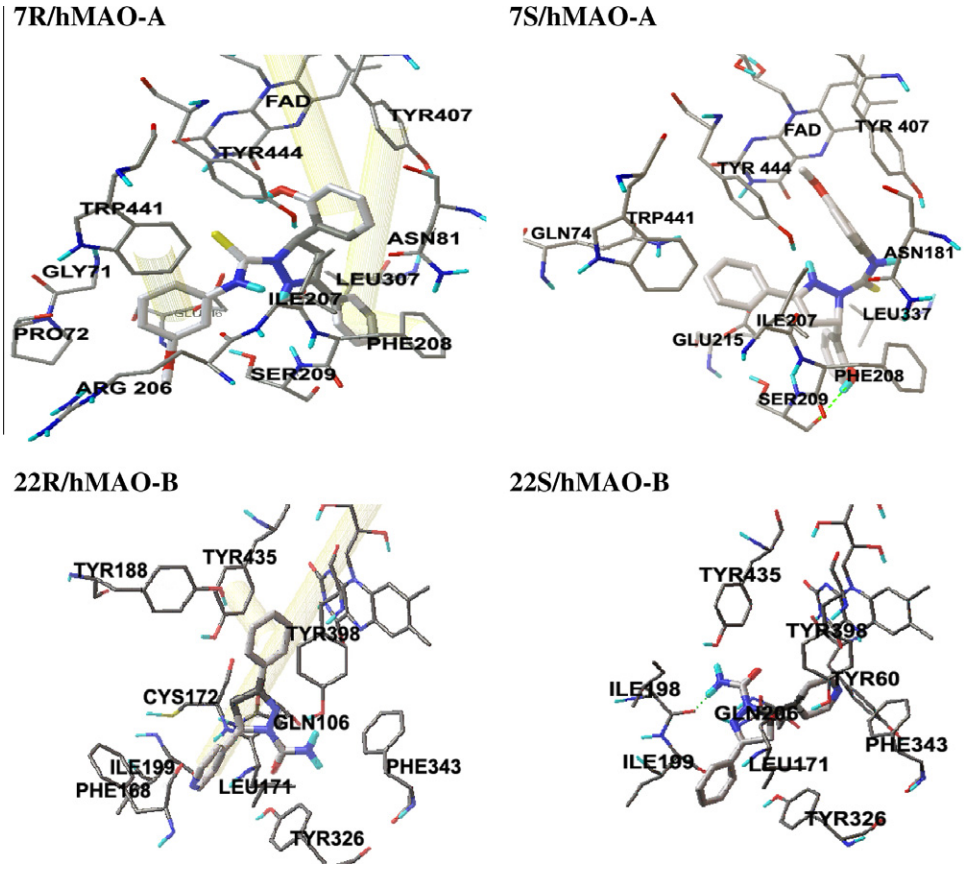


Figure 2. Interaction of **7**(*R*) and **7**(*S*) with hMAO-A (PDB Code: 2BXR) and **22**(*R*) and **22**(*S*) with hMAO-B (PDB Code: 2BYB).

kept away from P1. Only ring B displays one π→π interaction with PHE208. Absence of third ring (non-polar) and exposed –CO–NH_2 group (polar) in highly hydrophobic active site may be the reason for its reduced potency. *S*-conformer too keeps ring A in P2 and ring B in P3, but ring B was not positioned well to show any π→π interaction with PHE208, whereas ring A displays π→π interaction with PHE208. Moreover the pyrazoline N2 and carbamoyl NH displays two H-bonding interaction with TYR444 hydroxy H and O respectively. This additional H-bonding interaction keeps *S*-conformer slightly better than its *R*-conformer.

In MAO-B active site, the *R*-conformer of compound **22**, ring A was very well accommodated in Aromatic Cage (AC, FAD, TYR398 and TYR435) and displays π→π interaction with TYR435. The ring B and carbamoyl group were accommodated in Narrow Hydrophobic Cavity (NHC). In *S*-conformer the entire structure is accommodated in NHC with carbamoyl-NH establishing H-bond interaction with backbone carbonyl-O of ILE198. Both the conformers are equally active with different interaction within the active site of MAO-B. Compound **27**, both *R* and *S* conformers, keep ring A in P2 of MAO-A and none displayed any π→π interaction. But the carbamoyl-NH establishes H-bond interaction with Hydroxyl-O of SER209 (*R*-conformer) or TYR444 (*S*-conformer). This keeps ring C at the entrance of P1 (*R*-conformer, edge–face interaction) or in P3 (*S*-conformer, hydrophobic interaction). In MAO-B active site, ring A of both the conformers were well accommodated in AC and ring B along with thiocarbamoyl group is accommodated in NHC. The ring B displays π→π interaction with either FAD-adenine (*R*-conformer) or TYR188 and TYR326 (*S*-conformer). The interaction of **22**(*R*) and **22**(*S*) are shown in Figure 2.

The *N*-acyl[8,9,11] and *N*-thiocarbamoyl pyrazolines[10,12] were reported earlier with selective MAO-A inhibitory activity. Recently *N*-thiocarbamoyl pyrazolines carrying heteroaryl rings in 3 and 5 positions were reported to have selective MAO-B inhibitory activity at μM concentration.[12] *N*-Carbamoyl pyrazolines were not studied for their MAO inhibitory activity and **22** with selective MAO-B inhibitory activity provides an opportunity to further exploration.

The current study once again establishes the following facts: (i) presence of third aryl ring (ring C) increases potency and selectivity towards MAO-A, (ii) absence of third aryl ring drastically reduces the potency and selectivity towards MAO-A. Further modifications may reveal factors determining nonselectivity and selectivity towards MAO-B and (iii) carbamoyl substitution at N1 position of pyrazoline along with unsubstituted ring A and heterocyclic ring B (4-pyridyl) provides selectivity towards hMAO-B (though poor, this may serve as a starting point for further exploration). Further derivatives should be prepared to explore the possibility of getting a potent and selective hMAO-B inhibitor of this kind.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.05.057.

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