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Towards development of selective and reversible pyrazoline based MAO-inhibitors: Synthesis, biological evaluation and docking studies

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ABSTRACT

Ten novel 3,5-diaryl pyrazolines were synthesized and investigated for their monoamine oxidase (MAO) inhibitory property. All the molecules were found to be reversible and selective inhibitor for either one of the isoform (MAO-A or MAO-B). Further insights in the theoretical evaluation of the possible interactions between the compounds and monoamine oxidases (MAO-A or MAO-B) have been developed through docking studies. The theoretical values are in congruence with their experimental values.

Monoamine oxidase inhibitors (MAOIs) have shown therapeutic value in variety diseases especially neurodegenerative diseases.[1] Earlier, MAO-inhibitors introduced into clinical practice were abandoned due to adverse side-effects, such as hepatotoxicity, orthostatic hypertension and the so called 'cheese effect' characterized by hypertensive crises.[2] These side-effects were hypothesized to be related to nonselective and irreversible monoamine oxidase inhibition. Ability of new generations of inhibitors to selectively inhibit two isoforms (MAO-A and MAO-B) led to a renewed interest in the therapeutic potential of these compounds. These two forms of MAO are characterized by their different sensitivities to inhibitors and their different specificities to substrates.[3] MAO-A preferably metabolizes serotonin, adrenaline, and noradrenaline,[4] whereas β-phenylethylamine and benzylamine are predominantly metabolized by MAO-B.[5] Tyramine, dopamine, and some other important amines are common substrates for both the isoenzymes.[6] Nowadays, the therapeutic interest of MAOIs falls into two major categories. MAO-A inhibitors have been used mostly in the treatment of mental disorders, in particular depression and anxiety,[7–9] while MAO-B inhibitors could be used in the treatment of Parkinson's disease and Alzheimer's disease.[10,11] Efforts have been oriented toward the discovery of reversible and selective inhibitors of MAO-A/MAO-B leading to a new generation of compounds.

The classical period of the MAO-inhibitors started with hydrazine derivatives. Originally proposed as a tuberculostatic agent, their prototype, iproniazid, was the first modern anti-depressant. 2-Pyrazolines can be considered as a cyclic hydrazine moiety. Pyrazolines were reported to have MAO inhibitory and anti-depressant activity.[12–30] Our group has earlier reported thirty two new 3,5-diaryl carbothioamide pyrazolines with MAO inhibitory activity.[31,32] In an effort to develop selective inhibitors of MAO, ten novel analogues were synthesized and screened MAO inhibitory activity. Structure based docking was performed in order to gain more insight towards their binding mode.

The compounds were synthesized as reported earlier.[31,32] The synthetic route has been outlined in Scheme 1. Hydroxy chalcones **1** and **2** were prepared through Claisen–Schmidt condensation of 2-hydroxy acetophenone with either 2-hydroxy benzaldehyde or 4-hydroxy benzaldehyde. Compounds **3** and **4** were synthesized by the reaction of hydrazine hydrate with **1** and **2**, respectively in ethanol. Reaction of benzoyl chloride with compounds **3** and **4** in pyridine provided compounds **5** and **6**, respectively. Reaction of benzene sulfonyl chloride and *p*-toluene sulphonyl chloride with compound **3** and **4** in tetrahydrofuran provided compounds **7**–**10**. Reaction of thiosemicarbazide with **1** and **2** in ethanol and 2 M equivalent of sodium hydroxide provided compounds **11** and **12**, respectively. Compounds **11** and **12** upon treatment with methyl iodide and then with hydroxylamine in methanol provided compounds **13** and **14**, respectively. Structures, physico-chemical and spectral characterization of the synthesized compounds are given in Supplementary data.

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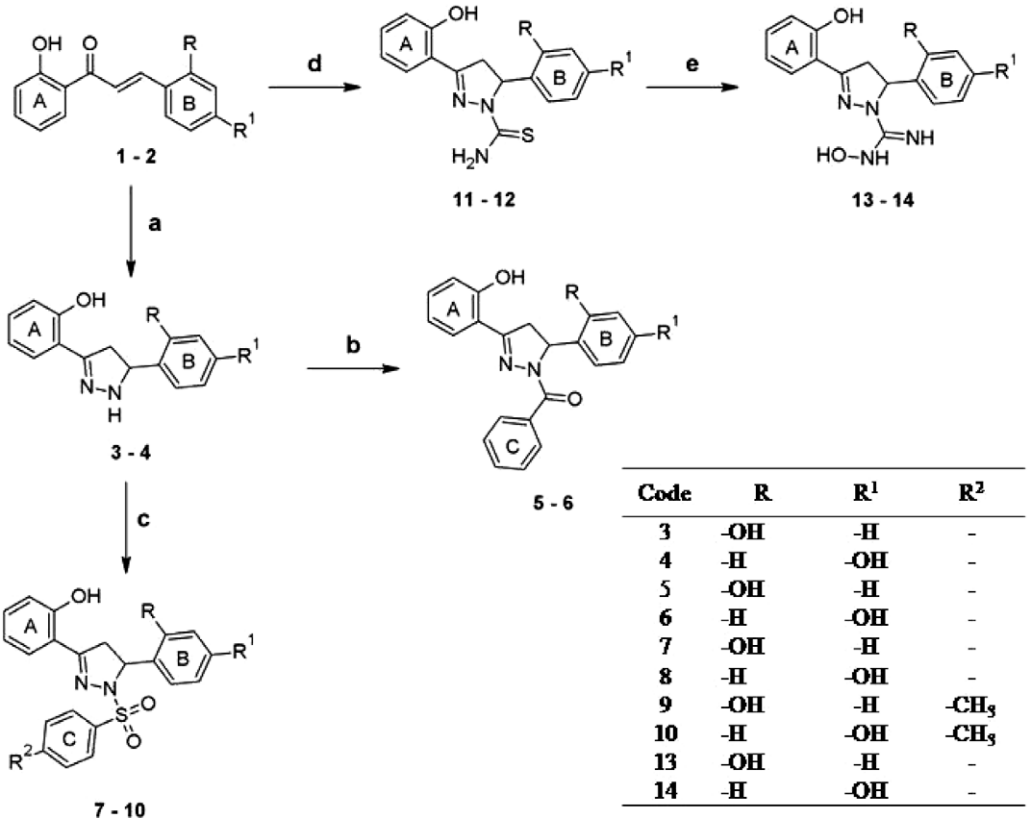

Code	R	R^1	R^2
3	-OH	-H	-
4	-H	-OH	-
5	-OH	-H	-
6	-H	-OH	-
7	-OH	-H	-
8	-H	-OH	-
9	-OH	-H	$-CH_3$
10	-H	-OH	$-CH_3$
13	-OH	-H	-
14	-H	-OH	-

Scheme 1. Synthesis of compounds **3**–**14**. Reagents and conditions: (a) $NH_2NH_2.H_2O$ (80%) excess, C_2H_5OH, reflux 3–6 h; (b) C_6H_5COCl, pyridine, reflux 3 h; (c) R^2-$C_6H_4SO_2Cl$, THF, stirring, 0.5–1 h; (d) thiosemicarbazide, C_2H_5OH, reflux 8–10 h; (e) CH_3I/NH_2OH, stirring 6–12 h.

MAO was purified from the rat liver and total MAO activity was measured spectrophotometrically according to the method of Holt.[33] Assay mixture contained a chromogenic solution consisted of vanillinic acid, 4-aminoantipyrin and peroxidase type II in potassium phosphate buffer (pH 7.6). Assay mixture was pre-incubated with substrate *p*-tyramine before addition of enzyme. The reaction was initiated by the addition of homogenate and increase in absorbance was monitored at 498 nm at 37 °C for 60 min. Molar absorption coefficient of 4654 M^{-1} cm^{-1} was used to calculate the initial velocity of the reaction. Results were expressed as nmol h^{-1} mg^{-1}. For selective measurement of MAO-A and MAO-B activities, homogenates were incubated with the substrate *p*-tyramine following the inhibition of one of the MAO isoform with selective inhibitors. After inhibition of homogenates with selective inhibitors, total MAO activity was determined by method of Holt. Newly synthesized compounds were dissolved in DMSO and used in the concentration range of 1–1000 μM. Inhibitors were then incubated with purified MAO at 37 °C for 0–60 min prior to adding to the assay mixture. Reversibility of the inhibition of MAO by these compounds was assessed by dilution. Kinetic data for interaction of the enzyme with these compounds were determined using Microsoft Excel package program. IC_{50} values were determined from plots of residual activity percentage, calculated in relation to a sample of the enzyme treated under the same conditions without inhibitor, versus inhibitor concentration [I].

All the synthesized compounds were selective and reversible inhibitor towards either MAO-A or MAO-B. Compounds **3** and **4** selectively inhibit MAO-B, with a selectivity index of 4.74 and 6.491, respectively. All the other analogues were selective towards MAO-A, with outstanding selectivity index in the magnitude of 10^{-4}–10^{-6} (**5**, **6** and **8**–**10**, Fig. 1). Their structure–activity relationship suggest that, any substitution at 1*N*-position of pyrazoline shifts the selectivity towards MAO-A. Substitution with toluene sulphonyl derivative (**9** and **10**) at 1*N*-position provided the most active and most selective MAO-A inhibitor.

In order to gain more insight on the binding mode of the compounds with MAO-A and MAO-B docking studies using AUTODOCK 4.0 (automated by separate UNIX script[34]) were employed. Marvin Sketch (Chemaxon), Protein Preparation Wizard (Schrodinger Inc.) and Open Babel were used for ligand and protein preparation. Top scoring molecules from the largest cluster were considered for interaction studies. The calculated inhibition constants (K_i) as well as their experimental values for each enzyme–inhibitor complex are shown in Table 1. Interestingly, the results obtained computationally are in good agreement with their experimental values. Ideally, any docking program should be capable of sorting the compounds according to their activity. Prediction of real binding affinities is an important aspect of any scoring function and their score (binding energy) should reflect their biological activity. Spearman's rank order correlation coefficient ρ (**1**) was previously employed[35–37] to evaluate docking and scoring protocols.

$$\rho = 1 - \frac{6\sum_{i}^{n}[r(x_i) - r(y_i)]^2}{n^3 - n} \quad (1)$$

Where n is the number of pairs $n = 10$ in our case, $r(x_i)$ and $r(y_i)$ are the rank of the activity and the interaction energy of the ith sample in the testing set. In theory, the Spearman correlation coefficient falls between −1 and +1, where +1 corresponds to a perfect correlation, −1 corresponds to a perfect inverse correlation, and zero corresponds to total disorder. An excellent rank order correlation coefficient for MAO-A, $\rho = 0.964$, and for MAO-B, $\rho = 0.936$ was obtained. Justifying the search algorithm and scoring function employed in the current study (Figs. 1 and 2a and b).

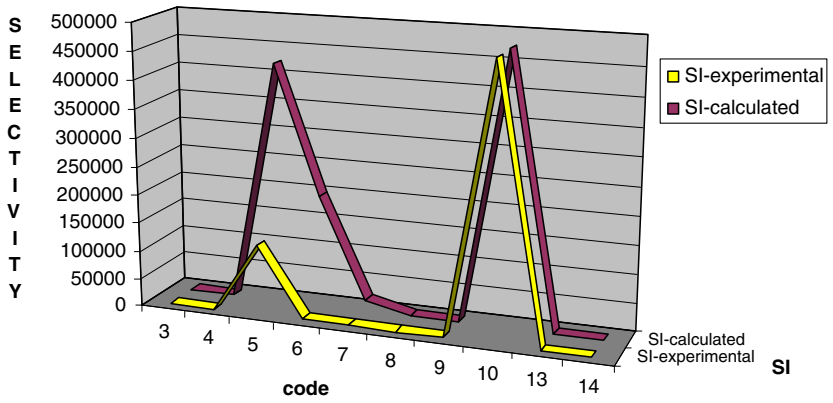

Figure 1. Experimental and calculated selectivity indices (calculated as K_i (MAO-A)/K_i (MAO-B)) of the newly synthesized pyrazoline derivatives show a very close correlation.

Table 1
Calculated and experimental K_i values corresponding to the inhibition of MAO isoforms by the newly synthesized pyrazoline derivatives

Code	[a]Experimental (K_i)		[b]Calculated (K_i)		Inhibition type	Reversibility	MAO inhibitory selectivity
	MAO-A (μM)	MAO-B (μM)	MAO-A (μM)	MAO-B (μM)			
3	3.7	0.78	1.94	0.54	Competitive	Reversible	Selective for MAO-B
4	3.9	0.60	2.65	0.60	Competitive	Reversible	Selective for MAO-B
5	0.80	99230	0.83	349410	Competitive	Reversible	Selective for MAO-A
6	5.77	20330	3.82	754150	Competitive	Reversible	Selective for MAO-A
7	0.91	1550	0.39	7300	Competitive	Reversible	Selective for MAO-A
8	0.68	700.2	0.16	209.72	Competitive	Reversible	Selective for MAO-A
9	0.36	620	0.28	462.23	Competitive	Reversible	Selective for MAO-A
10	0.60	285900	0.34	—	Competitive	Reversible	Selective for MAO-A
13	15.66	96.8	17.09	88.22	Competitive	Reversible	Selective for MAO-A
14	10.6	210	8.97	167.09	Competitive	Reversible	Selective for MAO-A
SEL	105.66	1.35	ND	ND	Competitive	Reversible	Selective for MAO-B
MOC	0.005	1.08	ND	ND	Competitive	Reversible	Selective for MAO-A

SEL-Selegeline, MOC-Moclobemide.
[a] 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 pre-incubated with the homoganates for 60 min at 37 °C. Each value represents the mean ± SEM of three independent experiments.
[b] Values obtained through AUTODOCK program.

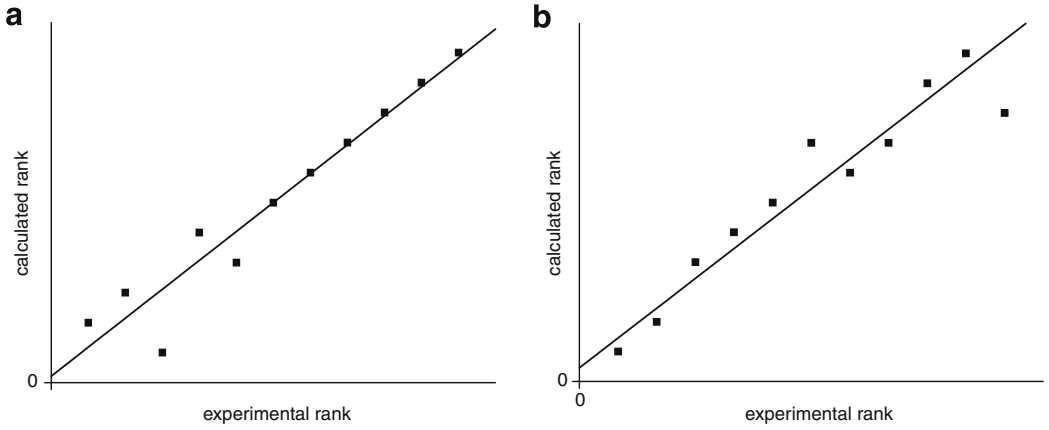

Figure 2. Plot of calculated versus experimental rank. (a) For MAO-A and (b) for MAO-B. Spearman's rank order correlation coefficient ρ MAO-A, $\rho = 0.964$, and for MAO-B, $\rho = 0.936$ was obtained.

Colibus et al.[38] meticulously depicted the structural comparison of human MAO-A and MAO-B. According to their findings MAO-A and MAO-B share a high sequence similarity of 72%, overall structure of human MAO-A is quite similar to that of MAO B (rms deviation of 1.2 Å, 488 equiv C-α atom). The only significant structural difference observed is the conformation of the cavity-shaping loop of MAO-A from 210 to 216 (201–207 in MAO-B) which results in Cα movements up to 6 Å.

Binding of these ligands to either MAO-A or MAO-B reveals the importance substitution in 1*N*-position of pyrazolines. Their subtle variation in this position can greatly influence both the selectivity as well as their potency.

In complex of compounds **7**–**10** with MAO-A, hydrogen bonding interaction was observed between the sulphonyl oxygen of the ligands and hydroxyl hydrogen of either TYR444 (compounds **7**–**8**) or TYR407 (compounds **9**–**10**). Due these Hydrogen bonding interactions ring B of compounds **7**–**8** and ring C of compounds **9**–**10** were positioned in the aromatic cage (FAD, TYR407 and TYR444, Pocket1). A para methyl substitution of ring C of compounds **9**–**10** considerably changes the orientation of the ligand in the active site that results in significant increase in potency as well as selectivity indices towards MAO-A compared with compounds **7**–**8**. The other two phenyl rings were well accommodated in pocket2 (ILE180, ILE335, LEU337, MET350, PHE352) and pocket3 (due to cavity-shaping loop, GLY74, ARG206, ILE207, PHE208, GLU216, TRP441). Interaction of compounds **4**, **6**, **8** and **10** with human MAO-A (2BXR) was given in Figure 3a.

In complex of compounds **3**–**4** with MAO-B, the hydrogen bonding interaction was found between 1*N* hydrogen of pyrazoline ring and hydroxyl oxygen of TYR435 while 2*N* hydrogen with carbonyl oxygen of ILE199. Due to this interaction the ring B and ring A of compounds **3** and **4** were positioned in the pocket1 (aromatic cage) and in narrow cavity (pocket 2 and 3 were reduced and merged to narrow cavity in MAO-B due to different orientation of the cavity forming loop, Figure 3b) respectively. Compounds **3**–**4** interacts with MAO-B better than MAO-A due to the interaction of ring B with catalytic pocket1 of MAO-B that is missing in case of MAO-A (Fig. 3a and b, compound **4** represented in blue colour). Compounds **5**–**10** with additional ring C experiences a significant steric hindrance in the narrow cavity that accounts for its poor interaction of these compounds with MAO-B. Interaction of compounds **4**, **6**, **8** and **10** with human MAO-B (2BYB) was given in Figure 3b.

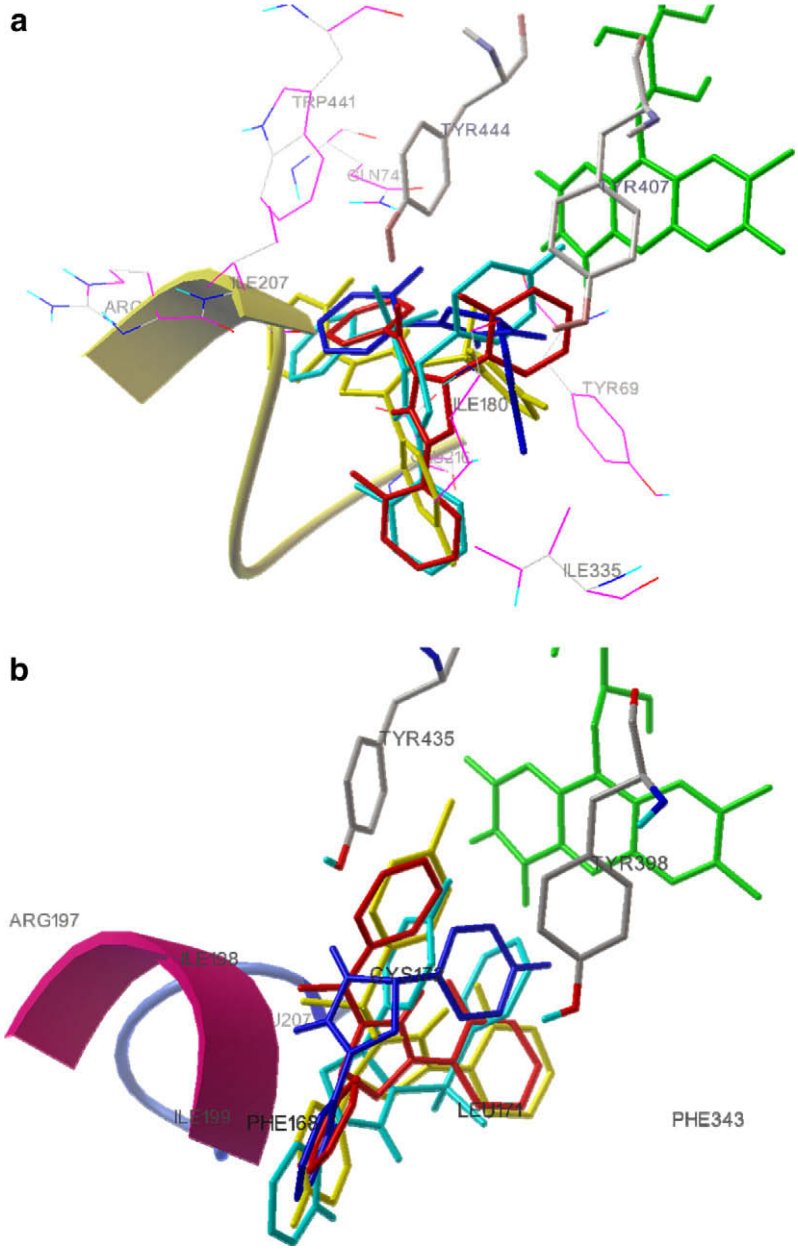

Figure 3. (a) Interaction of compounds **4**, **6**, **8** and **10** with MAO-A (2BXR), (b) interaction of compounds **4**, **6**, **8** and **10** with MAO-A (2BYB). FAD in green colour, compound **4** in dark blue colour, compound **6** in red colour, compound **8** in light blue colour and compound **10** in yellow colour. TYR of aromatic cage are in tube representation coloured by atom type and cavity-shaping loop residues of MAO-A and its equivalent in MAO-B are represented as ribbon.

From the above study we have successfully identified few compounds which are reversible and selective inhibitor of either MAO-A or MAO-B. A concise idea about their structure activity relationship was also drawn and importance of different side chain substitutions especially at 1*N*-position was understood. Presence any substitution at 1*N*-position shifts the selectivity towards MAO-A. There binding mode analysis with the help of molecular docking provided imperative insights about their molecular recognition process it also provided information which is helpful in designing other selective and reversible MAO-inhibitors in future.

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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.2009.11.015.

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