Letters

Monoamine Oxidase Isoform-Dependent Tautomeric Influence in the Recognition of 3,5-Diaryl Pyrazole Inhibitors

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Abstract: A series of 3,5-diaryl pyrazoles were prepared and assayed for their ability to inhibit reversibly monoamine oxidase-A (MAO-A) and monoamine oxidase B (MAO-B). Several compounds show inhibitory activity with concentration values in the nanomolar range. A computational work was carried out on the two most selective inhibitors that have tautomeric pyrazole forms. The binding free energies of these compounds for each MAO isoform were influenced by the tautomeric equilibria.

Monoamine oxidases (MAO, EC 1.4.3.4), widely distributed in all living organisms, are FAD-containing enzymes, tightly bound to the outer mitochondrial membrane. In mammals, two different isoforms of MAOs are present, namely, MAO-A and MAO-B. MAO-A is located predominantly in catecholaminergic neurons, while MAO-B is present in serotonergic neurons and glia.[1,2] Both isoforms are characterized by specific substrates and inhibitors. MAO-A has a higher affinity for serotonin and norepinephrine and is more sensitive to inhibition by clorgyline, while MAO-B preferentially deaminates β-phenylethylamines and benzylamine and is sensitive to low concentrations of L-deprenyl.[3,4] Dopamine, tyramine, and tryptamine are common substrates for both MAOs.

Efforts have been addressed to the design, synthesis, and study of new reversible and selective MAO inhibitors, leading to compounds such as toloxatone[5] and moclobemide.[6] These compounds selectively block MAO-A and do not form a covalent bond with the MAO enzyme, acting via a reversible inhibitory mechanism. These MAO-A inhibitors retain antidepressant effects in animal models and are devoid of the severe food and drug incompatibilities induced by the first generation of MAO inhibitors.

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Table 1. Chemical and Physical Data and Anti-MAO Activity of Pyrazole **3a**−**k**[a]

cmpd	R	R^1	mp (°C)	yield (%)	pIC_{50} MAO-A	pIC_{50} MAO-B	pSI[b] selectivity
3a	H	H	193−5	24	9.00	8.00	1.00
3b	H	4-Cl	217−9	35	6.52	8.22	−1.70
3c	H	4-F	196−8	25	5.39	7.52	−2.13
3d	4-Cl	4-Cl	240−2	35	8.69	5.22	3.47
3e	4-Cl	4-F	220−2	35	8.52	7.39	1.13
3f	4-F	4-F	182−4	29	5.20	4.00	1.20
3g	4-CH_3	H	193−5	43	5.82	9.00	−3.18
3h	4-CH_3	4-Cl	211−3	39	5.52	8.05	−2.53
3j	4-CH_3	4-F	215−7	27	5.64	7.00	−1.36
3k	4-CH_3	4-CH_3	171−4	35	7.85	6.35	1.50
MCL[c]					4.94	2.00	2.94
TOL[d]					6.42	4.82	1.60
SEL[e]					4.42	6.00	−1.58

[a] The data represent mean values of at least three separate experiments. [b] pSI = log selectivity index = pIC_{50} (MAO-A) − pIC_{50} (MAO-B). [c] Moclobemide. [d] Toloxatone. [e] Selegiline.

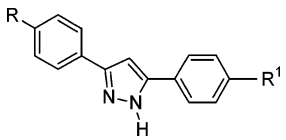

Figure 1. General formula of 3,5-diaryl pyrazole.

The B isoform is known to be important, especially after the discovery that MAO-B oxidizes a Parkinsonism-producing neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, to form an active neurotoxin, 1-methyl-4-phenylpyridinium ion.[7] Because L-deprenyl, a representative MAO-B inhibitor, may have neuroprotective activity especially for dopamine neurons, it is used in treatment of Parkinson's disease. MAO-B inhibitors are also currently used in clinical trials in the treatment of Alzheimer's disease because an increased level of MAO-B has been detected in the plaque-associated astrocytes of brains from Alzheimer's patients.[8]

Different families of heterocycles containing two or four nitrogen atoms have been used as scaffolds for synthesizing reversible and selective monoamino oxidase inhibitors (MAO-Is-A and MAO-Is-B). In particular, Wouters et al.[9] studied oxadiazolone, tetrazole, oxadiazinone, and indenopyridazine derivatives as potent, reversible, and selective MAO-B inhibitors. Altomare et al. studied the MAO inhibitory activity of isoquinoline derivatives and condensed pyridazines.[10,11] From the activities of diazine derivatives as MAO inhibitors, it emerges that most of the condensed pyridazines are reversible MAO-B inhibitors, with none or little effect on MAO-A, while the condensed pyrimidines are reversible inhibitors endowed with an appreciable selectivity toward MAO-A. Substituents on the diazine nucleus modulate the inhibitory activity. In contrast, the nature of the ring(s) condensed with diazine appears to affect selectivity toward MAO-A and MAO-B significantly.

With this in mind, and continuing in our study of reversible and selective pyrazole derivatives as inhibitors of MAO-A and MAO-B isoforms,[12] we here report the synthesis of a new series of compounds featuring the pyrazole ring, to verify if the planar conformation affects the inhibitory activity. The 3,5-diphenyl

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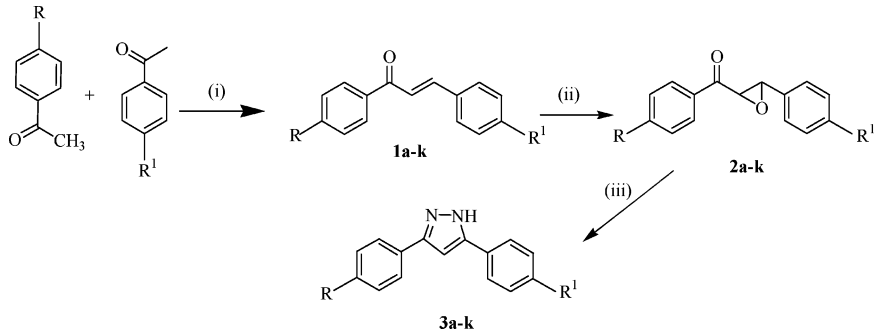
Scheme 1[a]



[a] Reagents and conditions: (i) $Ba(OH)_2$, EtOH 96%, 30 °C; (ii) H_2O_2 35%, EtOH dry, K_2CO_3; (iii) N_2H_4, *p*-toluenesulfonic acid, xylenes, refluxed.

pyrazole derivatives **3a**−**k**, listed in Table 1 and shown in Figure 1, were prepared according to the methods shown in Scheme 1.

Treatment of an appropriate acetophenone with benzaldehydes gave α,*β*-unsaturated ketones (chalcones) **1a**−**k** by a typical base-catalyzed aldol condensation[13] in 50−80% yields (Scheme 1). The reaction of 2-propen-1-ones **1a**−**k** with an excess of aqueous hydrogen peroxide in ethanol gave the corresponding chalcone epoxide **2a**−**k**; the reaction of suitable epoxide with hydrazine monohydrate 98% and *p*-toluenesulfonic acid monohydrate in boiling ethanol gave the corresponding 3,5-diphenyl pyrazoles **3a**−**k**.[14] The structures of compounds **3a**−**k** were confirmed by IR, mass spectra, and ^{1}H NMR analyses.

Pyrazoles **3a**−**k** were evaluated for their ability to inhibit MAO-A and MAO-B. The pIC_{50} values against the two MAO isoforms and the A-selectivity are summarized in Table 1. The A-selectivity has been expressed as pSI and represents the log selectivity index calculated as pIC_{50} (MAO-A) − pIC_{50} (MAO-B). The positive value means a MAOI-A and the negative one means a MAOI-B.

All the tested derivatives showed a reversible mode of action, because dialysis for 24 h in a cold room against a 0.1 M potassium phosphate buffer (pH 7.2) could restore 90 to 100% of the enzyme activity.

As reported in Table 1, some derivatives of **3** show inhibitory activity with concentration values in the nanomolar range. The best MAO-A inhibitors are **3a**, **3d**, and **3e**, with pIC_{50} values in the nanomolar range (8.52−9.00), while the best MAO-B inhibitors are **3a**, **3b**, **3g**, and **3h**, with pIC_{50} values in the nanomolar range (8.00−9.00). Compound **3a** shows good inhibitory activity against MAO-A and MAO-B but low selectivity (pIC_{50} MAO-A = 9.00, pIC_{50} MAO-B = 8.00, and pSI = 1.00). Compounds **3b** and **3c**, which bear a halogen atom on the 4-position of the 3,5-aryl moieties, show good inhibitory activity against MAO-B associated with good inhibitory selectivity. Compound **3d**, which bears the chloro substituent in the 4-position of the aryl moieties, shows good inhibitory activity against MAO-A associated with high selectivity.

Compound **3e**, which bears fluorine and chlorine atoms on the 4-position of the 3,5-aryl moieties, and compound **3f**, which bears the fluorine atoms on the 4-position of the aryl moieties, show lower inhibitory activity on both isoforms.

Finally, compounds **3g** (R = 4-CH_3, R^1 = H), **3h** (R = 4-CH_3, R^1 = 4-Cl), and **3j** (R = 4-CH_3, R^1 = 4-F) show good inhibitory activity against MAO-B associated with high selectivity.

Among all, compound **3g** is the most interesting inhibitor, with a good inhibitory activity on the MAO-B isoform (pIC_{50} MAO-B = 9.00) and a high selectivity (pSI = −3.18).

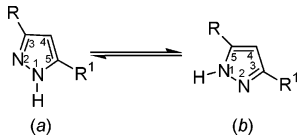


Figure 2. Tautomeric forms of pyrazole.

Taking into account that 1*H*-pyrazoles can exist in two tautomeric forms *a* and *b* (Figure 2) and that when the substituents at the 4-position of the 3- and 5-aryl moieties are different (R ≠ R^1), the two tautomers are nonequivalent,[15,16] we highlighted the effect of tautomerism of the most active and selective derivative **3g** on the interaction with the active site of the enzyme. The same procedure was repeated also for compound **3h**, which also exists in two tautomeric forms and shows good inhibitory activity against MAO-B associated with good selectivity.

Taking into account the large electronic delocalization of these 3,5-diaryl pyrazole derivatives, both *a* and *b* tautomeric isomers of **3g** and **3h** were built in planar conformation and energy evaluated by ab initio techniques.[17]

The computational study was carried out following our previously reported approaches,[12] focusing on the most active and selective inhibitors, **3g** and **3h**, which can undergo the tautomeric equilibrium shown in Figure 2.

Pretreatment of the enzyme models is summarized in the Supporting Information.

Thanks to the extreme simplicity of the compound in terms of free rotatable bonds, we could skip the conformational search. Energy optimization was carried out by the DFT B3LYP method with the 6-311G**++ basis set. The internal energy difference between the two optimized isomers proved to be lower than 0.08 kcal/mol, demonstrating that both are significantly populated at room temperature. Therefore, both *a* and *b* tautomers were included in the docking experiments using crystallographic MAO-A and MAO-B models,[18,19] available in the Protein Data Bank (PDB) as targets.[20] After pretreatment of the isoform models, the GLIDE approach[21] was used to generate configurations of the two tautomers in the enzymatic clefts using a box of about 110 000 Å^3 centered on the FAD N5 atom. The most stable 10 GLIDE poses of the 5000 generated were fully energy minimized with the AMBER* united atom force field in GB/SA water[22,23] and submitted to the MM-GBSA binding mode analysis.[24]

Each energy minimum pose was submitted to a refinement based on a 500 ps molecular dynamics run carried out at 300 °K with a 1.5 fs time step. Fifty conformations were regularly collected each 10 ps and energy minimized, in the same force field and solvation conditions, to obtain a final ensemble. The thermodynamic evaluation was performed on these optimized

Table 2. Thermodynamic Details of **3g** and **3h** Tautomers (Tau) into the Enzymatic Clefts: Relative Internal Energies (RIE) and Boltzmann Population (Prob %)

		MAO-A		MAO-B	
cmpd	Tau	RIE	prob %	RIE	prob %
3g	*a*	74.5	0	84.6	0
	b	0.0	100	0.0	100
3h	*a*	0.0	100	33.0	0
	b	17.7	0	0.0	100

Table 3. Number of Optimized Configurations (nconf) and Binding Free Energy ($\cdot G_{bind}$) in kcal/mol Computed at 300°K for Each **3g** and **3h** Tautomer (Tau) Complexed into the MAO-A and MAO-B Enzymatic Clefts

		MAO-A		MAO-B	
cmpd	Tau	nconf	$\cdot G_{bind}$	nconf	$\cdot G_{bind}$
3g	*a*	39	−47.16	45	−41.38
	b	41	−43.69	44	−46.60
3h	*a*	43	−42.24	43	−44.14
	b	44	−35.21	49	−44.34

configurations, considering the Boltzmann population at 300°K of the enzyme−ligand complex computed unifying *a* and *b* tautomer ensembles related to the same compound/isoform complex. Such an approach allowed us to obtain a single ensemble for each compound with respect to each enzyme. To identify the role of each tautomer in the MAO recognition, the probability was carried out using the all-energy contributions of the 1:1 complexes (Table 2).

Further details of modeling protocol are reported in the Supporting Information.

As shown in Table 2, a consistent effect in the tautomer recognition was highlighted by the RIE and Boltzmann population, with extreme values equal to 0 or 100%, derived from global complex internal energies.

Considering the binding free energies of the most populated configurations (Table 3), a good agreement can be appreciated with the experimental inhibition trend. Nevertheless, this agreement should be critically taken into account, at least due to the difficulty to evaluate, by molecular mechanics force fields specific interaction, such as the $\pi-\pi$ stacking of the side chain of aromatic residues and the rings of our ligands substituted with electron-different R and R^1 moieties. The graphical inspection and LigPlot analysis[25] of the energy-minimum complexes revealed the recognition patterns of **3g** and **3h** into the enzyme clefts mainly characterized by $\pi-\pi$ stacking and other contacts (see Supporting Information).

The most probable and stable complex of the tautomer *b* of **3g** in the MAO-B isoform showed contacts typical of many potent inhibitors: a double $\pi-\pi$ stacking of the R ring between Tyr398 and Tyr435 and other interactions in the hydrophobic site delimited by Tyr326, Leu328, Phe343, Ile199, Phe168, Leu171, and Cys172 (Figure 3).[26]

Other relevant contacts were found in two intermolecular hydrogen bonds established by the pyrazole, respectively, with the side chain of Tyr398 (phenolic oxygen) and Gln206 (amidic hydrogen).

In conclusion, this study highlights that two equally stable pyrazole tautomers undergo a large equilibrium perturbation induced by MAO isoform recognition. These results will be used to pursue our research in this field and to provide useful information on the rational design of novel potent MAO-B inhibitors.

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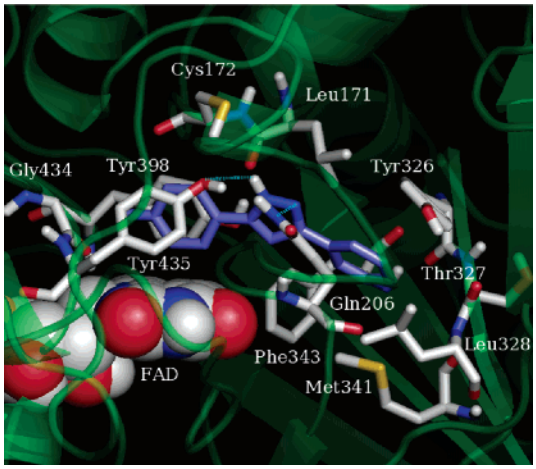

Figure 3. Most probable and stable complex of **3g** (tautomer *b*) and MAO-B. Interacting residues of the active site are shown in labelled polytubes, FAD in CPK rendering, the compound in blue carbon polytube, and other aminoacids in ribbon. Hydrogen bonds are displayed as dashed lines.

Supporting Information Available: ^{1}H NMR spectral data and elemental analyses of derivatives **3a**−**k**. Molecular modeling details about the computational protocol, one table, and seven figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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