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Synthesis and molecular modelling studies of prenylated pyrazolines as MAO-B inhibitors

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ABSTRACT

A series of *N*-substituted-3-[(2′-hydroxy-4′-prenyloxy)-phenyl]-5-phenyl-4,5-dihydro-(1*H*)-pyrazolines were synthesized and tested on human monoamine oxidase-A and -B isoforms. Structure–activity relationships and molecular modelling showed that some substitutions, such as benzyloxy or chlorine atom, improve the best interaction with active site of hMAO-B.

Monoamine oxidase (MAO, EC 1.4.3.4, amine–oxygen oxidoreductase) is a membrane-bound flavoenzyme responsible for the oxidative deamination of xenobiotic amines[1] and monoamine neurotransmitters such as serotonin (5-HT), norepinephrine (NE), and dopamine (DA).[2]

Mammals, including humans, contain two isozymes, MAO-A and MAO-B, distinguished by substrate and inhibitor selectivity[3,4] MAO-A preferentially catalyses the oxidation of serotonin and norephinephrine and is inhibited by clorgyline, whereas MAO-B catalyses the oxidation of phenyl ethylamine and benzyl amine and is inhibited by (*R*)(−)-deprenyl. Tyramine, dopamine, and tryptamine are substrates for both enzymes. Because of their role in the metabolism of monoamine neurotransmitters, the MAO-A and MAO-B are thought to be involved in psychiatric and neurological disorders such as depression and Parkinson's disease, respectively.[5] For this reason the identification of MAO inhibitors (MAO-Is) is of great interest in drug discovery[3,4] since inhibitors of MAO are used as antidepressants and neuroprotectants.[4,6] In particular, MAO-A inhibitors are useful as antidepressants,[7,8] and MAO-B inhibitors in the treatment of Parkinson's disease.[9–11] Inhibition of MAO-A results in increased brain levels of biogenic amines, including noradrenaline and serotonin which are pathologically decreased in depression. Inhibition of MAO-B leads to an increase in the neurotransmitter, dopamine. Dopamine is involved in reward reinforcement[12] and low levels of this neurotransmitter occur in Parkinson's disease.[9]

Bearing in mind what reported in the literature about heterocyclic monoamine oxidase inhibitors with pyrazole and pyrazoline structures[13] and pursuing our study in this field,[14–16] here we report the synthesis of new derivatives **3a–k** (Fig. 1), the biological data, and the molecular modelling study of the most active compounds.

The newly compounds bearing, isoprenoid chain, on aromatic group in C3, because as reported in literature the remarkable

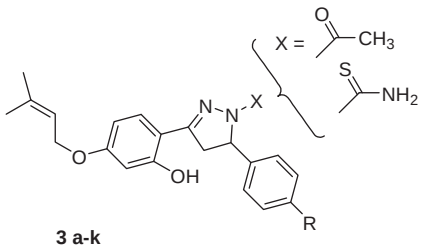

Figure 1. Derivatives **3a–k**: *N*-substituted-3(2′-hydroxy-4′-prenyloxy)phenyl-5-phenyl-4,5-dihydro-(1*H*)-pyrazoles.

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properties of some natural compounds as prenylated flavonoids are thought to reside in their enhanced interaction with biological membranes and increased affinity for target proteins compared with their non-prenylated analogues.[17–20] Moreover, in the past, we synthesized and tested some prenylated chalcones,[21] which showed a poor inhibitory activity on MAO-B; so this work would enable us to compare the MAO inhibition activities of the chalcones to that of the pyrazoles.

The biological activities on novel and reference compounds are reported in Table 1. As shown, the most of derivatives synthesized showed an interesting inhibitory activity on hMAO-B isoform with no efficacy towards hMAO-A, except derivative **3d**, which shows good inhibition for both isoforms. The more potent hMAO-B inhibitors are those with a benzyloxy group in *para* position at C5 (compounds **3d** and **3j**) and those with chlorine substituent in the same position (compounds **3e** and **3k**). The introduction of methyl and methoxy groups (**3b**, **3c**, **3g** and **3h**), in the same position, gave a significant decrease or loss in the inhibition. Furthermore, we can suppose that the different behaviour of the derivatives **3d**, **3j** and **3e**, **3k** is probably closely related to the substituents on N1 as well.

So, following our previously reported studies,[17,19] and to pointed out the importance of different substituents, we focused by molecular modelling techniques our attention on the interaction of both (*R*)- and (*S*)-stereoisomers of the most potent inhibitors **3d**, **3e**, **3j** and **3k** within the hMAO binding clefts as reported in Supplementary data. The interaction energy of complexation has been considered to evaluate the binding modes proposed for **3d**, **3e**, **3j** and **3k**. Theoretical results have been able to reproduce the experimental hMAO-B inhibition of all compounds but no recognition of the hMAO-A has been suggested. Actually all molecules, including the **3d**, highlighted unsatisfactory interaction to such an isoform. So our analysis has been focused onto the hMAO-B only. In this isoform tested compounds were ranked with interaction energies in the range from −11.10 to −13.20 kcal/mol with slight differences between the enantiomers (Table 2).

All compounds reported similar binding modes into the hMAO-B. In all cases the isoprenyloxy moiety was directed towards the FAD cofactor and the C5 pyrazoline substituents towards the outer side of the hMAO-B cleft. Solvent water molecules, by means of hydrogen bonds, have contributed to stabilize the recognition of (*R*)-**3d**, (*R*)-**3j**, (*S*)-**3j** and (*R*)-**3k** (see Supplementary data). Taking into account the potency and the chemical similarity we decided to, respectively, compare the most stable complexes of **3d** versus **3j** and of **3e** versus **3k** (Fig. 2).

Table 2
The experimental IC_{50} values (racemic mixture) and theoretical interaction energies of **3d**, **3e**, **3j** and **3k** with hMAO-B for all enantiomers.

Compound	hMAO-B interaction energy (kcal/mol)			Exp. pIC_{50}
	(*R*)	(*S*)	Average	
3d	−13.00	−13.20	−13.10	6.76
3e	−11.30	−11.10	−11.20	5.06
3j	−12.30	−13.20	−12.75	6.57
3k	−12.80	−12.90	−12.85	6.08

In both **3d** and **3j** cases the (*S*)-enantiomer showed a slightly better interaction energy with respect to the (*R*)-one. Interestingly the (*S*)-**3d** did not reported hydrogen bonds to the target, but it highlighted two π–π stacking contacts, respectively, involving the pyrazoline ring and Tyr326, and the benzyloxy moiety and Trp119. The quite chemically similar compound (*S*)-**3j** showed remarkable difference with respect to (*S*)-**3d**: the thioamide group established an hydrogen bond to the Ile199 backbone. This interaction promoted two hydrogen bonds among the pyrazole ring and the phenolic hydroxyl group to a water molecules bridging by the same interaction to Thr201 side chain. Such a network of productive interaction moved the (*S*)-**3j** far from the stacking involved residues reported for the (*S*)-**3d** and could be considered as the strongest recognition difference between these two analogues into the hMAO-B. The comparison between **3e** and **3k** showed a different scenario. First of all, the best interacting stereoisomer was (*R*)- for **3e** and (*S*)- for **3k**. The **3e**, the less potent among modelled inhibitors, was not able to produce significant interactions to hMAO-B, actually no hydrogen bond or stacking contacts were reported. The driving force the hMAO-B **3e** recognition can be addressed to hydrophobic interaction only: actually the pyrazole and the chloro-phenyl rings were located into a lipophilic cage delimited by Ile199, Ile316 and Tyr326 and the phenolic ring was surrounded by Leu171 and Ile198. The compound **3k**, showed a binding mode similar to **3j**, two hydrogen bonds were highlighted,

Table 1
Chemical structure and anti-MAO efficacy of derivatives **3a–k**. IC_{50} values and MAO-B selectivity ratios [IC_{50} (MAO-A)]/[IC_{50} (MAO-B)] for the inhibitory effects of test drugs (new compounds and reference inhibitors) on the enzymatic activity of human recombinant MAO isoforms expressed in baculovirus infected BTI insect cells.

Compound	R	X	mp (°C)	hMAO-A (pIC_{50})[a]	hMAO-B (pIC_{50})[a]
3a	H	$COCH_3$	274–275	**	**
3b	CH_3	$COCH_3$	248–249	**	**
3c	OCH_3	$COCH_3$	237–239	**	4.22
3d	OCH_2Ph	$COCH_3$	160–163	6.50	6.76
3e	Cl	$COCH_3$	175–177	**	5.06
3f	H	$CSNH_2$	205–207	**	4.28
3g	CH_3	$CSNH_2$	235–236	**	**
3h	OCH_3	$CSNH_2$	202–205	**	**
3j	OCH_2Ph	$CSNH_2$	175–176	**	6.57
3k	Cl	$CSNH_2$	186–189	**	6.08
Clorgyline[b]				8.35	4.21
Deprenyl[c]				4.17	7.70
Iproniazid[b]				5.18	5.12
Moclobemide[b]				3.44	*
Isatin[d]				4.25	5.10

All IC_{50} values (new compounds and references inhibitors) have been estimated in this work as described in Ref. 13 using *p*-tyramine (5 mM) as common substrate for hMAO-A and (5 mM) for hMAO-B (1 mM). Results are mean ± S.E.M. from five experiments. *Inactive at 1 mM (highest concentration tested). **Inactive at 100 μM (highest concentration tested).

[a] $pIC_{50} = -\log IC_{50}$.
[b] See Ref. [22].
[c] See Ref. [23].
[d] See Ref. [24].

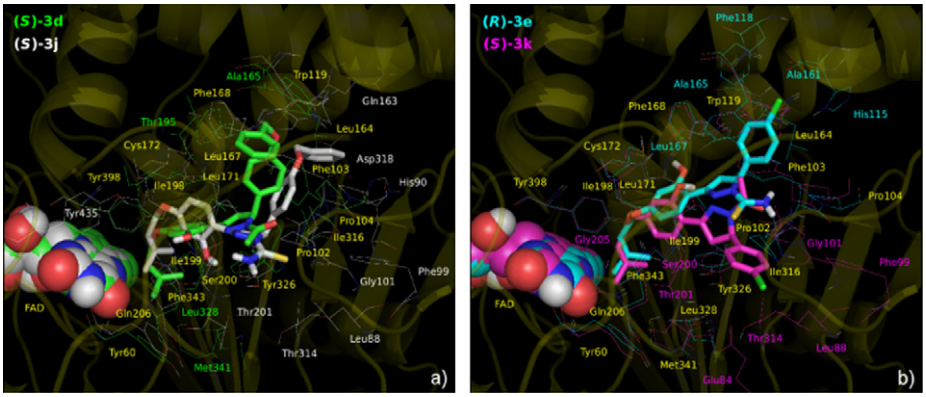
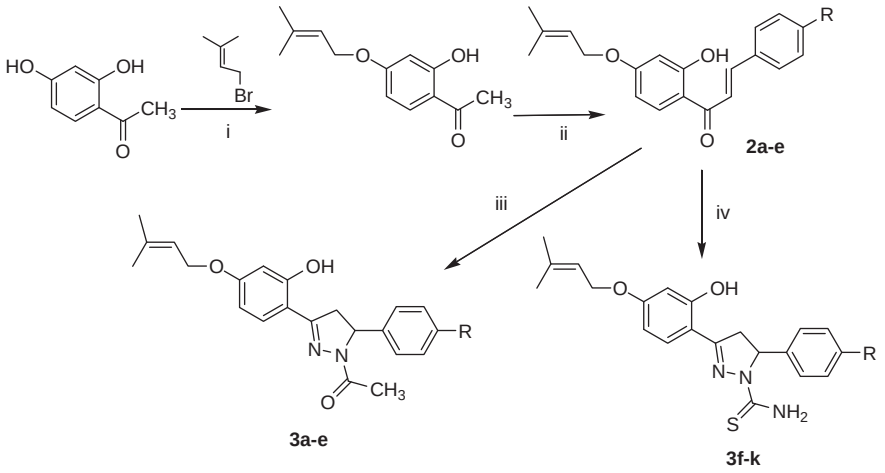


Figure 2. Comparison among the most stable hMAO-B theoretical complexes of a) **3d** (green carbons) and **3j** (white carbons) and b) **3e** (cyan carbons) and **3k** (magenta carbons). The enantiomers are reported in polytube. The enzyme is showed in transparent yellow cartoon, the FAD cofactor is displayed in spacefill rendering. Interacting residues are reported in wireframe and coloured according to the respective complex model. Residues interacting to both displayed enantiomers are labelled in yellow. Solvent molecules and hydrogen bonds are hidden for clarity.



Scheme 1. Reagents and condition: (i) K_2CO_3, acetone, rt; (ii) benzaldehyde, $Ba(OH)_2{\cdot}8H_2O$, EtOH 96%, 30 °C; (iii) NH_2–NH_2, CH_3COOH, EtOH dry, 78 °C; (iv) $NH_2NHCSNH_2$, EtOH dry; 78 °C.

respectively, between the phenol and the backbone of Phe168 and between the thioamide group and the Pro102. The chloro-phenyl substituent hydrophobically contacted Phe118, Leu164, Phe168, Trp119 and Pro104.

Therefore, our results and in particular molecular modelling studies interestingly pointed out that more substituents in the molecules as isoprenyloxy, benzyloxy, thioamide, and phenolic hydroxyl, are important for interaction with active site of hMAO-B, so our study opens new perspective for the design of a novel class of hMAO-B inhibitors.

Derivatives **3a–k**, were synthesized as reported in Scheme 1 (see Supplementary data). The newly synthesized derivatives **3a–k**, were characterised by elemental analysis and the ^{1}H NMR spectral studies (see Supplementary data). All pyrazolines synthesized were screened for inhibition on the two human isoforms of human monoamine oxidase. (Chemical and physical data, anti-MAO efficacy values are reported in Table 1).

The potential effects of the test drugs on MAO inhibition were investigated by measuring their effects on the production of hydrogen peroxide H_2O_2 from *p*-tyramine, using the Amplex® Red MAO assay kit (Molecular Probes, Inc., Eugene, Oregon, USA) and microsomal MAO isoforms prepared from insect cells (BTI-TN-5B1-4) infected with recombinant baculovirus containing cDNA inserts for human MAO-A or MAO-B (Sigma–Aldrich Química S.A., Alcobendas, Spain).

The production of H_2O_2 catalysed by MAO isoforms can be detected using 10-acetyl-3,7-dihydroxyphenoxazine (Amplex® Red reagent), a non-fluorescent and highly sensitive probe that reacts with H_2O_2 in presence of horseradish peroxidase to produce a fluorescent product, resorufin. In this study, MAO inhibition was evaluated using the above mentioned method, following the general procedure previously described by us.[25]

As reported in our recent communications,[14,15] molecular modelling techniques have been applied in order to rationalise the recognition of most potent inhibitors with respect to human MAO-A and MAO-B enzymes. The new, high resolution, Protein Data Bank[26] (PDB) crystallographic structures 2Z5X[27] and 2V60[28] were considered as receptor model of hMAO-A and hMAO-B, respectively.

The compounds **3d**, **3e**, **3j** and **3k** were the selected ligands for our computational study consisting in two main steps: (i) conformational search of all enantiomers and (ii) flexible docking simulation toward both hMAO isozyme. Details of the procedures followed in both steps are reported in the Supplementary data.

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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.2010.09.061.

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