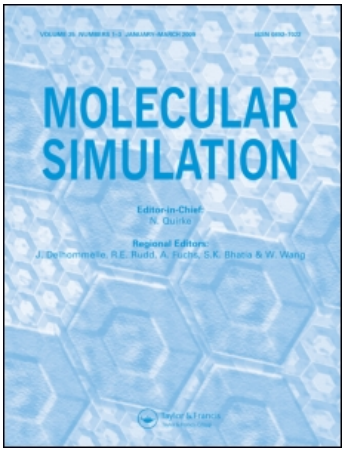

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MODELS OF MONOAMINE OXIDASE A AND B ACTIVE SITES OBTAINED BY USING 3D QSAR WITH CoMFA ANALYSIS

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The monoamine oxidase catalyses the oxidative deamination of neuroactive amines. This enzyme exists in two forms A and B, which differ by substrates preference and inhibitors specificity. Investigation of the structures of these enzymes and design new selective inhibitors are of greatly interesting since MAO A inhibitors are used in therapeutic practice as antidepressants and MAO B inhibitors – in the treatment Parkinson's diseases. The three dimension structures of monoamine oxidases are still unknown. Therefore, one of the most perspective approach to define significant features of structure active site is method based on analysis of structure-activity relationship (3D QSAR) with comparison of molecular fields analysis (CoMFA) allowing to get the spatial distribution of important properties affecting the activity.

In present study we investigate the structures of active sites MAO A and B using 16 pyrazinocarbazole derivatives in variant conformation. Majority of pyrazinocarbazole derivatives have a rigit conformation, but three of those is sufficiently flexible. The latters can be in two conformation types: long molecules (substitution accommodate along axis of main structure) and short molecules (substitution accommodate at acute angle about of main structure). Several 3D QSAR and CoMFA models of MAO A and B active sites were design for data sets containing various types of flexible molecules conformation. All obtained models are statistical reliable and have sufficient predictive power for tested compound tetrindole. The best MAO A model that include two flexible molecules in long conformations was obtained, and the longest one of those in short conformation. In contrast, for MAO B model containing all flexible molecules in the short conformations is more preferred.

On the basis of obtained data the schematic models of MAO A and B active sites structures are proposed. According to these models MAO A active site have the narrow long cavity that accommodate long molecules, while MAO B active site is broader and shorter.

Keywords: Monoamine oxidase; 3D QSAR; CoMFA analysis

*Corresponding author.

INTRODUCTION

The monoamine oxidase (MAO) catalyses the oxidative deamination of neuroactive amines. This enzyme exists in two forms A and B, which differ by substrates preference and inhibitors specificity [1]. It was shown that compounds in which the aromatic nucleus is separated by one or two carbons from the oxidizable nitrogen atom act as preferential MAO B substrates. When the distance between the aromatic nucleus and nitrogen atom is equivalent to three or more carbon atoms, the substrates bind selectively to MAO A [2]. This structural information allow to propose that the active sites of the two forms of MAO differ in term of their size. Moreover, lipophilicity appeared to be a common property of MAO B substrates while water soluble, electron rich aromatic amines are substrates of MAO A [3].

Investigation of the structures of these enzymes and designing of new selective inhibitors are greatly interesting since MAO A inhibitors are used in therapeutic practice as antidepressants and MAO B inhibitors – in the treatment Parkinson's diseases. Three-dimension (3D) structures of monoamine oxidases are still unknown. Therefore, one of the most perspective approach to define significant features of active site structure is the analysis of structure-activity relationship (3D QSAR) with comparison of molecular fields analysis (CoMFA) allowing to get the spatial distribution of important properties affecting the activity.

3D QSAR with CoMFA approach is sensitive to molecule conformations [4]. Therefore for accurate modelling it is proposed to use the rigid compounds and carry out the conformational analysis for flexible ones.

So in this investigation of the MAO A and B active site topology using 3D QSAR with CoMFA analysis we studied MAO A and B inhibitors with rigid basic structure pyrazinocarbazole derivatives. For flexible substituents the conformational analysis was carried out.

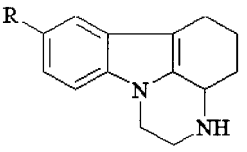
MATERIALS AND METHODS

Determination of MAO Activity and its Inhibition

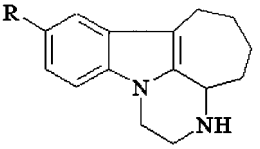
Rat liver mitochondria were used as a source of MAO A and MAO B. The activity of MAO was assayed radiometrically [5] with minor modifications [6] using 0.1 mM [^{14}C]-5-hydroxytryptamine (serotonin) and 5 μM [^{14}C]-phenylethylamine as substrates for MAO A and B respectively. These concentrations which are either close to or below K_m value allow

competitive inhibition to be detected. To obtain reliable comparative IC_{50} values (concentration required for 50% inhibition of enzymatic activity) rat liver mitochondria were incubated at 37°C for 30 min with all suspected MAO inhibitors and the enzymatic activity was measured thereafter by adding substrates of MAO A and B. Such approach is widely used for pilot studies of potential MAO inhibitors [7]. The inhibitory activities of pirlindole analogues are shown in Table I.

TABLE I Inhibitory activity (IC_{50}, mkM) of pirlindole analogues



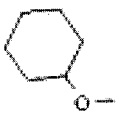
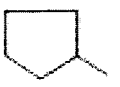
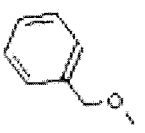
P1-P11, P13-P16



P12

Name	R	MAO-A Exp.	MAO-A Pred.	MAO-B Exp.	MAO-B Pred.
P1		0.11	0.11	≫100	97.7
P2		0.04	0.04	20	19.1
P3		12.6	13.0	> 178	182
P4		3.98	3.80	> 178	182
P5		0.071	0.08	158	182
P6		7.9	8.13	250	245
P7		0.79	0.83	180	174

TABLE I (Continued)

Name	R	MAO-A Exp.	MAO-A Pred.	MAO-B Exp.	MAO-B Pred.
P8		4.0	3.80	> 1000	912
P9		13.0	13.18	16.0	15.5
P10		0.26	0.24	12.5	11.75
P11		0.5	0.51	> 500	525
P12		0.53	0.5	29.0	30.2
P13		0.08	0.07	160	160
P14		0.13	0.14	7.5	7.5
P15		0.14	0.13	0.14	0.14
P16		0.03	0.03	79.4	79.4

Computer Modelling

The calculations were carried out on Silicon Graphics Workstation O_2 using Sybyl 6.5 software [8]. The molecular models were constructed and their geometries were optimised using the standard Tripos force field. The atomic charges calculation and geometry optimisation of molecules were done by semiempirical AM1 method. They were used in the subsequent analysis. Values of molecular hydrophobisity were calculated by method of Moriguchi *et al.* [9]. Conformational analysis was done using Random search program of Sybyl.

For 3D QSAR with CoMFA analysis the inhibitors were aligned by fitting indole parts of molecules atom by atom. The region was generated automatically by the program. Both steric and electrostatic fields were taken into consideration. The steric and electrostatic potentials were generated using a sp^3 carbon probe and +1 charge. QSAR analysis was carried out in two steps using PLS technique. In the first analysis, using 5 components and a number of cross-validation groups equal to a number of compounds, the optimal number of components was determined. The optimal number of components for the final 3D QSAR model was chosen as the number of components that corresponds to the minimum cross-validated standard error of estimate (s_{cv}) and R^2_{cv} 0.4. The second run was performed without cross-validation, using the optimal number of components previously determined. The results of the second analysis were used for drawing the coefficients' contour maps.

RESULTS

The most of used 16 pyrazinocarbazole derivatives had common basic structure and distinguished by substitution in 8 position (Tab. I). The substituents of pyrazinocarbazole derivatives have a rigid structures, except three of them which are sufficiently flexible (P02, P03, P15). The last ones can be in two types of conformation: long conformation (substituents accommodate along axis of the main structure) and short conformation (substituents accommodate at acute angle to main structure). Several 3D QSAR and CoMFA models of MAO A and B active sites were designed for data sets containing various types of flexible molecules conformations. All obtained models were statisticaly reliable and had sufficient predictive power for test compound tetrindole (Tab. II).

TABLE II 3D QSAR with CoMFA analysis for MAO A and MAO B

Model	*Type of flexible molecules conformation*	R^2_{cv}	s_{cv}	*m*	*n*	R^2	*s*	*F*	IC_{50} *pred.*
MAO A	Long	0.455	0.706	3	2	0.853	0.37	29	14×10^{-8}
	Long, excluding P15	0.477	0.764	0	4	0.945	0.25	48	10×10^{-8}
	Short	0.432	0.656	3	1	0.705	0.47	26	18×10^{-8}
MAO B	Long	0.591	0.443	2	2	0.875	0.25	38	5.6×10^{-5}
	Short	0.584	0.452	2	2	0.886	0.23	43	5.2×10^{-5}

R^2_{cv} – square of the multiple correlation coefficients in analysis with cross-validation,
s_{cv} – cross-validated standard error of estimate,
m – number of omitted molecules,
n – optimal number of components,
R^2 – square of the multiple correlation coefficients in analysis with no-validations,
s – standard error,
F – significance test,
IC_{50} pred. – predictive values of inhibitory activity for test compound tetrindole. Experimental values of inhibitory activity for tetrindole are 4.5×10^{-8} M and 6.3×10^{-5} M for MAO A and B respectively.

The model for MAO A with the set of molecules, where flexible ones were in the long conformations, the statisticaly significant square of the multiple correlation coefficients in analysis with cross-validation (R^2_{cv}) was obtained when 3 molecules were deleted. The distribution of the steric fields shown the presence of limitation region length for the molecules. The substituent of the longest and flexible molecule (P15) was placed in this region (Fig. 1). It allowed to propose that molecule P15 bind to active site in short conformation.

To validate this hypothesis one more set of molecules was made, where molecule P15 was in the short conformation. The obtained model was the best, since satisfactory (R^2_{cv}) was got without the molecule omitting. Besides, inhibitory activity of test compound (tetrindole) was better predicted than in all other models. Distribution of the steric fields is shown at Figure 2. Favourable steric fields can be seen along the X-axis where substituents with increased length demonstrated enhanced inhibition. Unfavourable steric regions can be seen on either side of substituents.

The 3D QSAR with CoMFA analysis for MAO A using the set of molecules with all flexible molecules in short conformations led to decreasing statistical values of cross-validation and no-validation analyses and predictive power for test compound (tetrindole).

Designing of models for MAO B was the next step of investigation. The models using both sets with short flexible compounds and long ones had similar statistics and predictive power for tetrindole. The distribution of the steric fields for sets with long flexible compounds is shown at Figure 3 and for sets with short flexible compounds at Figure 4. An unfavourable steric

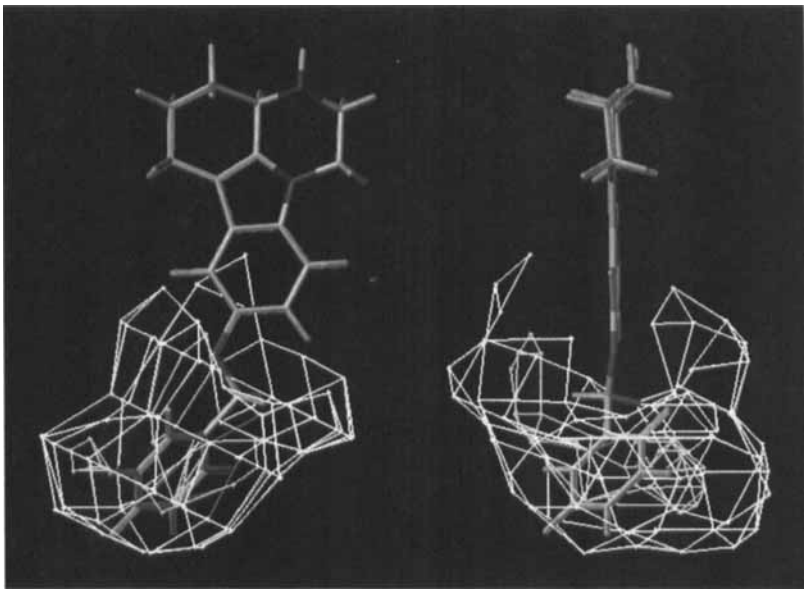


FIGURE 1 Standard dev*coefficients contour plot of CoMFA fields of MAO A model with the molecules set, where flexible molecules are in the long conformations. (*Sterically disfavoured areas (contribution level of 30%) are represented by yellow lines; sterically favoured areas (contribution level of 70%) – by green lines.) (See Color Plate XIV).

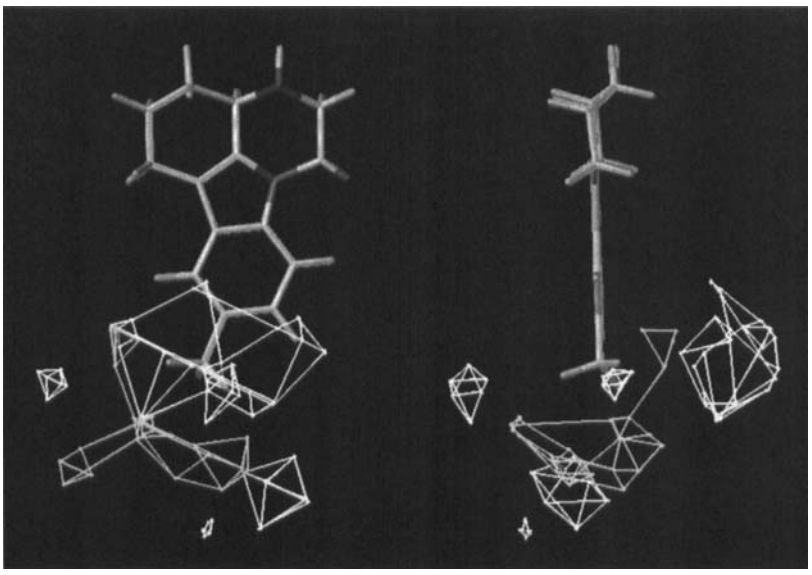


FIGURE 2 Standard dev*coefficients contour plot of CoMFA fields of MAO A model with the molecules set, where two flexible molecules are in the long conformations but molecule P15– in the short conformation. (*Sterically disfavoured areas (contribution level of 30%) are represented by yellow lines; sterically favoured areas (contribution level of 70%) – by green lines.) (See Color Plate XV).

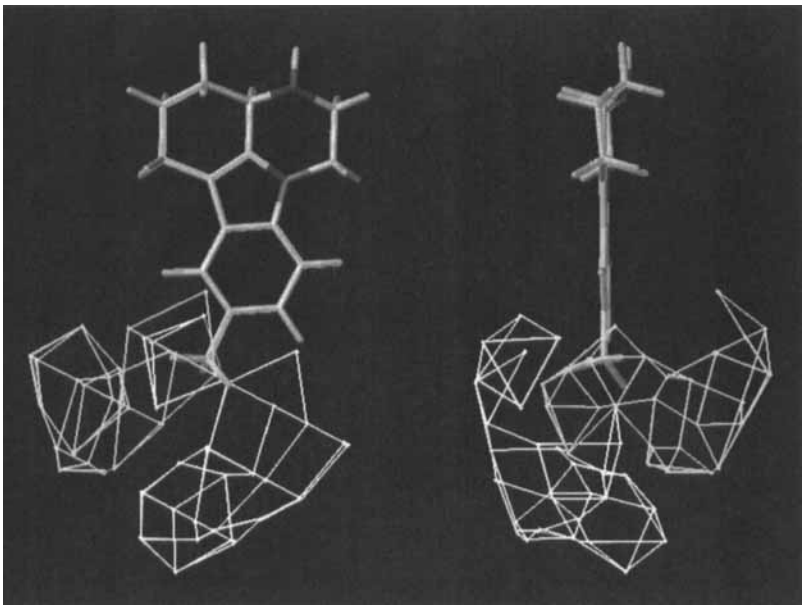


FIGURE 3 Standard dev*coefficients contour plot of CoMFA fields of MAO B model with the molecules set, where flexible molecules are in the long conformations. (*Sterically disfavoured areas (contribution level of 30%) are represented by yellow lines; sterically favoured areas (contribution level of 70%) – by green lines.) (See Color Plate XVI).

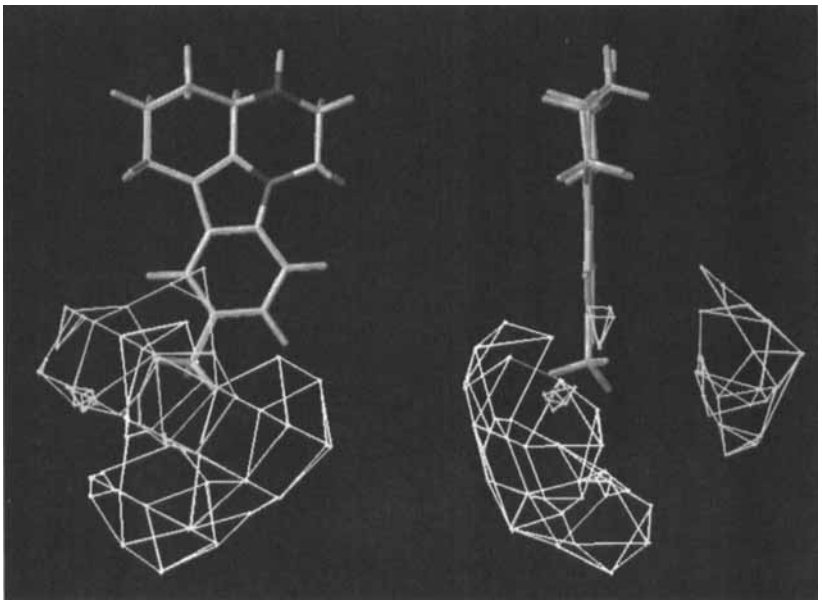


FIGURE 4 Standard dev*coefficients contour plot of CoMFA fields of MAO B model with the molecules set, where flexible molecules are in the short conformations. (*Sterically disfavoured areas (contribution level of 30%) are represented by yellow lines; sterically favoured areas (contribution level of 70%) – by green lines.) (See Color Plate XVII).

field limits the length of C-8 substituents in the MAO B model while a favourable steric region can be seen at an adjacent site.

DISCUSSION

Earlier using indole and isatin derivatives we proposed that MAO A and B active sites are quite different. Most of isatin analogues was shown to have planar structures and the length and width of most potent MAO A inhibitors did not exceed 14.0 and 6.0 angstroms, respectively, whereas selective MAO B inhibitors had smaller sizes (8.5 and 5.0 angstroms, respectively) [10, 11].

Our 3D QSAR with CoMFA models for MAO A and B provide further evidence for steric differences of the active sites of these enzymes. Comparison of steric fields of pirlindole analogues as MAO A and MAO B inhibitors suggests the existence of steric obstacle at C-8 (Figs. 3 and 4) in MAO B molecule, which might explain inability of long rigid pirlindole analogues to possess of the potent MAO B inhibitory activity. For MAO A inhibitors increase of the length of molecule in this region potentates their activity.

As was noted above, 3D QSAR is sensitive to molecule conformations used in analysis [4]. Therefore we used mainly rigid molecules revealing high inhibitory activity. The conformational analysis for flexible molecules was done and MAO A and B models where flexible analogues have various conformations was design. This procedure has allowed to describe more adequately the MAO A active site structure and propose the conformations of the flexible molecules in which they interact with the active site. Molecules P2 and P9 are appears to interact in the long conformations while the longest P15 interact in the short conformation.

The active site models for MAO B (with using all molecules both in the short and in the long conformations) reveal the same distribution of the steric fields. However, the designing both statisticaly significant models require to omit the longest flexible molecule (P15) with the best MAO B inhibitory activity. Furthermore, the substituents of the long molecules are placed in the unfavourable region. This indicates that flexible molecules are likely to interact with MAO B active site in the short conformation. This is in accordance with Krueger *et al.* data that the long and flexible oxodiazolones are very potent inhibitors of MAO B, whereas shorter and rigid analogues are less potent [12].

The better 3D QSAR with CoMFA model for MAO B was obtained when hydrophobicity of compounds was taken into consideration. This

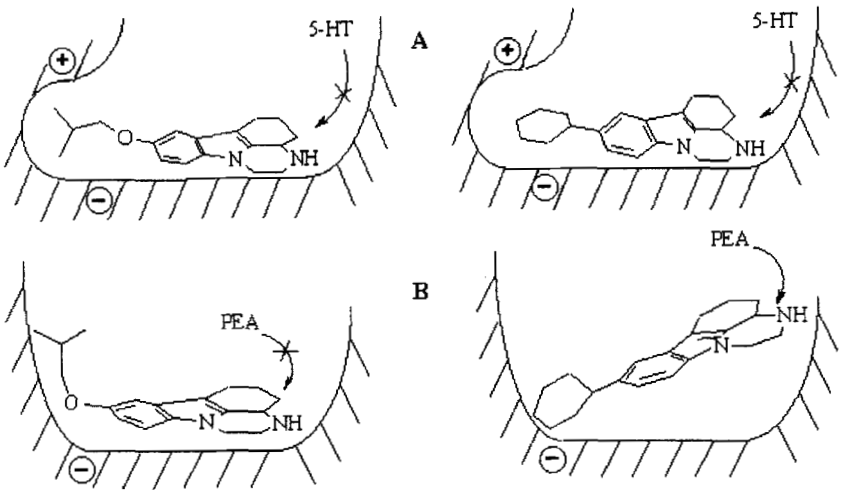


FIGURE 5 Schemes of MAO-A (**A**) and MAO-B (**B**) active site structures and putative positions of P2 (left) and tetrindole (right) molecules (*Arrows show that phenylethylamine (PEA) can displace tetrindole, but not P2. Serotonin (5-HT) can not displace both molecules.)

is in accordance with the general point of view that preferential MAO B substrates and selective inhibitors are more hydrophobic compounds than MAO A [13].

Comparison of steric fields of pirlindole analogues as MAO A and MAO B inhibitors suggests the existence of steric obstacle at C-8 in MAO B molecule, which might explain inability of long rigid pirlindole analogues to be potent inhibitors of MAO B. For MAO A inhibitors the increasing of the length of molecule in this region potentates their activity. This generally agrees with our previous studies on isatin analogues [10, 11]. On the basis of obtained results the schemes of MAO A and B active sites structures were designed (Fig. 5). MAO A active site is narrow slot, which has enough space to accommodate rather "long" rigid inhibitors. In contrast, the MAO B active site is broader and shorter and in this case flexible molecules interacting in the short conformations are good inhibitors this enzyme, while the long rigid molecules cannot be accommodated in active site.

The models obtained in this study can be used for further design of new generation of selective reversible inhibitors of MAO.

Acknowledgements

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