QSAR Analysis of Indole Analogues as Monoamine Oxidase Inhibitors

A. E. Medvedev, A. S. Ivanov,* A. V. Veselovsky, V. S. Skvortsov, and A. I. Archakov

Laboratory of Biogenic Amines and Laboratory "Computers in Biochemistry,"
Institute of Biomedical Chemistry, Russian Academy of Medical Sciences, Pogodinskaya Street 10,
Moscow, 119832, Russia

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The quantitative structure−activity relationship (QSAR) analysis with comparative molecular field analysis (CoMFA) of indole derivatives−monoamine oxidase (MAO) inhibitors were done. The pharmacophore model included four features: two hydrophobic rings, one donor atom, and one acceptor site. The predictive values (cross-validated r^2) of QSAR analysis for the inhibition of MAO-A and MAO-B were 0.743 and 0.603, respectively. The contributions of steric and electrostatic fields in the interaction between inhibitors and enzymes were equal. The three-dimensional arrangement of these fields for MAO-A and MAO-B suggests that structures of active site for both enzymes are considerably differed from each other.

INTRODUCTION

Monoamine oxidase (E.C. 1.4.3.4), an integral protein of the outer mitochondrial membrane, catalyses a reaction of oxidative deamination of neurotransmitter monoamines in central nervous system and peripheral tissues.[1] The enzyme exists in two forms, MAO-A and MAO-B, initially distinguished by sensitivity to low concentrations of acetylenic inhibitors, clorgyline and deprenyl, respectively. Strong evidence now exists that these forms of MAO are different proteins, encoded by two different genes.[1,2]

In spite of essential progress in elucidation of peculiarities of primary structure, biosynthesis of MAO and mechanisms of its insertion into the outer mitochondrial membrane,[3,4] precise information about steric structures of substrate biding sites of MAO-A and B is still absent. This stimulates computer-modeling studies based on experimental data of MAO interaction with substrates and inhibitors. One of the first computer analysis of series of potent MAO inhibitors revealed that common arrangement of these inhibitors includes aromatic ring and a side chain with an amino group located in the ring's plane.[5] The distance between the center of nitrogen and the ring was 0.50−0.55 nm.[6] The discovery of MAO-mediated bioactivation of MPTP to neurotoxin MPP[7] which causes a development of major symptoms of parkinsonism in experimental animals gave new rise for quantitative structure−activity relationship studies with relevance to some structural features influencing the binding to active sites of MAO-A and MAO-B.[8,9]

Indoles are a large group of nitrogen-containing heterocyclic compounds widely distributed in living world and in its chemical environment. Some indole amines as serotonin and tryptamine are substrates of MAO. Recently it has been shown that isatin (2,3-dioxindole) acts as a relatively selective inhibitor of MAO-B than MAO-A.[10] 5-Hydroxyisatin exhibited selective MAO-A inhibition, whilst indole and other isatin analogues were less potent MAO-A and B inhibitors.[11]

In the present report we have analyzed our experimental data on inhibition of MAO-A and MAO-B by indole and isatin analogues obtained in our and other laboratories.[10−13] The action of these inhibitors was readily reversible.[12]

Previous pilot QSAR analysis of their inhibitory potency revealed that molecules of selective MAO A and B inhibitors had different sizes: 11.5 × 5.6 × 1.8 and 8.5 × 5.1 × 1.8 Å, respectively. Here we have investigated further their quantitative structure-activity relationship (QSAR) with molecular field analysis (CoMFA).

MATERIALS AND METHODS

Experimental data of MAO inhibitors were taken from previous reports.[11−13]

Computer Modeling. The study was carried out using Sybyl 6.1 software (Tripos GmbH, Munich, Germany) running on Silicon Graphics workstation Indigo-2 (R4400, XZ). The molecular models were constructed, and their geometry were minimized using the standard Tripos force field. The atomic charges were calculated by Gasteiger−Huckel method. They were used in the subsequent analysis.

PowerSearch program for Windows (Tripos GmbH, Munich, Germany) was employed for the conformation search for flexible bonds of molecules. In our study we have used the conformers with the lowest energy as the most probable conformation.

The correctness of atomic charges' distribution, calculated by empirical Gasteiger−Huckel method, was also validated for some molecules by quantum mechanical AM1, MNDO, and MINDO3 methods.[14]

CoMFA Analysis. The inhibitors were aligned by fitting the indole common structure atom by atom. Initial pharmacophore model was constructed using the DISCO program.[15] This model was made by matching all structures of inhibitors, with a range of tolerance from 0.5 to 5.0 by 0.5 and features requirement from 3 to 7. Then the CoMFA analysis was carried out in QSAR option of Sybyl. The region was generated automatically by the program. Both steric and electrostatic fields were taken into investigations. The steric and electrostatic potentials were generated by using an sp^3 carbon probe and a +1 charge. QSAR analysis was made using PLS technique in two steps. At first using 10 components and cross-validation groups equal to a number of compounds the optimal number of components was determined. The second step was made with the determined optimal number of components and without cross-validation.

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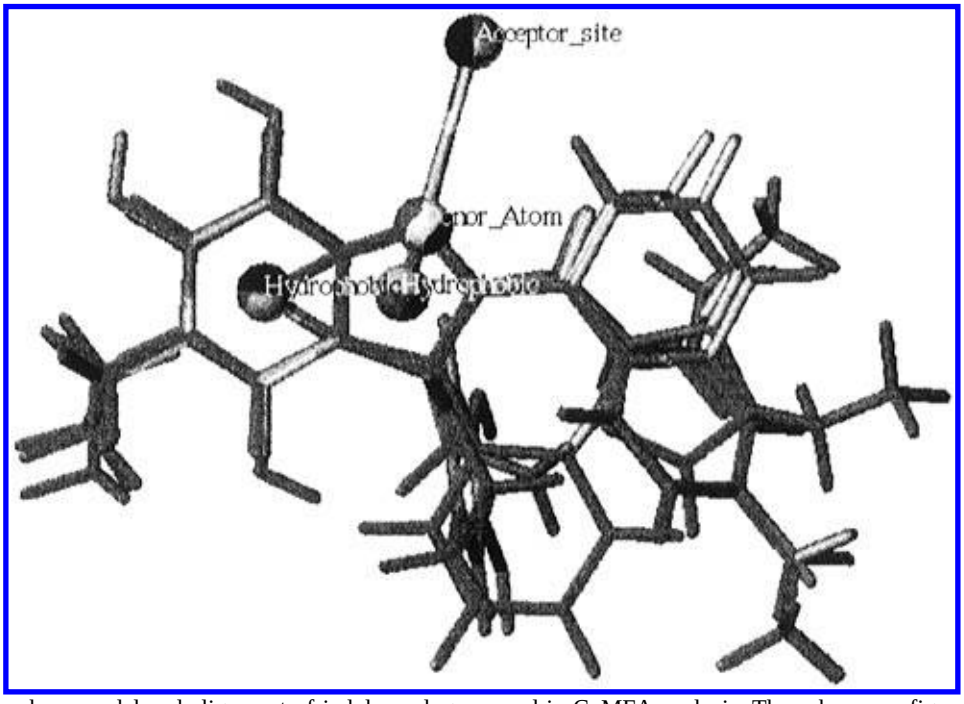


Figure 1. Pharmacophore model and alignment of indole analogues used in CoMFA analysis. The spheres on figure are the features of pharmacophore.

The last results were taken for drawing the coefficients contour maps. The values of inhibitory activity of compounds were taken in QSAR analysis as log (IC_{50}).

RESULTS

Pilot analysis of structure−inhibitory activity relationship for a set of indole and isatin derivatives as inhibitors MAO-A and MAO-B was done previously.[12] It revealed that selective inhibition of MAO-A requires coplanar structure of substitutes at C_2 and C_3 of indole ring or presence of hydroxy group at C_5 of isatin, whereas the distribution of electron density in the molecules is important for MAO-B inhibition. Sizes of molecules were also essential for the selective inhibition of both enzymes.[12]

In this study we have used the computer 3-D QSAR analysis with CoMFA module. This approach usually allows to determine both exact relationships between structure of compounds and their inhibitory activities and propose essential steric and electrostatic regions on them enzyme molecule important for the manifestation of biological effects of the compounds.

Figure 1 shows pharmacophore model constructed using DISCO program. This model includes four features: two hydrophobic centers (both condensed aromatic ring of the heterocyclic indole nucleus), one donor atom, and one acceptor site that are the common features of all inhibitors used. The resultant pharmacophore model is consistent with a notion that the aromatic ring is an important element in the structures of substrates and inhibitors of MAO.[5,16,17]

Initial QSAR analysis was begun with cross-validation, and a number of optimal components was determined. In both cases (inhibition of MAO-A and MAO-B) the numbers of components for the final analyses were 10. The second run was done without cross-validation with chosen sets of components.

The results of QSAR analysis for MAO-A and MAO-B are summarized in Tables 1 and 2. The model can explain about 92% and 99% of the inhibitory potency of the compounds regarding MAO-A and MAO-B, respectively. Variations of experimental data had good predictive ability: cross-validative r^2 values were 0.743 and 0.603 for MAO-A and MAO-B, respectively (Table 3). The influences of steric and electrostatic fields on inhibitory activity of the compounds used were equal for both enzymes.

Three-dimensional contour map of coefficients of steric and electrostatic fields shows regions where differences in molecular fields are associated with differences in biological activity of the compounds. Figure 2 represents such maps for steric and electrostatic regions of MAO-A inhibitors. For better visualization a molecule with high inhibitory potency was added. The region of steric fields where QSAR predicted increasing inhibitory activity of compounds (favorable region) is near the aromatic substituents at C_2 position of the indole nucleus. There were six unfavorable regions, decreasing inhibitory activity: three of them were near the favorable region, and three others were near C_5 and C_6 positions (Figure 2a). The region where negative charges of compounds increase the interaction with target (Figure 2b) is located in the area of the aromatic substituents, and there were a few regions for positive charges surrounding whole molecules.

Figures 3 and 4 show the CoMFA fields for a weak (compound **21**) and a potent (compound **17**) MAO-B inhibitors, respectively. Figures 3a and 4a represent the steric contour maps. Four favorable regions for steric interaction have been recognized: one is near C_5 position of indole ring and three others surround aromatic substituents at C_2. Unfavorable regions included *ortho*-position at the additional aromatic ring two positions between C_5 and C_7 of indole ring. Figures 3b and 4b show the electrostatic CoMFA map for MAO-B inhibitory activity. The regions where negative charges enhance activity were found near C_4−C_7 of indole ring and in the region of aromatic substituents at C_2 of indole ring. The major regions where positive charges increase inhibitory activity surround C_5 of indole are also located near indole nitrogen and at *para*-position phenyl substituents at C_2. Comparison of the steric and electrostatic fields for MAO-A and MAO-B inhibitors shows the existence of some

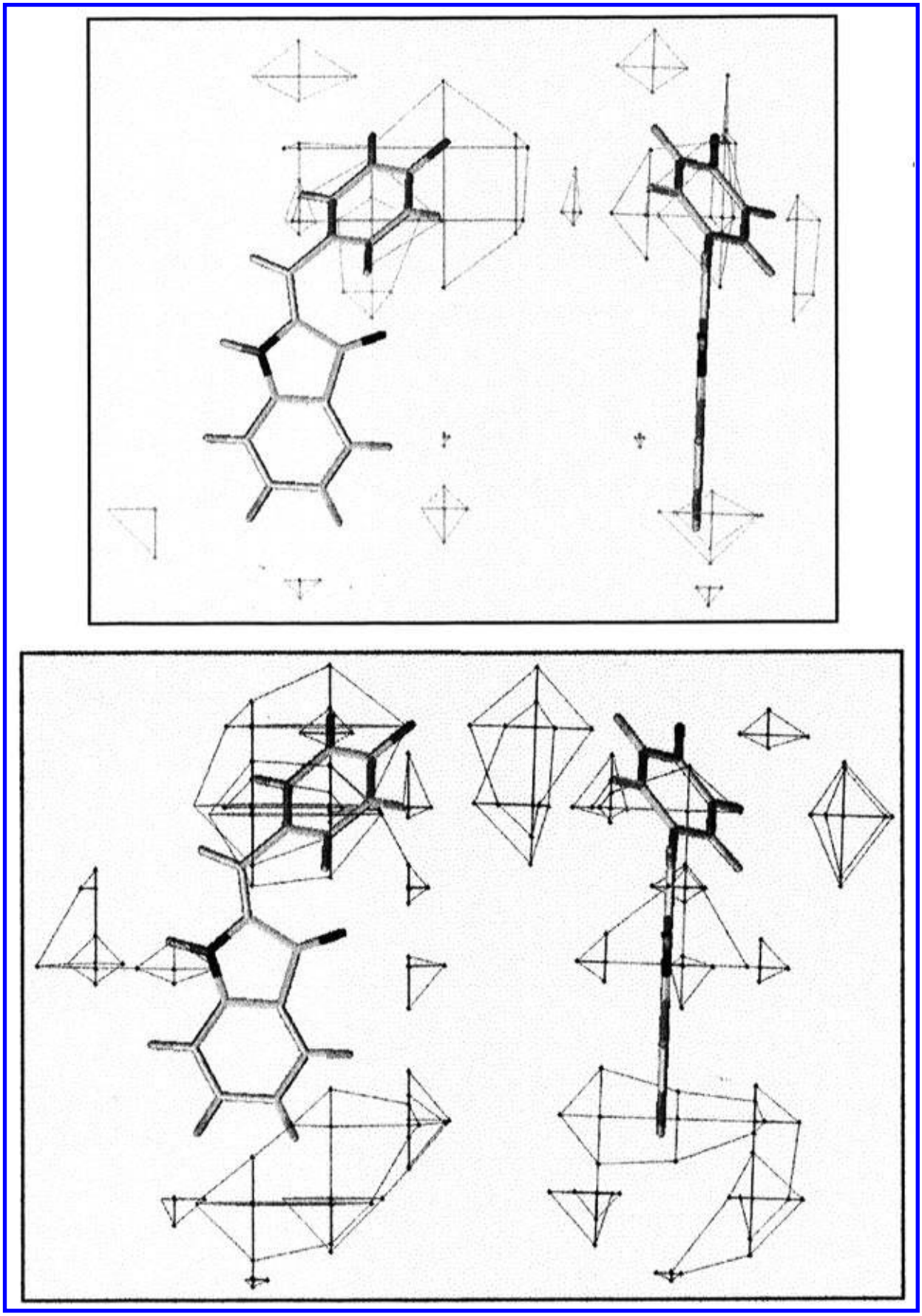


Figure 2. Coefficient contour plot of CoMFA fields of a potent inhibitor of MAO-A (compound 21). A (top) Steric field. Grey contours surround regions where low steric interaction increases inhibitory activity (unfavorable region), white—higher steric interaction increases activity (favorable region). B (bottom) Electrostatic field. Grey contours surround regions where positive charges increase inhibitory properties, white—for negative charge.

common regions in both groups of the compounds that determine the inhibitory properties. For steric interaction the favorable region was placed near aromatic substituents at C_2, whereas unfavorable regions were found near the

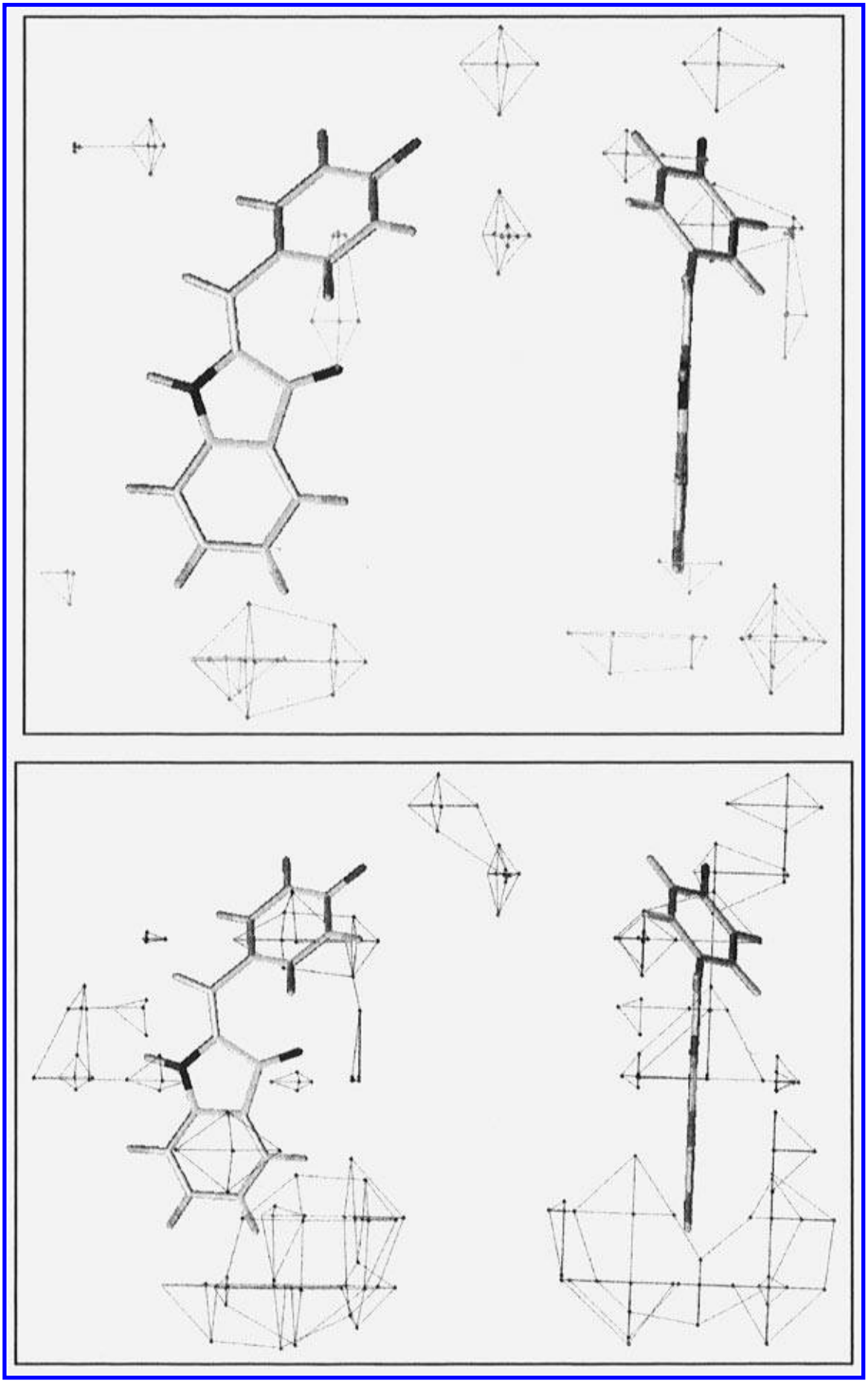


Figure 3. Coefficient contour maps of steric (A, top) and electrostatic (B, bottom) fields. Legend as for Figure 2.

ortho-position of phenyl substituents and between C_{5-6} and C_{6-7} of indole ring. Two regions were found for the electrostatic fields: (i) the region for negative charges was placed near aromatic ring of the substituents and (ii) and a

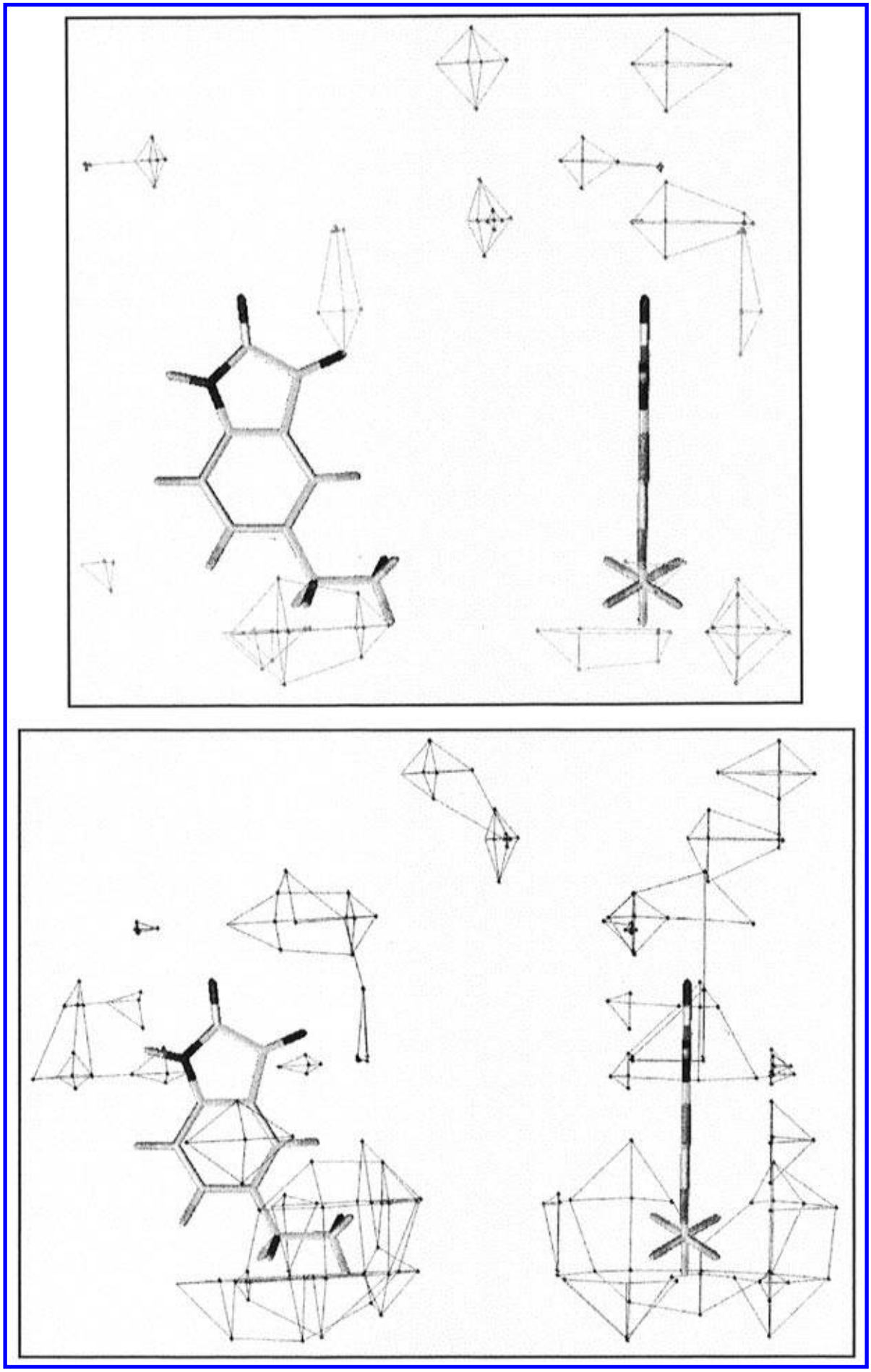


Figure 4. Coefficient contour plot of steric (A, top) and electrostatic (B, bottom) CoMFA fields. Molecule **17** is displayed. Legend as for Figure 2.

Table 1. Experimental and Predicted MAO IC_{50} for Indole and Isatin Analogues

no.	names and structures of compounds	MAO-A (IC_{50}) exper.	MAO-A (IC_{50}) pred.	MAO-B (IC_{50}) exper.	MAO-B (IC_{50}) pred.
1	indole $R_2 = R_3 = R_4 = R_5 = H$	200	245	100	170
2	2-oxindole $R_1 = R_3 = R_3' = R_5 = R_6 = R_7 = H$; R_2, $R_2' = O$	>100	81	73	78
3	isatin $R_1 = R_5 = R_6 = R_7 = H$; R_2, $R_2' = R_3$, $R_3' = O$	56	19	8	5
4	2-phenylindole $R_3 = R_4 = R_5 = H$; $R_2 = C_6H_5$	4	13	20	12
5	3-phenylindole $R_2 = R_4 = R_5 = H$; $R_3 = C_6H_5$	4	5	20	21
6	4-OH-indole $R_2 = R_3 = R_5 = H$; $R_4 = OH$	700	676	1000	759
7	5-OH-indole $R_2 = R_3 = R_4 = H$; $R_5 = OH$	600	550	600	600
8	5-OH-isatin $R_1 = R_6 = R_7 = H$; R_2, $R_2' = R_3$, $R_3' = O$; $R_5 = OH$	8	24	>100	141
9	5-OH-2-phenylisatin $R_1 = R_6 = R_7 = H$; $R_5 = OH$; R_3, $R_3' = O$; $R_2 = C_6H_5$	71	20	>1000	708
10	6-OH-isatin $R_1 = R_5 = R_7 = H$; $R_6 = OH$; R_2, $R_2' = R_3$, $R_3' = O$	>100	95	>100	87
11	7-OH-isatin $R_1 = R_5 = R_6 = H$; $R_7 = OH$; R_2, $R_2' = R_3$, $R_3' = O$	>100	126	>100	87
12	*N*-methylisatin $R_1 = CH_3$; $R_5 = R_6 = R_7 = H$; R_2, $R_2' = R_3$, $R_3' = O$	95	102	14	15
13	2-phenylmethyleneisatin $R_1 = R_5 = R_6 = R_7 = H$; R_3, $R_3' = O$; R_2, $R_2' = CH\text{-}C_6H_5$	14	13	>1000	912
14	dihydrazone-isatin $R_1 = R_5 = R_6 = R_7 = H$; R_2, $R_2' = R_3$, $R_3' = N\text{-}NH_2$	17	17	417	389
15	dopamine-isatin $R_1 = R_5 = R_6 = R_7 = H$; R_2, $R_2' = O$ R_3, $R_3' = -NHC_2H_4C_6H_2(OH)_2-$	210	210	>500	500
16	5-methylisatin $R_1 = R_6 = R_7 = H$; $R_5 = CH_3$; R_2, $R_2' = R_3$, $R_3' = O$	16	9	1	1
17	5-ethylisatin $R_1 = R_6 = R_7 = H$; $R_5 = C_2H_5$; R_2, $R_2' = R_3$, $R_3' = O$	35	10	1	1
18	5-butylisatin $R_1 = R_6 = R_7 = H$; $R_5 = C_4H_9$; R_2, $R_2' = R_3$, $R_3' = O$	6	4	54	59

large region for positive charges surrounded C_5 of indole ring.

However, selective inhibitors of MAO A and B have distinct patterns of both electrostatic and steric fields. Perhaps this is a reason for some well-known differences in substrate specificity and inhibitor selectivity between these enzymes.

Recently Rajesh et al.[18] found that condensation of isatin with acetone or acetophenone, which removes 3-oxo group of isatin decreased inhibitory properties regarding MAO-B. We have used these data for the further examination of the predictive property of our model. The predicted IC_{50} values of these compounds for the inhibition of MAO-B were 6 and 20 times higher compared with isatin. Consequently our model reflects the inhibitory properties. The results of our analysis also suggest that the distribution of electrostatic and steric properties in the indole derivatives may be crucial for the manifestation of MAO-A and MAO-B inhibition.

DISCUSSION

At the first step of our study the pharmacophore model has been constructed using DISCO program. This model reflects common features of all compounds that may be important for their inhibitory activity. However, Figure 1 shows only the indole nucleus as the common structure.

CoMFA fields' maps reflect the essential sites for inhibitory activity of the set of compounds. Data of Table 3 indicate that steric and electrostatic fields had nearly equal contribution to the inhibitory activity of the compounds studied. This is in contrast to previous data with MPTP analogues,[8,19] where an electrostatic field was shown to have no influence on the interaction of MAO-B with these compounds. The reasons for this discrepancy could be too little variance in the electrostatic properties of MPTP analogues taken in that investigation or a different position of indole and MPTP analogues in the active site. Lack of any information about this location does not allow for comparison of the CoMFA fields' maps of both types of these compounds. It should be stressed that MPTP analogues are substrates of MAO, whereas compounds used in the present computer analysis are not metabolized by MAO. Our data on the significance of electrostatic fields for MAO-A inhibition are consistent with results of Thull et al.[20] Similar to the present report they also found the influence of steric and electrostatic fields on activity of MAO inhibitors.

Comparison of pharmacophore model and electric fields of QSAR model (Figures 1, 2b, 3b, and 4b) revealed some

Table 2. Experimental and Predicted MAO IC_{50} for C_2-Substituted Isatins

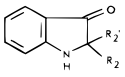
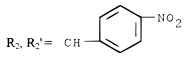
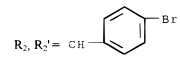
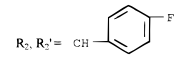
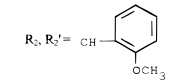
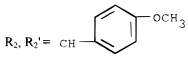
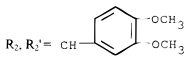
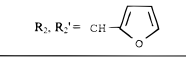
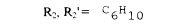
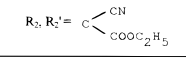
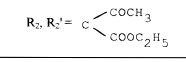
No	Structures of compounds	MAO-A (IC_{50})		MAO-B (IC_{50})	
		Exper.	Pred.	Exper.	Pred.
19		9	9	1884	2239
20		9	9	501	513
21		4	4	>1000	1148
22		89	87	209	209
23		4	4	16	12
24		45	45	20	23
25		4	4	447	372
26		36	35	191	178
27		14	14	100	115
28		562	575	562	589

Table 3. 3D-QSAR Analysis with CoMFA for MAO-A and MAO-B

	MAO-A	MAO-B
r^2_{cw}	0.743	0.603
optimal no. of components, used in final analysis	10	10
r^2	0.922	0.990
S	0.250	0.110
F	20.1	168.3
CoMFA (Steric)		
normalized coefficients	3.05	3.57
relative contribution	0.514	0.455
CoMFA (Electrostatic)		
normalized coefficients	2.88	4.28
relative contribution	0.486	0.545

interesting peculiarities. The pharmacophore model proposes the presence of the acceptor site, which probably has a positive charge. The CoMFA model predicts the existence of a region near this acceptor site where positive charges increase inhibitory potency of the compounds. The reason of this discrepancy is not clear. The calculation of atomic charges by quantum mechanical methods (AM1, MNDO, and MINDO3) show that nitrogen atom in the indole derivatives has negative charge. The general distribution of charges in molecules remains constant. So some differences between DISCO and CoMFA models are not the artifact of atomic charge's calculation by Gasteiger−Huckel method.

The most clear differences in the potency of MAO-A and MAO-B inhibition have been found for the compounds **9**, **13**, **19**−**21**, and **25**. They are potent inhibitors of MAO-A and poor inhibitors of MAO-B (Tables 1 and 2). Previously we found that the reason of different efficacy of MAO-A and MAO-B inhibition could be explained by differences in sizes of molecules. Present results show, that for MAO-A it also depends on the interaction of substituents at C_2 with favorable steric field. For MAO-B these substituents are in unfavorable region of steric field, while there is the region where increasing negative charge enhances biological activity. Perhaps, variation of substituents in this part of inhibitor indole molecule (C_2) is the most perspective for design of selective inhibitors of MAO-A and MAO-B among this basic structure.

Thus present QSAR with CoMFA analysis of MAO-A and -B inhibitors suggest that besides some common regions of CoMFA fields the 3D structure of active sites of enzymes may be different. The developed model can be used in molecular databases for searching perspective lead compounds effectively inhibit any type of MAO. At the present time our screening of Cambridge Structural Database is in progress.

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