Pergamon

Biochemical Pharmacology, Vol. 50, No. 6, pp. 869–877, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)00220-0

INHIBITION OF MONOAMINE OXIDASE BY ISOQUINOLINE DERIVATIVES

QUALITATIVE AND 3D-QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS

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(*Received* 3 *March* 1995; *accepted* 17 *May* 1995)

Abstract—A series of isoquinolines, N-methyl-1,2-dihydroisoquinolines, N-methyl-1,2,3,4-tetrahydroisoquinolines, 1,2,3,4-tetrahydroisoquinolines, and N-methylisoquinolinium ions were tested as inhibitors of monoamine oxidases A and B. All compounds were found to act as reversible and time-independent MAO inhibitors, often with a distinct selectivity towards MAO-A. As a class, the N-methylisoquinolinium ions were found to be the most active MAO-A inhibitors, with N-methyl-6-methoxyisoquinolinium ion emerging as a potent (IC_{50} = 0.81 μM) and competitive MAO-A inhibitor. Comparative molecular field analysis (CoMFA, a 3D-QSAR method) of MAO-A inhibition was performed using the data reported here and in the literature. Using the steric and lipophilic fields of the inhibitors, quantitative models with reasonable predictive power were obtained that point to the importance of steric, lipophilic, and polar interactions in modulating MAO-A inhibitory activity.

Key words: isoquinolines; isoquinolinium ions; monoamine oxidase (MAO); MAO-A; CoMFA; reversible inhibitors

There has been in recent years a considerable renewal of interest in monoamine oxidase (MAO; EC 1.4.3.4.), an FAD-containing enzyme of the outer mitochondrial membrane that plays a key role in the degradation of catecholamines and serotonin [1]. MAO exists as two isoenzymes, MAO-A and MAO-B, which differ in their substrate specificity and sensitivity to inhibitors [2]. Although the amino acid sequence of the two isozymes is known [3], practically everything remains to be discovered about their three-dimensional structure and especially the topographical differences between their active sites [4].

One reason for the current interest in MAO is linked to the discovery that the neurotoxin MPTP¶ causes the death of dopaminergic neurons and induces symptoms very similar to Parkinson's disease in humans [5]. The first step in this toxicity is the oxidation of MPTP by MAO to the $MPDP^+$ and then to the 1-methyl-4-phenylpyridinium cation (MPP^+) [6, 7]. Whereas MPTP is selectively oxidized by MAO-B, a number of analogues of MPTP are substrates of MAO-B and/or of MAO-A.

MAO is also of interest because it is the target of novel, reversible inhibitors able to act as antidepressants without causing hypertensive crises through inhibition of tyramine breakdown [1]. The reversible MAO-A inhibitor moclobemide is an example of this new class of antidepressive drugs [8].

There are two reasons why isoquinolines and analogues are of interest in the context of MAO. First, a number of these compounds are formed endogenously as metabolites of neurotransmitters, and may be neurotoxic [9, 10]. Their mode of interaction with MAO-A and MAO-B may therefore be a significant factor towards understanding the etiology of Parkinson's disease, should it be demonstrated that their *in vivo* mechanism of neurotoxicity closely resembles that of MPTP. A few 1,2,3,4-tetrahydroisoquinoline (THIQ) derivatives (e.g. N-methyl-THIQ) have indeed been reported to be weak MAO substrates [11]. In contrast, a number of quinoline and quinaldine derivatives are inhibitors of both forms of MAO (e.g. several THIQs and their N-methylated derivatives) [12, 13]. The reversible nature of this inhibition has been demonstrated for a large series of isoquinoline (IQ) derivatives [14].

Because of such inhibitory effects, quinoline and isoquinoline derivatives may be of interest as lead compounds in the search for novel medicinal MAO inhibitors. From a more fundamental perspective, their interaction with MAO-A or MAO-B could prove informative enough to allow specific insights into the topographical features of the active sites of the two isoenzymes.

The first aim of our study was to determine the MAO inhibitory activity of various tetrahydroisoquinolines, dihydroisoquinolines, isoquinolines, and N-methylisoquinolinium ions substituted in the 6- and/or 7-positions

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¶ Abbreviations: CoMFA, Comparative Molecular Field Analysis; 3D-QSAR, three-dimensional QSAR; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; $MPDP^+$, 1-methyl-4-phenyl-2,3-dihydropyridinium cation; MPP^+, 1-methyl-4-phenylpyridinium cation; THIQ, tetrahydroisoquinoline; IQ, isoquinoline; MLP, Molecular Lipophilicity Potential; CPC, Centrifugal Partition Chromatography; PLS, Partial Least Squares.

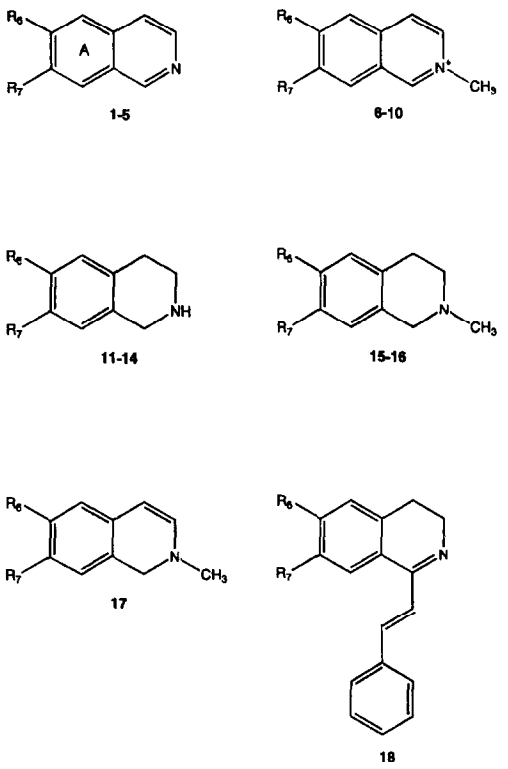

Fig. 1. Structures of compounds **1–18** (series I).

(series I; see Fig. 1). Secondly, the inhibitory activities of these compounds, together with those of other analogues (series II; see Fig. 2) investigated by Bembenek *et al.* [14], were submitted to a three-dimensional QSAR (3D-QSAR) approach using comparative molecular field analysis (CoMFA) and according special attention to the MLP of these inhibitors. In this study, the 3D-QSAR approach proved successful enough to reveal important topographical features characterizing the active site of MAO-A.

MATERIALS AND METHODS

Chemicals

The substituted isoquinolines (**2–5**) were prepared according to published methods [15, 16] as reported elsewhere [17]. N-methylisoquinolinium iodides (**6–10**), substituted 1,2,3,4-tetrahydroisoquinolines (**12–14, 16**) and N-methyl-6-methoxy-1,2-dihydroisoquinoline (**17**) were prepared by classical procedures as described separately [18]. 6,7-dimethoxy-1-styryl-3,4-dihydroisoquinoline hydrochloride (**18**) was a kind gift from Prof. H. D. Höltje (Freie Universität, Berlin, Germany). Isoquinoline (**1**), THIQ (**11**), and N-methyl-THIQ (**15**) were purchased from Merck (Darmstadt, Germany). The chemical structure and purity of the compounds were verified by ^{1}H-NMR, IR, elemental analysis, HPLC, and GC-MS.

The following products were obtained from commercial sources and used without further purification: Na_2HPO_4, KCl, KH_2PO_4, DMSO, and sucrose (Fluka Chemie AG, Buchs, Switzerland), kynuramine and clorgyline (Sigma Chemical Co., St. Louis, MO, U.S.A.), and selegeline (RBI Inc., Natick, MA, U.S.A.).

Preparation of rat brain mitochondria

Rat brain was isolated according to the modified method of Clark and Nicklas [19] as previously described [20]. The protein content of the washed mitochondrial fraction was determined with bovine albumin as a standard [21].

MAO assay

MAO inhibitory activities were measured as previously described [20, 22] using harmane as a standard [23]. A continuous fluorimetric assay [24, 25] was used to evaluate the MAO substrate activity of THIQ and N-THIQ.

3D-QSAR

All calculations were run on Silicon Graphics Personal Iris 4D-35 and Silicon Indigo R4000 workstations. The starting geometries of the isoquinoline derivatives used in the CoMFA study were taken from the fragment library of the SYBYL 6.03 molecular modeling package (Tripos Associates, St. Louis, MO, U.S.A.).

CoMFA was performed with the QSAR option of SYBYL. AM1 charges and default settings were used [26] except for the option "drop-electrostatic," which was set to "No," which means that the electrostatic field is explicitly calculated at grid points with high steric interactions (Δ 30 kcal/mol). The common isoquinoline skeletons of all analogues within a chemical series were aligned atom by atom. Sampling of the steric and elec-

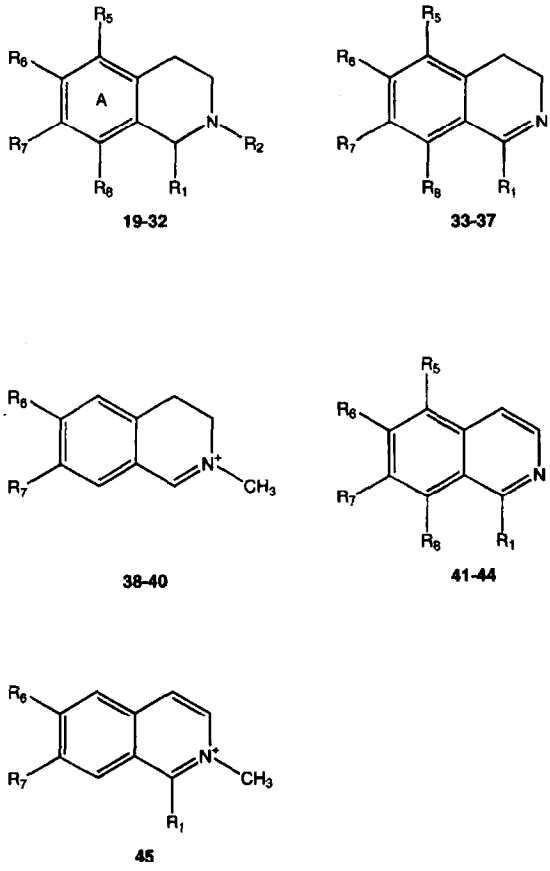

Fig. 2. Structures of compounds **19–45** (series II).

Table 1. Structure and MAO inhibitory activities of isoquinolines, N-methylisoquinolinium ions, 1,2,3,4-tetrahydroisoquinolines, N-methyl-1,2,3,4-tetrahydroisoquinolines, and N-methyl-1,2-dihydroisoquinolines (for structures, see Fig. 1)

			Inhibitory activities			Prediction*
			MAO-A		MAO-B	MAO-A
No.	R_6	R_7	IC_{50} [μM]	pIC_{50}	IC_{50} [μM]	pIC_{50}
1	H	H	62.9 ± 2.1†	4.20	47.9 ± 2.5†	3.96
2	OCH_3	H	34.7 ± 2.6	4.46	22.7 ± 0.8	4.51
3	H	OCH_3	113 ± 10	3.95	134 ± 12	3.79
4	OCH_3	OCH_3	128 ± 8	3.89	>100‡	4.26
5	$-O-CH_2-O-$		49.9 ± 3.4	4.30	10.1 ± 0.6	4.33
6‖	H	H	32.7 ± 2.4	4.49	160 ± 2.5	4.96
7‖	OCH_3	H	0.81 ± 0.07	6.09	36.2 ± 2.8	5.51
8‖	H	OCH_3	10.9 ± 0.4	4.96	135 ± 6	4.77
9‖	OCH_3	OCH_3	4.0 ± 0.3	5.40	96.2 ± 16.9	5.32
10‖	$-O-CH_2-O-$		12.3 ± 1.5	4.91	19.9 ± 3.4	5.31
11	H	H	90.4 ± 1.9	4.04	47.8 ± 6.9	4.15
12	OCH_3	H	28.9 ± 1.5	4.54	85.8 ± 6.5	4.69
13	H	OCH_3	52.6 ± 2.4	4.28	26.9 ± 1.3	3.98
14	$-O-CH_2-O-$		23.4 ± 0.8	4.63	132 ± 6	4.50
15	H	H	60.1 ± 2.7	4.22	93.9 ± 7.8	4.43
16	OCH_3	H	6.7 ± 0.6	5.17	269 ± 34	4.93
17	OCH_3	H	18.1 ± 0.9	4.74	>20 μM§	4.77
18	OCH_3	OCH_3	53.9 ± 2.3	4.27	38.2 ± 7.1	4.36

* Predicted with model **A5.**
† Extrapolated values, solubility = 40 μM.
‡ 17% inhibition at 100 μM.
§ Inactive at 20 μM.
‖ Compounds as iodides.

trostatic fields was taken at the nodes of a 3D grid of 1.5 Å resolution. The same was true for the third molecular field introduced into CoMFA, namely the Molecular Lipophilicity Potential (MLP) [27]. This new tool in 3D-QSAR is based on the atomic lipophilic fragmental system of Broto and Moreau [28], slightly modified to take into account atoms (e.g. N^+) not described in the original system. In an iterative process, these novel fragmental values were optimized to approximate as closely as possible the experimental log P values of MPP^+ derivatives [29] and isoquinoline derivatives [18].

Two series of compounds were submitted to a CoMFA study: the 18 compounds whose inhibitory activities (IC_{50} values) were measured in this study (series I), and 31 compounds (K_i values) reported by Bembenek *et al.* [14] (series II), leaving out the two compounds that also appear in series I as compounds **1** and **15.** In a first step, a Partial Least Squares (PLS) analysis was carried out. If the correlation coefficient was greater than 0.4, a complete CoMFA was undertaken.

RESULTS

MAO inhibitory activities

The IC_{50} values of the 18 compounds in series I are reported in Table 1. The values are in the range 0.8 μM to 128 μM for MAO-A and 10 μM to 268 μM for MAO-B. For the most active compounds, complete enzyme kinetics was examined, and the inhibition found to be a competitive type (results not shown). All derivatives seem to act in a reversible and time-independent manner. These findings indicate that the tested compounds are not substrates of MAO-A or MAO-B, as verified here for THIQ and N-methyl-THIQ.

N-methyl-6-methoxy-isoquinolinium (**7**, IC_{50} = 0.81 μM towards MAO-A) emerges as the most active inhibitor examined. In fact, the N-methylisoquinolinium ions (N-Me-IQs^+, compounds **6–10**) as a group are the most active and selective MAO-A inhibitors, whereas the more lipophilic and uncharged isoquinolines (IQs, compounds **1–5**) are weak MAO-A inhibitors, displaying poor or no selectivity towards MAO-B.

The THIQ derivatives are more active towards MAO-A than the IQ derivatives. Such a difference could be due to the higher lipophilicity of the isoquinoline derivatives (log P values in the range 1.68–2.08) compared to that of the THIQ derivatives (calculated and experimental log P values in the range 1.03–1.71). The lipophilicity of most of the isoquinoline derivatives examined here has been accurately measured by Centrifugal Partition Chromatography (CPC) [30], and the results will be reported separately [18].

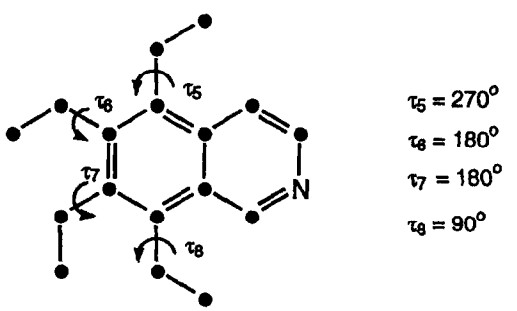

Fig. 3. Torsion angles of the hydroxy and methoxy substituents in positions 5–8 as fixed for the CoMFA studies: substituents 6 and 7 are in the plane of the ring system, while substituents 5 and 8 are perpendicular to the plane and above it.

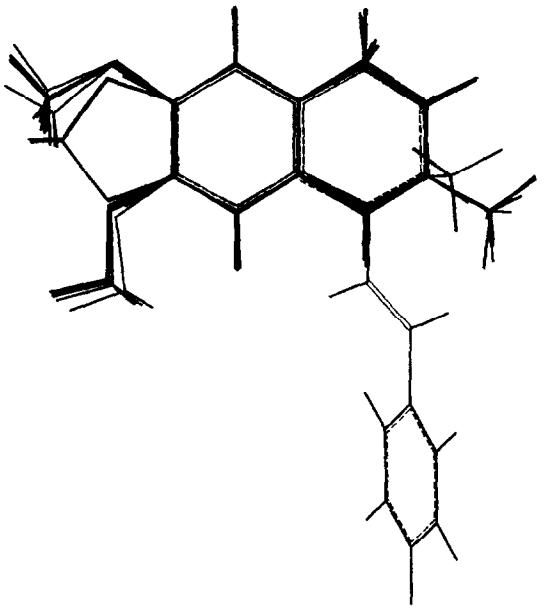

Fig. 4. Superposition of 18 compounds of series I.

The results in Table 1 further suggest that the active site of MAO-A, in contrast to that of MAO-B, can bind positively-charged amines with high affinity, and consequently that the basicity of amines can play a significant role in enhancing their selectivity towards MAO-A. Indeed, the amino acid sequences of the two MAO isoenzymes suggest that their active sites are quite different, with the active site of MAO-B displaying a higher hydrophobic character [3].

With regard to MAO-A inhibitory activity, substitution in position 6 and/or 7 with electron-donating methoxy or methylenedioxy groups usually produces an increase in MAO-A inhibitory activity. Globally, substitution in position 6 is more favourable than in position 7. Such trends are particularly clear in N-methyl-IQ$^+$ derivatives. Based on their electronic conjugation deduced from the UV absorption spectra (results not shown), the positive charge must be more delocalized in N-methyl-6-methoxy-IQ$^+$ than in the 7-methoxy isomer due to the contribution of a para-quinoid mesomeric structure in the former isomer. Thus, charge delocalization may also be a factor leading to increased MAO-A inhibitory activity.

Table 2. Structure and MAO-A inhibitory activities of isoquinolines and tetrahydroisoquinolines reported by Bembenek *et al.* [14] (series **II** in this study) (for structures, see Fig. 2)

							MAO-A	
No.	R_1	R_2	R_5	R_6	R_7	R_8	$pK_{i(measured)}$	$pK_{i(predicted)}$*
***R*-19**	CH_3	H	H	OH	OH	H	4.51	4.06
***S*-19**	CH_3	H	H	OH	OH	H	3.55	3.35
20	H	CH_3	H	OH	OH	H	2.89	3.37
21	CH_3	CH_3	H	OH	OH	H	4.44	4.26
22	CH_3	H	H	OCH_3	OH	H	4.47	4.85
***R*-23**	CH_3	H	H	OH	OCH_3	H	4.11	4.22
***S*-23**	CH_3	H	H	OH	OCH_3	H	3.80	3.52
24	CH_3	CH_3	H	OH	OCH_3	H	4.15	4.42
***R*-25**	CH_3	H	H	OCH_3	OCH_3	H	5.22	4.95
***S*-25**	CH_3	H	H	OCH_3	OCH_3	H	3.73	4.18
26	H	CH_3	H	OCH_3	OCH_3	H	4.57	4.21
***R*-27**	CH_3	CH_3	H	OCH_3	OCH_3	H	5.70	5.33
***S*-27**	CH_3	CH_3	H	OCH_3	OCH_3	H	3.99	4.13
28	CH_3	H	H	OCH_3	OCH_3	OCH_3	3.77	3.62
29	CH_3	CH_3	H	OCH_3	OCH_3	OCH_3	3.80	4.15
30	H	H	OCH_3	OCH_3	OCH_3	OCH_3	3.15	3.14
31	H	CH_3	OCH_3	OCH_3	OCH_3	OCH_3	3.59	3.26
32	CH_3	H	OCH_3	OCH_3	OCH_3	OCH_3	3.00	3.07
33	CH_3	H	H	OCH_3	OH	H	4.89	5.01
34	CH_3	H	H	OH	OCH_3	H	4.10	4.30
35	CH_3	H	H	OCH_3	OCH_3	H	5.40	5.20
36	CH_3	H	H	OCH_3	OCH_3	OCH_3	5.70	5.76
37	CH_3	H	OCH_3	OCH_3	OCH_3	OCH_3	5.00	5.07
41	CH_3	H	H	OH	OH	H	3.89	3.84
42	CH_3	H	H	OH	OCH_3	H	3.89	4.00
43	H	H	H	OCH_3	OCH_3	OCH_3	4.39	4.46
44	CH_3	H	OCH_3	OCH_3	OCH_3	OCH_3	4.77	4.78
45	CH_3	CH_3	H	OH	OCH_3	H	3.82	3.75

			MAO-A			
No.	R_6	R_7	$pK_{i(measured)}$	$pK_{i(predicted)}$*	$pIC_{50measured}$†	$pIC_{50predicted}$‡
38	H	H	3.89	4.04	3.59	4.76
39	OCH_3	H	5.40	5.15	5.10	5.32
40	OCH_3	OCH_3	5.22	5.34	4.92	5.13

* Predicted with model **B1.**

† $pIC_{50} = p(2 \times K_i)$ for $(S = K_m)$.

‡ Predicted with model **A5.**

Table 3a. Statistical results of a CoMFA study of 18 MAO-A inhibitors (series I) (see Table 1)

Model	Field	q^2*	N†	r^2	Relative contribution		
					ste	ele	lipo
A1	steric field (ste)	0.36	–	–	–	–	–
A2	electrostatic field (ele)	0.36	–	–	–	–	–
A3	lipophilicity field (lipo)	0.34	–	–	–	–	–
A4	ste and ele	0.49	2	0.76	0.60	0.40	–
A5	ste and lipo	0.51	2	0.77	0.56	–	0.44
A6	ele and lipo	0.35	–	–	–	–	–
A7	ste, ele, and lipo	0.50	2	0.76	0.53	0.17	0.30

* Cross-validated correlation coefficient q^2.
† Optimal number of principal components used in the final analysis.

Table 3b. Statistical results of a CoMFA study of 31 MAO-A inhibitors (series II) (see Table 2)

Model	Field	q^2*	N†	r^2	Relative contribution		
					ste	ele	lipo
B1	steric field (ste)	0.51	6	0.89	1	–	–
B2	electrostatic field (ele)	0.33	5	–	–	–	–
B3	lipophilicity field (lipo)	0.28	4	–	–	–	–
B4	ste and ele	0.44	5	0.86	0.65	0.35	–
B5	ste and lipo	0.44	5	0.85	0.60	–	0.40
B6	ele and lipo	0.37	5	–	–	–	–
B7	ste, ele, and lipo	0.44	3	0.77	0.46	0.26	0.28

* Cross-validated correlation coefficient q^2.
† Optimal number of principal components used in the final analysis.

All 18 compounds displayed only modest activity towards MAO-B. Although no clear qualitative structure-activity relationships can be derived for MAO-B inhibition, some trends are apparent. THIQ derivatives with a 6-methoxy **(12, 16)** or a methylenedioxy group **(14)** are significantly less active towards MAO-B than the corresponding IQ and isoquinolinium derivatives **(2, 5, 7, 10),** whereas 7-methoxy-THIQ **(13)** is more potent than 7-methoxyisoquinoline **(3)** and 7-methoxyisoquinolinium ion **(8).** Furthermore, no significant difference in activity is seen (as is the case for MAO-A inhibition) when the isoquinolines **(1–5)** and the corresponding isoquinolinium ions are compared **(6–10).**

3D-QSAR of MAO-A inhibition

Conditions of study. CoMFA (comparative molecular field analysis), a particularly useful tool in three-dimensional quantitative structure-activity relationships [26], was used in this study to gain insight into the structural requirements modulating MAO-A inhibitory activity. The MAO-B data presented here are not suitable for a QSAR analysis because of relatively modest differences in activity (Table 1). Similarly, most of the compounds reported by Bembenek *et al.* [14] were not active enough on MAO-B for a CoMFA study to be performed.

Some of us have recently described a molecular lipophilicity field (MLP) and shown its considerable interest as a third field in CoMFA in addition to the traditional steric and electrostatic fields [27]. Since the MLP previously described [27] is not parametrized for the quaternary nitrogen atom in its various molecular environments, we derived these values from the experimental log P values of inhibitors **6–9** [18] and of ten model compounds [29], all experimentally determined by CPC [30].

A critical step in any CoMFA study is the superposition of all compounds in a biologically relevant conformation. The compounds investigated here are relatively rigid and straightforward to superimpose. Their minimal energy conformation was calculated by the semi-empirical AM1 method, and the hydroxy or methoxy substituents in positions 5–8 were fixed in conformations shown in Fig. 3. Their ring system was then superimposed atom by atom. The superposition of the 18 compounds of series I is shown in Fig. 4. Two series of compounds were submitted to a CoMFA study, namely the 18 inhibitors investigated here (series I), and a series of compounds reported by Bembenek *et al.* [14] (series II) (Table 2). Unfortunately, it was impossible to merge the two series of inhibitors in a single analysis for two reasons. First, Bembenek *et al.* used an enzyme preparation (human placenta MAO-A) different from the one used here. And second, their results are expressed as K_i values, not always transformable into IC_{50} values.

Series I. The statistical results obtained with series I are reported in Table 3a. Models based on a single field (models **A1, A2,** and **A3**) are of insufficient statistical quality, the cross-validated correlation coefficient (q^2) being smaller than 0.4. In contrast, models based on the steric field plus either the electrostatic field (model **A4**) or the lipophilicity field (model **A5**) are statistically acceptable. No improvement is obtained with a model based on the three fields (model **A7**). The final model for series I is thus model **A5,** which expresses the contribu-

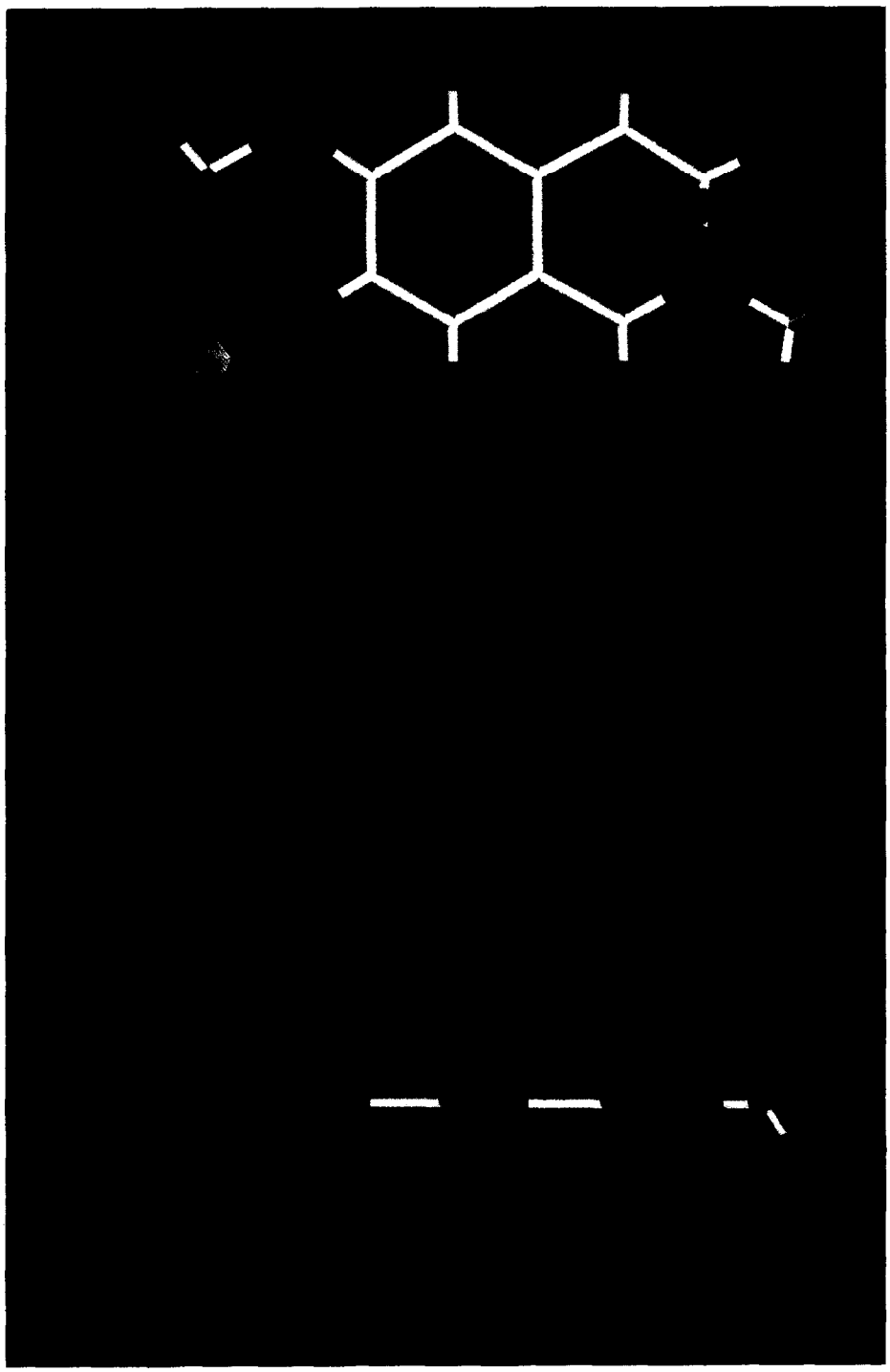

Fig. 5. Graphical representation of model **A5** derived for the MAO-A inhibition of compounds in series I. The colour code is as follows: Sterically favourable zones are in green, sterically unfavourable zones in red; zones where lipophilicity is favourable are in yellow, and zones where hydrophilicity (polarity) is favourable are in cyan. The view is from above and from the side, with the most active compound (**7**) positioned in the model. A 6-methoxy substituent is shown to point in a sterically favourable zone (green), whereas a bulky substituent in position 7 would be unfavourable (red). A favourable hydrophilic domain (cyan) is seen above and below the positively charged nitrogen atom. A lipophilic substituent in position 7 and/or 6 increases MAO-A inhibitory activity (yellow).

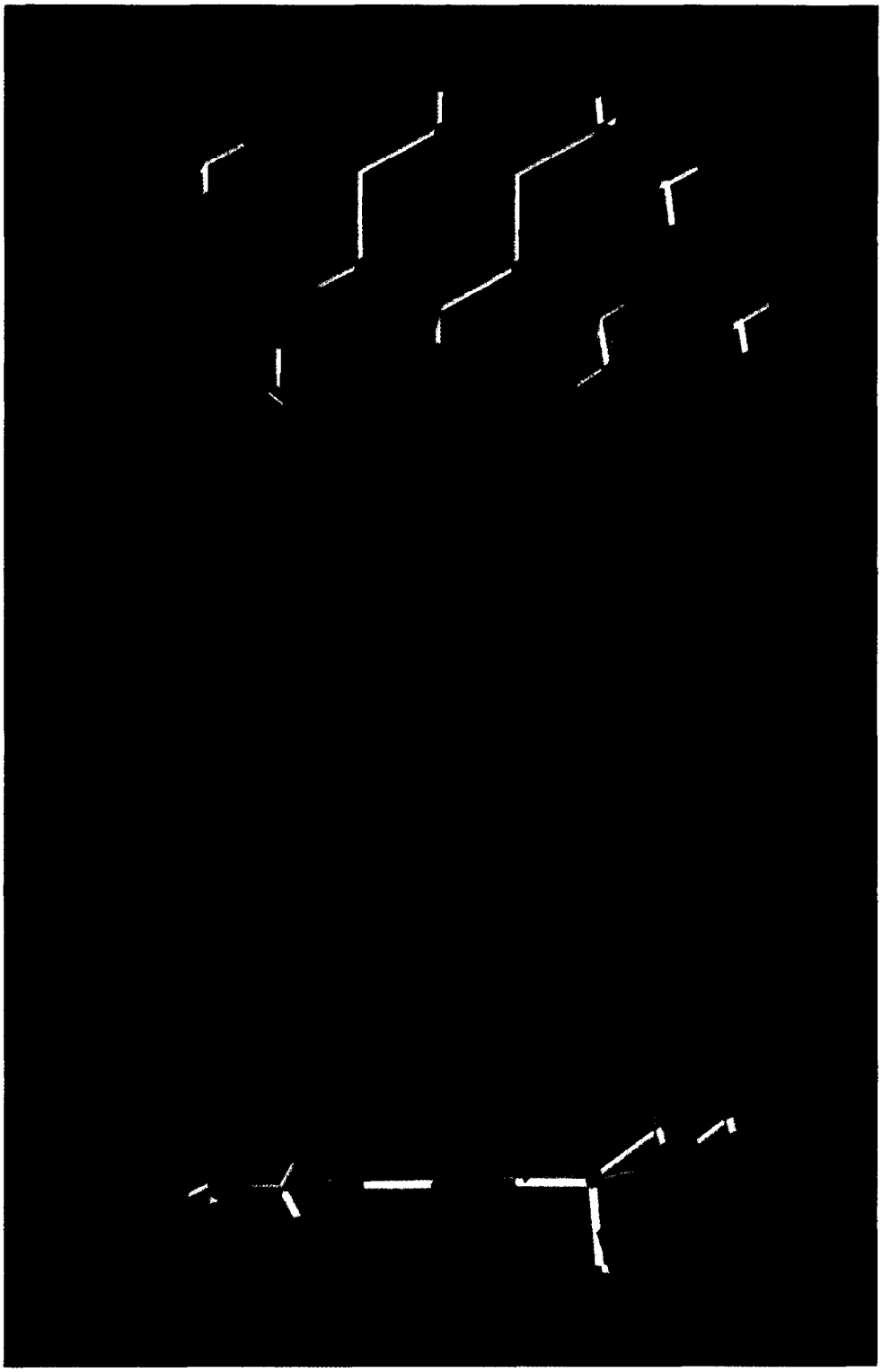

Fig. 6. Graphical representation of model **B1** derived for the MAO-A inhibition of compounds in series II. The model incorporates only the steric field, whose colour code is as in Fig. 2. The view is from above and from the side, with one of the most active compounds (***R*-27**) positioned in the model. The figure shows a broad unfavourable steric zone (red) above positions 1 *S* and 8. A few favourable steric zones (green) are apparent, mainly close to positions 1*R* and 6.

tions of the steric and lipophilicity fields. Table 1 compares the experimental data with the values predicted by model **A5.**

Model **A5** is represented graphically in Fig. 5, which shows the steric influence of substituents (i.e., the regions where a substituent has a favourable steric influence (green) or an unfavourable one (red)). Lipophilic influences are also depicted in Fig. 5, there being one region where a lipophilic substituent is favourable (yellow), and two regions above and below the nitrogen atom where the favourable influence of hydrophilicity (polarity) is felt (cyan). As shown in Fig. 5, a substituent in position 6 is sterically favourable, and a substituent in position 7 can make both a favourable lipophilic contribution and an unfavourable steric contribution. In model **A4** (see Table 3a), the lipophilicity field is replaced by an electrostatic field. Although the statistical quality of model **A4** and **A5** is similar, model **A5** seems, however, more informative than model **A4** because of the additional lipophilic signal around the nitrogen. In model **A4** the variation of substituents in position 6 is described by both steric and electrostatic fields. Moreover, the electrostatic signal is localized around the substituent in position 6, suggesting a correlation between the electrostatic and steric fields (results of model **A4** not shown).

To test the extrapolative capacity of model **A5,** the MAO-A inhibitory potency of the three charged N-methyl-3,4-dihydroisoquinolinium ions in series II was predicted. The activity of the two 6,7-disubstituted compounds **39** and **40** was well predicted (Table 2), but the prediction was off by one order of magnitude for the unsubstituted compound **38.** This poor prediction is not surprising considering that the activity of **38** is low relative to those of the charged compounds in series I.

Series II. The results of the CoMFA study of series II are summarized in Table 3b. The best model was obtained with the steric field alone (model **B1**), and the other two fields taken alone did not yield an acceptable correlation coefficient. Models combining the steric field plus one (**B4** and **B5**) or the two others (**B7**) are satisfactory, but do not improve on model **B1**. The latter was thus selected as the final model. The experimental and predicted values are compared in Table 2. A graphical representation of model **B1** is given in Fig. 6, showing two major regions of favourable steric influence near the 1*R*- and 6-positions. A favourable 1*R* substituent is found in the active MAO-A inhibitors ***R*-19, *R*-23, *R*-25,** and ***R*-27**. Compounds with a 1*S* substituent are less active, and the model indeed shows an unfavourable steric influence in this region. A minor unfavourable region is seen above position 8, indicating a rather distant (approx. 4 Å) influence.

DISCUSSION

This study offers novel rationalizations into the three-dimensional structure-activity relationships of MAO-A inhibition. The models presented here increase our topographical understanding of the binding site of this enzyme, and as such may prove useful in the design of novel MAO inhibitors. In addition to previous studies [31, 32] that pointed out that hydrophobic interactions influence the affinity of MAO-B substrates, we show here that beside electrostatic forces, steric, lipophilic, and hydrophilic interactions also play an important role in modulating MAO-A inhibitory activity.

The two 3D models **A5** and **B1** may appear different at first sight. However, their close analogy is worth stressing. Both models reveal a sterically favourable region around position 6, and complementary unfavourable regions close to positions 7 and 8. Furthermore, both models explore the 1- and 2-positions, but the information of model **B1** is steric (positions 1*R* and 1*S*), whereas that of model **A5** is hydrophilic. This discrepancy may be only an artifact due to the different composition of the two data sets. In fact, whereas the hydrophilic N-methylisoquinolinium ions are well represented in set **A** (5 out of 18 compounds), only 4 out of 45 quaternary IQ^+ are present in series II. Consequently, electrostatic and/or hydrophobic interactions exerted by CH_3-N^+ are not well accounted for by model **B1,** which in contrast explores the effect of the methyl group in position 1 better than model **A5.** For these reasons, models **A5** and **B1** are considered to be quite helpful, providing as they do complementary insight into the topography of the MAO-A binding site.

The experimental results reported here have another, more physiological interest. By extending the number of isoquinolines and analogues known to inhibit MAO-A and/or MAO-B, they bring to the forefront the question of the physiological (and perhaps pathological) significance of endogenous MAO inhibitors. Considering that a number of isoquinoline analogues are indeed formed endogenously, a physiological role may be postulated in the control of neurotransmitter function. Another possibility worth exploring is a protective effect in preventing MAO-mediated toxication of endogenous and/or exogenous MPTP-like neurotoxins.

Acknowledgements—PAC and BT acknowledge the support of the Swiss National Science Foundation. The Italian authors gratefully acknowledge the support of M.U.R.S.T. and C.N.R. (Rome, Italy).

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