Biochemical Pharmacology, Vol. 40, No. 4, pp. 783–792, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

TOXICATION OF MPTP (1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE) AND ANALOGS BY MONOAMINE OXIDASE

A STRUCTURE–REACTIVITY RELATIONSHIP STUDY

GERARD MARET,* NABIL EL TAYAR,* PIERRE-ALAIN CARRUPT,* BERNARD TESTA,*† PETER JENNER‡ and MARK BAIRD§
*Institut de Chimie Thérapeutique, Ecole de Pharmacie, Université de Lausanne, Place du Château 3, CH-1005 Lausanne, Switzerland; ‡Department of Pharmacology, King's College Chelsea Campus, Manresa Road, London SW3 6LX, U.K.; and §Department of Organic Chemistry, University of Newcastle, Framlington Place, Newcastle upon Tyne NE1 7RV, U.K.

(*Received* 30 *November* 1989; *accepted* 17 *March* 1990)

Abstract—MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) elicits motor deficits similar to those observed in Parkinson's disease. Before exerting its neurotoxic action, MPTP must be activated by brain monoamine oxidase (MAO) to the neurotoxic metabolite MPP^+ (1-methyl-4-phenylpyridinium). MPTP derivatives differ in their reactivity as MAO substrates and in their neurotoxicity. A structure–reactivity relationship study based on literature data was undertaken in order to determine the key features in the structure of MPTP and analogs that are responsible for the reactivity towards MAO. Thirty-three MPTP derivatives (including MPTP itself) were included in the study. To explain the reactivity towards MAO of the 33 MPTP analogs, different statistical methods (principal component analysis, multiple linear regression analysis) as well as the CoMFA (Comparative Molecular Field Analysis) approach, a new tool in structure–activity correlations, were used. Linear regression analysis failed to yield any predictive model, but suggested some trends. In contrast, the CoMFA approach was successful in correlating structural features and MAO reactivity. Coefficient contour maps showed where differences in the steric field (van der Waals' interactions) are most highly associated with differences in MAO reactivity. Several positive (in the *ortho*- and *meta*-position of the phenyl group) and negative (in the *para*-position of the phenyl group; beyond the *N*-methyl group) interaction regions were identified. Some structural features of the MAO active site could be postulated. First, the *N*-methyl group has the ideal size and elicits ideal interactions within the MAO active pocket, while smaller or larger groups are less favorable; second, *para*-substituent on the phenyl ring produce steric hindrances and are unfavorable to reactivity; third, *ortho*- and *meta*-substituents may have stabilizing interactions within the active pocket and are favorable to the reactivity. Moreover the model derived by CoMFA allowed us to make succesful predictions of reactivity towards MAO for several additional tetrahydropyridines.

Motor deficits similar to those observed in Parkinson's disease were reported in young drug addicts following self-administration of 1-methyl-4-phenyl-4-propionoxypiperidine (MPPP‡) (Fig. 1, I) a meperidine analog which was contaminated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Fig. 1, II) [1–4]. Administration of MPTP to monkeys elicited symptoms identical to those observed in humans exposed to MPTP, as consequence of a degeneration of nigrostriatal dopaminergic neurons [5, 6]. The same degeneration is observed in mice; rodents however are less senstitive to MPTP than primates [7–10].

The molecular basis of MPTP neurotoxicity causing degeneration of the nigrostriatal system has been studied intensively. The cascade of events responsible for MPTP neurotoxicity and dopaminergic neuron death can be summarized as following: first, MPTP is oxidized to the neurotoxin which causes cell death, MPP^+ (1-methyl-4-phenylpyridinium) (Fig. 1, IV) [11–14]; second, MPP^+ is transported and accumulated in dopamine (DA) neurons via the DA uptake system [15–18]; third, MPP^+ is transported and concentrated in the matrix of mitochondria [19, 20] where it inhibits the NADH dehydrogenase [21–26], leading to cessation of oxidative phosphorylation and ATP depletion, and resulting in neuron death [27].

The toxication of MPTP is mediated by monoamine oxidase (MAO, amine:oxygen oxidoreductase (deaminating) (flavin containing), EC 1.4.3.4), principally MAO type B, and to a lesser extent MAO-A. This enzyme catalyses the oxidation of MPTP to the intermediate $MPDP^+$ (1-methyl-4-phenyl-2,3-dihydropyridinium) (Fig. 1, III) which then spontaneously disproportionates to form MPTP and MPP^+. Brain MAO is the *key enzyme* that activates

---

† To whom correspondence should be addressed.

‡ Abbreviations: CoMFA, Comparative Molecular Field Analysis; DA, dopamine; $\Delta(\Delta H)$, protonation enthalpy; MAO, monoamine oxidase; MAO-A, MAO-B, monoamine oxidase type A, type B; MEP, molecular electrostatic potentials; $MPDP^+$, 1-methyl-4-phenyl-2,3-dihydropyridinium; MO, molecular orbital; MPP^+, 1-methyl-4-phenylpyridinium; MPPP, 1-methyl-4-phenyl-4-propionoxypiperidine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PLS, partial least-squares; QSAR, quantitative structure–activity relationships.

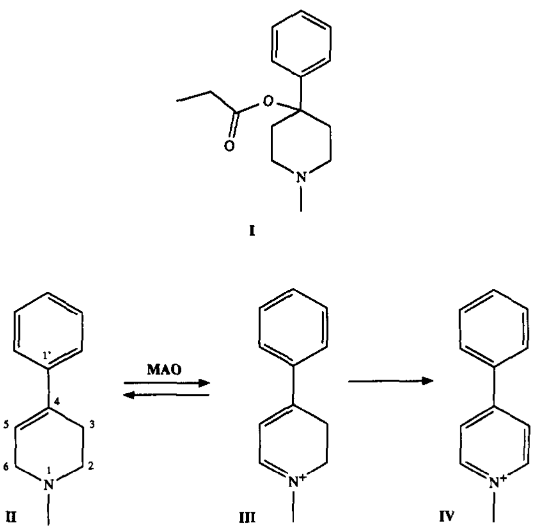


Fig. 1. Some compounds discussed in the text. I, MPPP (1-methyl-4-phenyl-4-propionoxypiperidine); II, MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; III, MPDP$^+$, 1-methyl-4-phenyl-2,3-dihydropyridinium; IV, MPP$^+$, 1-methyl-4-phenylpyridinium.

MPTP to the neurotoxic metabolite MPP$^+$. Oxidation of MPTP and analogs by MAO appears as a necessary (but not sufficient) condition for neurotoxicity: a MPTP analog first has to be a MAO substrate in order to be neurotoxic.

A number of MPTP analogs have been prepared and tested for their neurotoxicity [26–39], with the goal of determining the basic structural features (i) necessary to enhance reactivity as MAO substrate, and (ii) responsible for causing neurotoxicity [27, 32, 36–40]. The MAO reactivity of these analogs was then rationalized in terms of the presence or absence of a substituent, but no correlations with physicochemical properties are available.

The present work was designed to identify the key features in the structure of MPTP and analogs responsible for their reactivity towards MAO and to correlate this reactivity with structural physicochemical properties. Thirty-three MPTP derivatives (Table 1) were included in the study. Their substrate reactivity towards MAO were collected from the literature [27, 29, 31–37, 39–42]. As all sets of MAO reactivity values were incomplete and not directly comparable, we used qualitative indicators with three different levels to describe the MAO reactivity of these analogs.

To correlate reactivity with structural features, we used traditional QSAR (quantitative structure–activity relationships) methods, as well as the CoMFA (Comparative Molecular Field Analysis) approach; CoMFA represents molecules by their steric and electrostatic fields, sampled at the intersection of a three-dimensional lattice [43, 44]. A table is constructed from the field values (steric and electrostatic) of all the compounds to be analysed [43]. Partial least-squares (PLS) statistics [45] are used to determine a linear expression ("three-dimensional QSAR") from this unusually proportioned data table, with many more columns (field values) than rows (compounds). This PLS model is cross-validated, i.e. its predictive value is checked, and a final model derived. The results are represented graphically as a three dimensional "coefficient contour" map, which represents isocontour surfaces of the variable being contoured. This final model allows the regions in the steric and electrostatic fields most pertinent for activity to be found, and the regions in the contour map essential for or incompatible with reactivity to be visualized.

Whereas traditional QSAR analyses using different statistical methods (principal component analysis, multiple linear regression analysis) failed to give any predictive model, we were able using the CoMFA approach to correlate structural features and MAO substrate reactivity.

MATERIALS AND METHODS

Compounds and structural calculations. Molecular models of the 33 MPTP derivatives (Table 1) were constructed using the SYBYL software (Tripos Associates) running on a VAX-8550 computer; the geometry of each derivative was minimized using the standard Tripos force field [46, 47]; the space associated to the inter-ring torsional angle was searched on a 15° grid, and the final structure was minimized again. For each compound, only the geometry of minimal energy was retained. Determination of the minimal energy of the geometry obtained with SYBYL was made using full geometry optimization with the semi-empirical AM1 quantum mechanical program method [48] (program AMPAC, QCPE No. 506), also running on a VAX-8550 computer; the molecular orbitals and the electronic densities of the derivatives were also calculated by the program AM1. In recent studies [49, 50], AM1 was proved to be superior to MINDO/3 and MNDO parameterization for predicting molecular geometries, in particular, for the heat of formation and the rotational barrier.

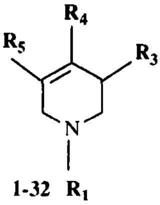
The protonation enthalpy $\Delta(\Delta H)$ is correlated with the pK_a [51]; it was calculated by subtracting the formation enthalpy of the non-protonated form from the formation enthalpy of the protonated form; the enthalpies were the ones calculated by AM1.

Molecular and H_2O-accessible surface areas were calculated with the program MOLSV (QCPE No. 509) running on an Apollo DN 3000 workstation. van der Waals' radii were taken from [52], and the H_2O molecule was assumed to be a sphere of radius 1.5 Å. The H_2O-accessible surface area was calculated by the program as being the ensemble of all the points occupied by the centre of the H_2O sphere when it rolls on the molecular surface.

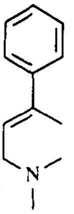
Statistical analysis. Principal component analyses were performed using the SIMCA program [53]. In these analyses, we described the MAO substrate reactivity of the 33 MPTP derivatives using a 3-level scale (level 1 for low or very low reactivity, level 2 for intermediate reactivity, and level 3 for marked reactivity, i.e. similar or higher than that of MPTP). To describe structural features, the following parameters were used:

— electronic densities on selected atoms;

Table 1. Structure and MAO reactivity of the MPTP analogs used in this study



1-32



33

	R_1	R_3	R_4	R_5	MAO reactivity*	Ref.
1	Me	H	Phenyl	H	3	†
2	H	H	Phenyl	H	3	33
3	Me	Me	Phenyl	H	2	37
4	Me	H	Phenyl	Me	2	37
5	Me	H	2'-Methylphenyl	H	3	27
6	Me	H	3'-Methylphenyl	H	3	27
7	Me	H	4'-Methylphenyl	H	2	27
8	Me	H	2'-Methoxyphenyl	H	3	27
9	Me	H	3'-Methoxyphenyl	H	2	27
10	Me	H	2'-Fluorophenyl	H	3	39
11	Me	H	3'-Fluorophenyl	H	3	39
12	Me	H	4'-Fluorophenyl	H	2	27, 39
13	H	H	4'-Fluorophenyl	H	2	41
14	Me	H	2'-Trifluoromethylphenyl	H	3	39
15	Me	H	3'-Chlorophenyl	H	3	27
16	Me	H	4'-Chlorophenyl	H	2	31
17	H	H	4'-Chlorophenyl	H	2	41
18	Me	H	Cyclohexyl	H	3	33
19	Me	H	2'-Thienyl	H	2	32
20	Me	H	Benzyl	H	3	27
21	Me	H	Tert-butyl	H	2	27
22	Me	H	Ethyl carboxylate	H	2	41
23	Me	H	Et	H	2	36
24	Et	H	Phenyl	H	2	27
25	2-Hydroxy-Et	H	Phenyl	H	2	41
26	Isopropyl	H	Phenyl	H	1	36
27	Propyl	H	Phenyl	H	1	27
28	Butyl	H	Phenyl	H	1	40
29	Cyclopropylmethyl	H	Phenyl	H	1	40
30	Allyl	H	Phenyl	H	1	40
31	Benzyl	H	Phenyl	H	1	40
32	Et	H	H	H	2	41
33		*N*,*N'*-Dimethylcinnamylamine			3	37

* The levels are: 1, low or very low reactivity; 2, moderate reactivity (<MPTP); 3, marked reactivity (≥MPTP).

† All references in the table.

- the H_2O-accessible surface of the $C(5)H–C(6)H_2$ group (C5–C6 edge);
- the protonation enthalpy $\Delta(\Delta H)$ of the nitrogen atom (as indicator of the pK_a).

Multiple linear regression analysis was performed using as physicochemical parameters the energy of the molecular orbital (MO) containing the nitrogen lone pair (= MO energy) an indicator of the ionization potential, the protonation enthalpy $\Delta(\Delta H)$ of the nitrogen atom, and the H_2O-accessible surface of the C5–C6 edge in the tetrahydropyridine ring.

CoMFA. The AM1 geometries and AM1 charges were used in the CoMFA approach. Both steric and electrostatic fields were considered. A "region" was defined with the automatic mode "molecular volume", that creates a single grid overlapping each molecule by at least 4 Å along all axes; the grid size was 2 Å along all axes; the "probe atom" had the van der Waals' properties of a sp^3 carbon and a +1.0 charge [43]. All analogs were aligned (congruently positioned in reference to a three dimensional frame) using the least-squares fitting of N(1) C(2), C(4), C(5), C(6), and C(1') of each molecule to the corresponding atoms of MPTP (Fig. 1, II) [43].

CoMFA was performed in two successive steps. In a first analysis, using five components and seven cross-validation groups, the optimal number of components was determined. The second run was performed without cross-validation, using the optimal number of component determined in the first analysis; the results of this second run were used for interpretation, drawing of the coefficients contour maps, and prediction of MAO reactivities for new derivatives. The coefficient contour maps were represented using the multiplication of standard deviations and coefficients. In a preliminary inves-

Table 2. CoMFA analysis: search for the optimal number of components (33 compounds, 7 cross-validation groups)

	Number of components				
	1	2	3	4	5
r^2	0.473	0.511	0.536	0.566	0.560
s	0.584	0.562	0.547	0.529	0.533
F	4.843	5.642	6.250	7.040	6.868

tigation, two different analyses were performed using two different alignment rules of the analogs, i.e. least-square fitting and automatic "field fit" procedures. The differences were not statistically significant.

RESULTS

Statistical analysis

Traditional QSAR analyses using different statistical methods such as principal component analysis and multiple linear regression analysis failed to give any predictive model.

Principal component analyses using structural parameters (electronic densities, H_2O-accessible surface, protonation enthalpy $\Delta(\Delta H)$) and a 3-level indicator of biological activity gave only non-significant results, probably because the variations of physicochemical parameters were too small and above all because biological activity was not described with quantitative values.

For the 33 compounds in Table 1, multiple linear regression analysis led to a relationship between reactivity towards MAO and the three structural parameters protonation enthalpy $\Delta(\Delta H)$, H_2O-accessible surface and energy of the MO associated to nitrogen lone pair (MO energy); this relationship is expressed by Eqn 1.

$$\begin{aligned}\text{MAO substrate reactivity} &= 0.185\,(\pm 0.107)\,\Delta(\Delta H)\\ &\quad - 2.41\,(\pm 1.47)\,\text{MO energy}\\ &\quad + 0.016\,(\pm 0.021)\,H_2O\text{-accessible surface}\\ &\quad - 4.09\,(\pm 16.93), \qquad (1)\end{aligned}$$

$(N = 33;\ r^2 = 0.477;\ s = 0.584;\ F = 8.85)$.

The correlation coefficient is quite low, and the regression equation explains only about 50% of the variance; moreover, the third term of the equation (H_2O-accessible surface) is not significant, the 95% confidence limit on the coefficient being greater than the coefficient itself. However, this equation is interesting in that it indicates some trends. Thus, the protonation enthalpy $\Delta(\Delta H)$ and the energy of the MO associated with the nitrogen lone pair contribute mainly to the correlation, the better MAO substrates having a lower MO energy and a higher protonation enthalpy. Other physicochemical parameters such as molar refractivity (*MR*), Hammett electronic constant (σ) and the hydrophobic substituent constant (π) were also proved unsuccessful (equations not shown).

CoMFA

CoMFA was more successful than traditional QSAR in correlating structural features and MAO substrate reactivity of MPTP derivates. The analysis allowed a predictive model to be derived.

As described in Materials and Methods, CoMFA was performed using cross-validation to determine the optimal number of principal components. The statistical evaluation of such analysis is represented in Table 2. We can see that the statistical parameters improve up to four components, but deteriorate when a fifth component is added. In consequence, the optimal number of components is four. The second run was performed without cross-validation, using the optimal number of components determined in the first step. The statistical parameters were as follows: $N = 33$; $r^2 = 0.926$; $s = 0.211$ and $F = 87.78$.

Of the several possible graphical representations of CoMFA results we chose to represent the contour maps of the multiplication of standard deviations and coefficients, because they most clearly show where the differences in fields are best associated with differences in target activity. Figure 2 shows colour stereoscopic views of such maps, for the steric aspect only, of the MAO substrate reactivity. The electrostatic maps do not contain pertinent information (see later) and are not shown. To help in visualization, the molecule of a very good substrate of MAO (2′-Me-MPTP) is added to the map in Fig. 2a and that of an unmetabolized analog (1-propyl-PTP) is added in Fig. 2b. The coloured polyhedra in each map surround all lattice points where the QSAR strongly associates changes in the steric field of MPTP analogs with changes in reactivity towards MAO. More specifically, the polyhedra surround lattice points where the scalar products of the associated QSAR coefficients and the standard deviation of all values of MAO substrate reactivity correspond to a given percentage of the data range. Colour is used to code the type of these interactions. In these maps, *green* polyhedra surround steric regions associated with an increase of MAO substrate reactivity (positive regions), whereas *red* polyhedra surround regions associated with a decrease in reactivity (negative regions). We note two major negative regions, one in the *para*-region of the phenyl group and one in the region of the *N*-methyl group of MPTP; indeed all known derivatives with a *para*-substituent on the phenyl ring have a reduced MAO reactivity compared to MPTP, and derivatives with a *N*-substituent longer than ethyl are inactive, the *N*-ethyl analog being less active than its *N*-methyl congener. A third negative region is located above the plane of the phenyl ring; it corresponds to the region occupied by the aliphatic side chain when the phenyl group is replaced by an aliphatic group; those derivatives with aliphatic chain have a reduced reactivity towards MAO. The major positive regions are located in the *ortho*- and *meta*-regions of the phenyl ring; indeed, the MPTP analogs that are the best MAO substrate described to date have an *ortho*-substituent on the phenyl ring, while those having a *meta*-substituent are also good MAO substrates.

The model allows us to make predictions of reactivity towards MAO for analogs that are not included in the study (for example newly tested compounds).

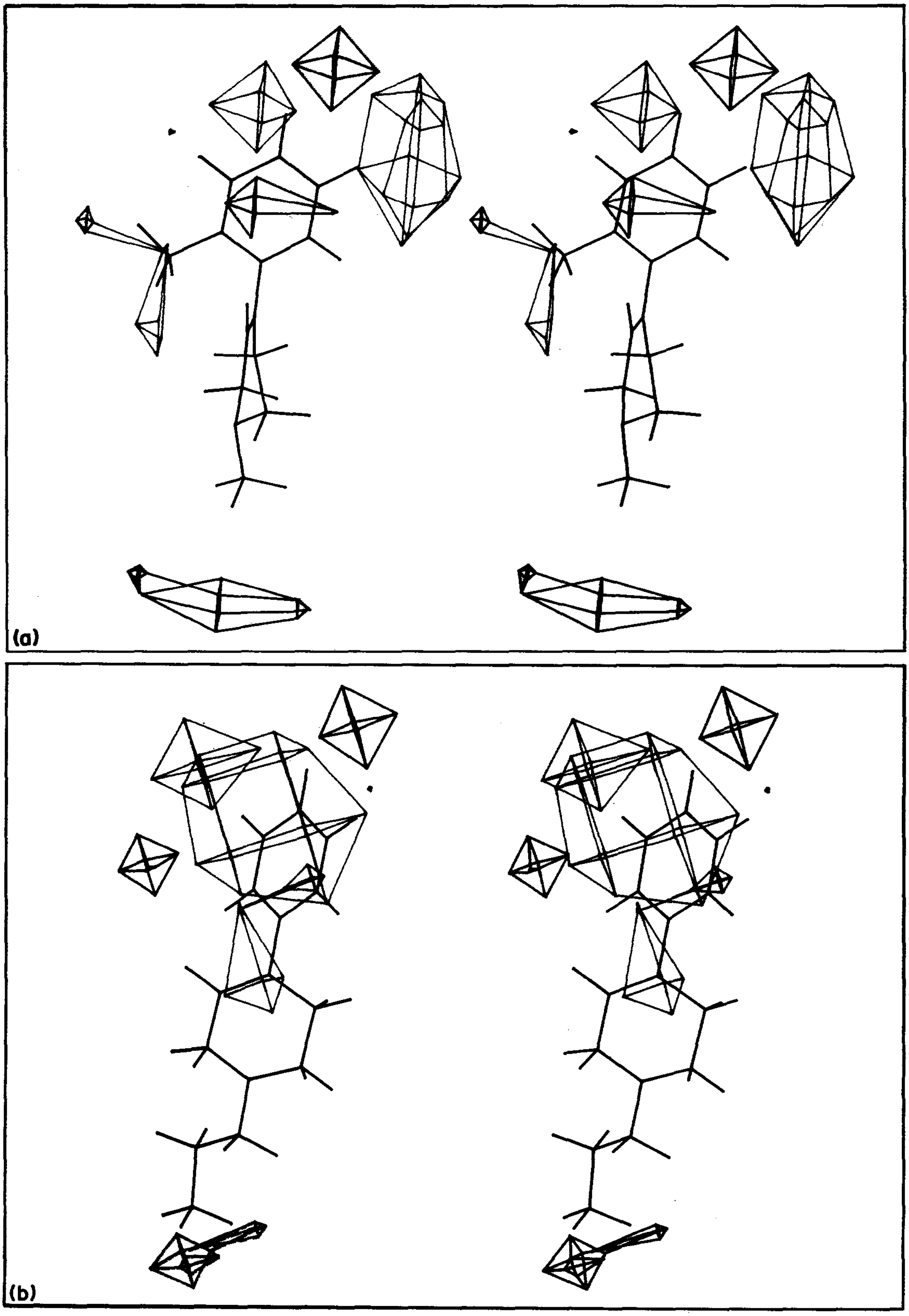
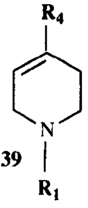
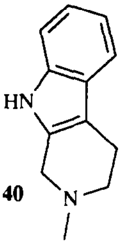


Fig. 2. Stereoscopic views of the contour map showing the major steric features influencing MAO substrate reactivity. Green contours surround regions where a higher steric interaction increases reactivity (the contours represent 70% of the range of the standard deviation coefficient). Red contours represent regions where a higher steric interaction decreases reactivity (30% of the standard deviation range). (a) and (b) stereoscopic views represent the same contours, the added molecule also being different. In (a), the represented molecule (1-methyl-4-(2′-methylphenyl)-1,2,3,6-tetrahydropyridine) is a good MAO substrate; in (b), the molecule (1-propyl-4-phenyl-1,2,3,6-tetrahydropyridine) is not a MAO substrate.

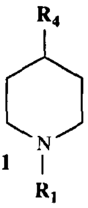
Table 3. Values predicted by CoMFA for MAO reactivity of MPTP analogs not included in the analysis



34-39



40



41

	R_1	R_4	Predicted value	Experimental value*	Ref.
34	Me	2′-Ethylphenyl	3.21	3	27
35	Me	2′-Chlorophenyl	2.66	3	27
36	Me	3′-Bromophenyl	3.05	3	27
37	Me	2′,6′-Dimethylphenyl	3.14	3	27
38	Me	1′-Methylpyrrol-2′-yl	2.71	3	30
39	4′-Cyanobutan-1′-one	Phenyl	1.07	1	41
40†	Me	Phenyl	1.80	2	62
41‡	Me	Phenyl	2.63	1	33

* Qualitative indicator; the levels are: 1, low or very low reactivity; 2, moderate reactivity (<MPTP); 3, marked reactivity (≥MPTP).

† 2-Methyl-1,2,3,4-tetrahydro-β-carboline, rigid analog of MPTP where carbon C5 and C2′ are connected by a N-bridge.

‡ Piperidine analog of MPTP.

Eight such compounds were available; their experimental and CoMFA-predicted reactivities (both described by qualitative indicators) are listed in Table 3; except for compound **41** (1-methyl-4-phenylpiperidine), the prediction corresponds to the experimental value. The experimental values for compounds **34–41** had been set aside from the beginning with the precise goal of testing the predictive capacity of the model.

DISCUSSION

Monoamine oxidase is the enzyme that transforms MPTP to the neurotoxic entity MPP^+ [11–14]; to be neurotoxic, a MPTP analog has to be a MAO substrate. This stresses the importance of the MAO in the toxication mechanism of MPTP. For this reason, we decided to evaluate the relation between the structure of the analogs and their reactivity as MAO substrates, in order to propose a structure–reactivity model and make some predictions on new MPTP derviatives.

It is not surprising that traditional QSAR methods failed to give any pertinent information. First, these methods are less appropriate when the biological activity is described by qualitative indicators as in our case. Second, the physicochemical parameters used to describe the structural features of the analogs showed very little variation, as the analogs investigated make up an homogenous series (Table 1); all the analogs are tetrahydropyridines, and the modifications occur mainly on the phenyl ring (substitution on the phenyl group or replacement of the phenyl ring) and on the nitrogen substituent.

The most striking feature of this work is the successful application of the CoMFA approach to the structure-reactivity study of MPTP analogs. Indeed, the CoMFA overcame the two problems that were obstacles in a classical QSAR analysis, leading to a model with predictive character and allowing us to make reactivity predictions for new compounds. In the CoMFA analysis, cross-validation was used to maximize the likelihood that the results have predictive validity.

Molecular electrostatic potentials (MEP) have proven a powerful tool for characterizing the essential electronic features of chemicals and their stereoelectronic complementarity with receptor sites [54–59]. The results of MEP calculations are represented graphically by means of isopotential curves plotted in some adequate plane. Comparing the maps of a set of analogs having superimposable skeletons and the same mechanism of action, it is possible to define a stereoelectronic pharmacophore [60], but the subjectivity of the *visual comparison* is a great drawback and the method has no QSAR applicability [61]. In contrast, the CoMFA method is based on the energies of the steric and electrostatic interactions between the tested compounds and a "probe atom" placed at the intersections of a regular three-dimensional lattice (called "region") and simulating all possible interactions with a receptor. CoMFA allows the *mathematical comparison* of the fields (steric and electrostatic) of a set of compounds, and three dimensional visualization of the regions that are favorable or detrimental to activity. Although the MEP approach is different from the CoMFA approach, both allow insight into features governing the specific interactions between a ligand and its receptor, CoMFA having the great advantage that it takes much more structural information into account and that it *compares mathematically the fields*.

As far as electrostatic fields are concerned, the present CoMFA analysis led to no conclusion. This is not surprising since the analogs examined here show too little variations in their electronic properties. The derived model is thus purely a steric one, and characteristically it fails to predict correctly the reactivity of compound **41**, an hexahydropyridine analog.

The interpretation of the steric maps stresses the importance of phenyl substituents and of the *N*-methyl group for MAO reactivity of MPTP derivatives. Phenyl substituents in *ortho*- or *meta*-position have a positive effect on the reactivity, whereas substituents in *para*-position have a negative effect. Indeed, all analogs with a *para*-substituent tested to date have, if any, a low reactivity towards MAO; in contrast some analogs with an *ortho*-substituent are better MAO substrates than MPTP itself, and most *ortho*- and *meta*-derivatives prepared to date are good MAO substrates. The *N*-methyl group is important for reactivity; *N*-H, *N*-ethyl and *N*-ethanol MPTP analogs are less good MAO substrates than MPTP, whereas longer alkyl chains abolish reactivity towards MAO. Moreover, there is a third negative region above the plane of the phenyl ring.

These results lead us to postulate some structural features of the MAO active site. MPTP analogs bearing an *N*-methyl moiety fit optimally in the active site of MAO; this group appears to have an ideal size and to elicit ideal interactions, presumably a hydrophobic interaction within the active pocket. *N*-H analogs are less active than the *N*-methyl derivatives; an *N*-ethyl or an *N*-ethanol moiety still fits into the active site, these groups being relatively small and quite flexible, but they induce steric hindrances impeding stabilizing interactions and resulting in reduced reactivity. *N*-Alkyl chains longer than *N*-ethyl are too hindered, impeding binding in the active site. The phenyl group also plays an important role in the reactivity, as obvious from the fact that it is surrounded of negative and positive regions. *para*-Substituents on the phenyl ring produce steric hindrances reducing considerably the reactivity. On the other hand, *ortho*- and *meta*-substituents may have stabilizing interactions within the active pocket; as in the case of the *N*-methyl, there must be an optimal size for these substituents, the *ortho-H* and the *ortho*-ethyl analogs being less reactive than the *ortho*-methyl; longer alkyl chain have not been tested.

The present results are still fragmentary, and further work is in progress to determine the conformational features eliciting MAO-A and MAO-B selectivity.

Acknowledgements—Support by the Swiss National Science Foundation (Grant 3.508-86) is gratefully acknowledged. Peter Jenner thanks the Parkinson's Disease Society, the Medical Research Council and the Research Funds of the Bethlem Royal and Maudsley Hospitals, and King's College Hospital.

Supplementary material—AM1 geometry in Cartesian coordinates for all compounds are available from the authors.

REFERENCES

1. Davis GC, Williams AC, Markey SP, Ebert MH, Caine ED, Reichert CM and Kopin IJ, Chronic Parkinsonism secondary to intravenous injection of meperidine analogues. *Psychiatry Res* **1**: 249–254, 1979.
2. Langston JW, Ballard P, Tetrud JW and Irwin I, Chronic product of meperidine-analog synthesis. *Science* **219**: 979–980, 1983.
3. Ballard PA, Langston JW, Tetrud J and Burns RS, Chemically induced chronic parkinsonism in young adults: clinical and neuropharmacologic aspects. *Neurology* **33**(Suppl 2): 90, 1983.
4. Langston JW and Ballard P, Parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydorpyridine (MPTP): implications for treatment and the pathogenesis of Parkinson's disease. *Can J Neurol Sci* **11**: 160–165, 1984.
5. Burns RS, Chiueh CC, Markey SP, Ebert MH, Jacobowitz DM and Kopin IJ, A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc Natl Acad Sci USA* **80**: 4546–4550, 1983.
6. Kolata G, Monkey model of Parkinson's disease. *Science* **220**: 705, 1983.
7. Heikkila RE, Hess A and Duvoisin RC, Dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice. *Science* **224**: 1451–1453, 1984.
8. Hallman H, Lange J, Olson L, Stromberg I and Jonsson G, Neurochemical and histochemical characterization of neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine on brain catecholamine neurones in the mouse. *J Neurochem* **44**: 117–127, 1985.
9. Sundstrom E, Stromberg I, Tsutsumo T, Olson L and Jonsson G, Studies on the effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on central catecholamine neurons in the C57B1/b mouse. Comparison with three other strains. *Brain Res* **405**: 26–38, 1987.
10. Melamed E, Rosenthal J, Globus M, Cohen O, Frucht Y and Uzzan A, Mesolimbic dopaminergic neurons are not spared by MPTP neurotoxicity in mice. *Eur J Pharmacol* **114**: 97–105, 1985.
11. Levitt P, Pintar JE and Breakfield XO, Immunocytochemical demonstration of monoamine oxidase B in brain astrocytes and serotonergic neurons. *Proc Natl Acad Sci USA* **79**: 6385–6389, 1982.
12. Heikkila RE, Manzino L, Cabbat F and Duvoisin R, Protection against the dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine by monoamine oxidase inhibitors. *Nature* **311**: 467–469, 1984.
13. Chiba K, Trevor A and Castagnoli N, Metabolism of the neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. *Biochem Biophys Res Commun* **120**: 574–578, 1984.
14. Chiba K, Peterson LA, Castagnoli KP, Trevor AJ and Castagnoli N, Studies on the molecular mechanism of bioactivation of the selective nigrostriatal toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Drug Metab Dispos* **13**: 342–347, 1985.
15. Chiba K, Trevor AJ and Castagnoli Jr N, Active uptake of MPP$^+$, a metabolite of MPTP, by brain synaptosomes. *Biochem Biophys Res Commun* **128**: 1228–1232, 1985.
16. Naoi M, Suzuki H, Kiuchi K, Takahashi T and Nagatsu T, Effect of *N*-methyl-4-phenylpyridinium ion on monoamine oxidase in a pheochromocytoma cell line, PC12h. *J Neurochem* **48**: 1912–1916, 1987.
17. Takahashi T, Naoi M and Nagatsu T, Uptake of *N*-methyl-4-phenylpyridinium ion (MPP$^+$) into PC12h pheochromocytoma cells. *Neurochem Int* **11**: 89–93, 1987.

18. Javitch JA, d'Amato RJ, Strittmatter SM and Snyder SH, Parkinsonism-inducing neurotoxin, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: uptake of metabolite *N*-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proc Natl Acad Sci USA* **82**: 2173–2177, 1985.
19. Ramsay RR and Singer TP, Energy-dependent uptake of *N*-methyl-4-phenylpyridinium, the neurotoxic metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, by mitochondria. *J Biol Chem* **261**: 7585–7587, 1986.
20. Ramsay RR, Dadgar J, Trevor AJ and Singer TP, Energy-driven uptake of *N*-methyl-4-phenylpyridinium by brain mitochondria mediates the neurotoxicity of MPTP. *Life Sci* **39**: 581–588, 1986.
21. Nicklas WJ, Vyas I and Heikkila RE, Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4-phenylpyridine, a metabolite of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Life Sci* **36**: 2503–2508, 1985.
22. Vyas I, Heikkila RE and Nicklas WJ, Studies on the neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: inhibition of NAD-linked substrate oxidation by its metabolite, 1-methyl-4-phenylpyridinium. *J Neurochem* **46**: 1501–1507, 1986.
23. Singer TP, Castagnoli Jr N, Ramsay RR and Trevor AJ, Biochemical events in the development of parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *J Neurochem* **49**: 1–8, 1987.
24. Ramsay RR, McKeown KA, Johnson EA, Booth RG and Singer TP, Inhibition of NADH oxidation by pyridine derivatives. *Biochem Biophys Res Commun* **146**: 53–60, 1987.
25. Rollema H, de Vries JB, Damsma G, Westerink BHC, Kranenborg GL, Kuhr WG and Horn AS, The use of *in vivo* brain dialysis of dopamine, acetylcholine, aminoacids and lactic acid in studies of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Toxicology* **49**: 503–511, 1988.
26. Mizuno Y, Saitoh T and Sone N, Inhibition of mitochondrial NADH-ubiquinone oxidoreductase activity by 1-methyl-4-phenylpyridinium ion. *Biochem Biophys Res Commun* **143**: 294–299, 1987.
27. Youngster SK, Sonsalla PK, Sieber B-A and Heikkila RE, Structure–activity study of the mechanism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity. I. Evaluation of the biological activity of MPTP analogs. *J Pharmacol Exp Ther* **249**: 820–828, 1989.
28. Fuller RW and Hemrick-Luecke SK, Persistent depletion of striatal dopamine in mice by *m*-hydroxy-MPTP. *Res Commun Chem Pathol Pharmacol* **53**: 167–172, 1986.
29. Zimmerman DM, Cantrell BE, Reel JK, Hemrick-Luecke SK and Fuller RW, Characterization of the neurotoxic potential of m-methoxy-MPTP and the use of its *N*-ethyl analogue as a means of avoiding exposure to a possible parkinsonism-causing agent. *J Med Chem* **29**: 1517–1520, 1986.
30. Fuller RW and Hemrick-Luecke SK, Persistent depletion of striatal dopamine and its metabolites in mice by TMMP, an analogue of MPTP. *J Pharm Pharmacol* **39**: 667–669, 1987.
31. Fuller RW, Hemrick-Luecke SK and Robertson DW, Comparison of 1-methyl-4-(*p*-chlorophenyl)-1,2,3,6-tetrahydropyridine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and *p*-chloroamphetamine as monoamine depletors. *Res Commun Chem Pathol Pharmacol* **50**: 57–65, 1985.
32. Fuller RW, Robertson DW and Hemrick-Luecke SK, Comparison of the effects of two 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine analogs, 1-methyl-4-(2-thienyl)-1,2,3,6-tetrahydropyridine and 1-methyl-4-(3-thienyl)-1,2,3,6-tetrahydropyridine, on monoamine oxidase *in vitro* and on dopamine in mouse brain. *J Pharmacol Exp Ther* **240**: 415–420, 1987.
33. Heikkila RE, Manzino L, Cabbat FS and Duvoisin RC, Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and several of its analogues on the dopaminergic nigrostriatal pathway in mice. *Neurosci Lett* **58**: 133–137, 1985.
34. Sonsalla PK, Youngster SK, Kindt MV and Heikkila RE, Characteristics of 1-methyl-4-(2′-methylphenyl)-1,2,3,6-tetrahydropyridine-induced neurotoxicity in the mouse. *J Pharmacol Exp Ther* **242**: 850–857, 1987.
35. Youngster SK, Saari WS and Heikkila RE, 1-Methyl-4-cyclohexyl-1,2,3,6-tetrahydropyridine (MCTP): an alicyclic MPTP like neurotoxin. *Neurosci Lett* **79**: 151–156, 1987.
36. Youngster SK, Sonsalla PK and Heikkila RE, Evaluation of the biological activity of several analogs of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *J Neurochem* **48**: 929–934, 1987.
37. Fries DS, de Vries J, Hazelhoff B and Horn AS, Synthesis and toxicity towards nigrostriatal dopamine neurons of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) analogues. *J Med Chem* **29**: 424–427, 1986.
38. Johannessen JN, Savitt JM, Markey CJ, Bacon JP, Weisz A, Hanselman DS and Markey SP, The development of amine substituted analogues of MPTP as unique tools for the study of MPTP toxicity and Parkinson's disease. *Life Sci* **40**: 697–704, 1987.
39. Riachi NJ, Arora PK, Sayre LM and Harik SI, Potent neurotoxic fluorinated 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine analogs as potential probes in models of Parkinson's disease. *J Neurochem* **50**: 1319–1321, 1988.
40. Brossi A, Further explorations of unnatural alkaloids. *J Natural Products* **48**: 878–893, 1985.
41. Gibb C, Willoughby J, Glover V, Sandler M, Testa B, Jenner P and Marsden CD, Analogues of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine as monoamine oxidase substrates: a second ring is not necessary. *Neurosci Lett* **76**: 316–322, 1987.
42. Heikkila RE, Kindt MV, Sonsalla PK, Giovanni A, Youngster SK, McKeown KA and Singer TP, Importance of monoamine oxidase A in the bioactivation of neurotoxic analogs of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc Natl Acad Sci USA* **85**: 6172–6176, 1988.
43. Cramer III RD, Patterson DE and Bunce JD, Comparative molecular field analysis (CoMFA). 1. Effect of shape on binding of steroids to carrier proteins. *J Am Chem Soc* **110**: 5959–5967, 1988.
44. Marshall GR and Cramer III RD, Three-dimensional structure–activity relationships. *Trends Pharm Sci* **9**: 285–289, 1988.
45. Lindberg W, Persson J-A and Wold S, Partial least-squares method for spectofluorimetric analysis of mixtures of humic acid and ligninsulfonate. *Anal Chem* **55**: 643–648, 1983.
46. Motoc I, Dammkoelher RA, Mayer D and Labanowski J, Three-dimensional quantitative structure–activity relationships. 1. General approach to the pharmacophore model validation. *Quant Struct–Act Relat* **5**: 99–105, 1986.
47. Labanowski J, Motoc I, Naylor CB, Mayer D and Dammkoelher RA, Three-dimensional quantitative structure–activity relationships. 2. Conformational mimicry and topographical similarity of flexible molecules. *Quant Struct–Act Relat* **5**: 138–152, 1986.
48. Dewar MJS, Zoebisch EG, Healy EF and Stewart JJP, AM1: a new general purpose quantum mechanical molecular model. *J Am Chem Soc* **107**: 3902–3909, 1985.

49. Fabian WMF, AM1 calculations of rotation around essential single bonds and preferred conformation in conjugated molecules. *J Comput Chem* **9**: 369–377, 1988.
50. Kass SR, Hydrocarbon acidities calculated with MINDO/3, MNDO and AM1. *J Comput Chem* **11**: 94–104, 1990.
51. Taagepera M, Hehre WJ, Topsom RD and Taft RW, Calculation of polar substituent parameters by ab initio molecular orbital methods. Proton affinities of substituted primary amines. *J Am Chem Soc* **98**: 7438–7440, 1976.
52. Testa B and Seiler P, Steric and lipophobic components of the hydrophobic fragmental constant. *Arzneim-Forsch (Drug Res)* **31**: 1053–1058, 1981.
53. Dunn III WJ and Wold S, Relationships between chemical structure and biological activity modelled by SIMCA pattern recognition. *Bioorg Chem* **9**: 505–513, 1980.
54. Petrongolo C, Quantum chemical study of isolated and interacting molecules with biological activity. *Gazz Chim Ital* **108**: 445–478, 1978.
55. Goldblum A, Structure–activity relationships of cholinesterase inhibitors. I. Quantum mechanical study of affinities of phenyl *N*-methyl carbamates. *Mol Pharmacol* **24**: 436–442, 1983.
56. Loew GH, Nienow JR and Poulsen M, Theoretical structure–activity studies of benzodiazepine analogues. *Mol Pharmacol* **26**: 19–34, 1984.
57. Kocjan D, Hodoscek M and Hadzi D, Dopaminergic pharmacophore of ergoline and its analogues. A molecular electrostatic potential study. *J Med Chem* **29**: 1418–1423, 1986.
58. Van de Waterbeemd H, Carrupt P-A and Testa B, Molecular electrostatic potential of orthopramides: implication for their interaction with the D-2 dopamine receptor. *J Med Chem* **29**: 600–606, 1986.
59. El Tayar N, Carrupt P-A, Van de Waterbeemd H and Testa B, Modeling of β-adrenoceptors based on molecular electrostatic potential studies of agonist and antagonists. *J Med Chem* **31**: 2072–2081, 1988.
60. Martin M, Sanz F, Campillo M, Pardo L, Pérez J and Turmo T, Quantum chemical study of the molecular patterns of MAO inhibitors and substrates. *Int J Quantum Chem* **23**: 1627–1641, 1983.
61. Tute MS, Limitations and prospects for the Hansch approach to SAR. In: *Physical Chemical Properties of Drugs* (Eds. Yalkowski SH, Sinkula AA and Valvani SC), pp. 141–161. Marcel Dekker, New York, 1980.
62. Naoi M, Matsuura S, Parvez H, Takahashi T, Hirata Y, Minami M and Nagatsu T, Oxidation of *N*-methyl-1,2,3,4-tetrahydroisoquinoline into the *N*-methylisoquinolinium ion by monoamine oxidase. *J Neurochem* **52**: 653–655, 1989.