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A 3D QSAR Study of Monoamino Oxidase-B Inhibitors Using the Chemical Function Based Pharmacophore Generation Approach

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A molecular modelling study was performed using the CATALYST software package on a dataset of 100 thiosemicarbazide and thiazole derivatives acting as MAO-B irreversible inhibitors in order to, (i) better elucidate the possible role of the ligand features which are significant for binding and (ii) generate chemical features based pharmacophore models which were subsequently used as 3D queries for database searching.

Based on known MAO-B inhibitors, pharmacophore hypotheses were created in order to find similarities between the thiazoles and thiosemicarbazides and identify the key sub-structures most likely to be significant for high MAO-B inhibitory activity.

Keywords: Catalyst software, Thiazole and thiosemicarbazide derivatives, MAO-B Inhibitors, 3D database searching

INTRODUCTION

The pattern of steric, electronic, and hydrophobic properties of a drug molecule determines its interactions with the binding site of a receptor. In addition, overall hydrophobic properties as well as the ionization state and dissociation rate are responsible for its transport and distribution within the biological system. QSAR (Quantitative Structure-Activity Relationships) models describe biological properties of drug-like molecules by correlating them with different physicochemical properties. Compounds with similar physicochemical properties are expected to exhibit similar biological activities. Nevertheless different structural motifs can lead to the same biological effect. It is also well accepted that bioactive ligands that bind to a common receptor must fulfill certain chemical and geometric criteria. In the case where the 3D structure of the receptor is known, it is straightforward to search for ligands that match the complimentary features of the receptor so that they can interact effectively with

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the binding site. However, the lack of precise data on the binding site calls for the definition of a pharmacophore. Conclusively, it is important to have enough information about both the receptor and its ligands (spanning a wide range of activity).

The progress in drug discovery leads to the need for automated pattern recognition systems suitable for the analysis of structure-activity data of large numbers of compounds, because the chemist's ability to recognize *similarities* between a large number of structurally different molecules is limited.

Such systems can be viewed as an extension of the traditional QSAR methods that go beyond attempts to relate physico-chemical properties of compounds with activity by incorporating more key substructures as pharmacophores into the analysis.[1]

For this purpose a crude description of the 3D pharmacophore is used as a criterion for 3D searching in large databases. Chemical features based pharmacophore models have been proposed to be useful for considering such effects.[2] Considered chemical features can be H-bond acceptors (HBA) and donors (HBD), aliphatic and aromatic hydrophobic groups, positive and negative charges, positive and negative ionizable groups or aromatic planes. Within the software package Catalyst,[3] *pharmacophore hypotheses* can be generated automatically or manually starting from a known conformation of a lead compound. These hypotheses can be used as queries to search databases for retrieving structures that fit the hypotheses, or as models to estimate the activities of new compounds. 3D database search experiments can return compounds structurally similar to those already known and satisfying the chemical and geometrical requirements, as well as structurally diverse compounds that also possess the features necessary for favourable ligand–receptor interactions and exhibiting higher to unknown biological effects.

The hit-lists produced by the 3D search consist of molecules that fit all the features of the query. Hit lists from pharmacophore searches can be very large, but the large number of hits is necessary to guarantee that no interesting compounds are missed in the search.

Monoamine oxidase (MAO) is an ubiquitous, FAD-containing enzyme, which is particularly abundant in the liver and brain. It is located in the outer mitochondrial membrane and catalyzes the oxidative deamination of endogenous neurotransmitters (biogenic monoamines) such as dopamine and serotonin and a variety of xenobiotics. It plays a central role in the regulation of intracellular level of these amines.[4–7]

Based on the specificity for substrates, sensitivity to inhibitors and amino acid sequence, two enzymatic forms of MAO, called MAO-A and MAO-B, have been identified. For instance, serotonin and norepinephrine are specifically deaminated by the MAO-A isoform and inhibited by moclobemide, while β-phenylethylamine and benzylamine are relatively specifically deaminated by the MAO-B isoform which is irreversibly inactivated by L-deprenyl. It has been shown that the two isoenzymes are distinct with about 70% homology in their primary sequence and are coded by two different genes with similar structure located on the human X chromosome.[8–11]

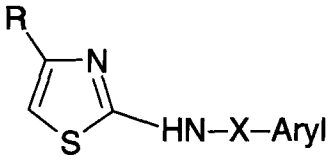
Mutagenesis experiments have identified a key amino acid responsible for substrate selectivity. Substitution of Phe-208 in MAO-A by the corresponding Ile-199 in MAO-B changed the selectivity profile of MAO-A to MAO-B, and *vice versa*. Thus, aromatic interactions might be responsible for MAO-A binding, whereas pure hydrophobic van der Waals' interaction might be involved in MAO-B binding.[12,13]

The three-dimensional structures of MAO-A and MAO-B are not known and the structural features of the active sites are not well defined. SAR (Structure-Activity Relationships) studies have been pursued on different chemotypes in order to define the contribution of steric, electronic and polar properties to the interactions

of substrates and inhibitors with each of the MAO isoform as well as the most relevant sub-structures for molecular recognition.[14–25] Recently important information has been reported by Morón *et al.*[13]

MAO is of considerable pharmacological interest because of its key role in the α-carbon oxidation metabolism of monoamine neurotransmitters like serotonin, catecholamines and dopamine and its possible involvement in many neuropsychiatric disorders.[26–30] Therefore it represents also an interesting target for therapeutic agents. In humans, MAO-B inhibitors (e.g. selegiline) are useful as co-adjuvants in the treatment of Parkinson's disease.[31–34] and under certain circumstances also in the treatment of Alzheimer's disease,[29,30,35] whereas MAO-A inhibitors are valuable antidepressant and antianxiety agents (e.g. moclobemide).[8]

TABLE I Structural formulae of Thiazole derivatives **1–45**

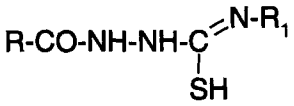


Compound	R	Aryl
X = —CO—NH—		
1	H	Trimethoxyphenyl
2	C_6H_5	Trimethoxyphenyl
3	p-C_6H_4—CH_3	Trimethoxyphenyl
4	p-C_6H_4—OCH_3	Trimethoxyphenyl
5	CH_3	Trimethoxyphenyl
6	H	Methylendioxyphenyl
7	C_6H_5	Methylendioxyphenyl
8	p-C_6H_4—CH_3	Methylendioxyphenyl
9	p-C_6H_4—OCH_3	Methylendioxyphenyl
10	CH_3	Methylendioxyphenyl
11	H	Dimethoxyethoxyphenyl
12	C_6H_5	Dimethoxyethoxyphenyl
13	p-C_6H_4—CH_3	Dimethoxyethoxyphenyl
14	p-C_6H_4—OCH_3	Dimethoxyethoxyphenyl
15	CH_3	Dimethoxyethoxyphenyl
X = —CH=N—		
16	H	Trimethoxyphenyl
17	C_6H_5	Trimethoxyphenyl
18	p-C_6H_4—CH_3	Trimethoxyphenyl
19	p-C_6H_4—OCH_3	Trimethoxyphenyl
20	CH_3	Trimethoxyphenyl
21	H	Methylendioxyphenyl
22	C_6H_5	Methylendioxyphenyl
23	p-C_6H_4—CH_3	Methylendioxyphenyl
24	p-C_6H_4—OCH_3	Methylendioxyphenyl
25	CH_3	Methylendioxyphenyl
26	H	Dimethoxyethoxyphenyl
27	C_6H_5	Dimethoxyethoxyphenyl
28	p-C_6H_4—CH_3	Dimethoxyethoxyphenyl
29	p-C_6H_4—OCH_3	Dimethoxyethoxyphenyl
30	CH_3	Dimethoxyethoxyphenyl
X = —CH_2—NH—[a]		
31	H	Trimethoxyphenyl
32	C_6H_5	Trimethoxyphenyl
33	p-C_6H_4—CH_3	Trimethoxyphenyl
34	p-C_6H_4—OCH_3	Trimethoxyphenyl
35	CH_3	Trimethoxyphenyl
36	H	Methylendioxyphenyl
37	C_6H_5	Methylendioxyphenyl
38	p-C_6H_4—CH_3	Methylendioxyphenyl
39	p-C_6H_4—OCH_3	Methylendioxyphenyl
40	CH_3	Methylendioxyphenyl
41	H	Dimethoxyethoxyphenyl
42	C_6H_5	Dimethoxyethoxyphenyl
43	p-C_6H_4—CH_3	Dimethoxyethoxyphenyl
44	p-C_6H_4—OCH_3	Dimethoxyethoxyphenyl
45	CH_3	Dimethoxyethoxyphenyl

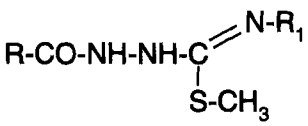
[a] See note at the end of the article.

The aim of this work was to generate common feature hypotheses for hydrazinothiazole and hydrazinothiosemicarbazide derivatives (Table I and Table II), both being characterized by the key "diazo" (hydrazine) *N-N* pharmacophoric substructure.[17,18] Moreover, starting from MAO-B inhibitors belonging to other different chemical classes present in the Derwent World Drug Index (WDI), it was envisaged to define additional pharmacophore hypotheses in order to find similarities between these compounds and the thiazoles/thiosemicarbazides[17,18] and work out the structural requirements for high MAO-B inhibitory activity. Finally it was planned to carry out virtual screening within the WDI using our pharmacophore hypotheses as 3D database queries in order to detect other known molecules possibly acting as MAO-B inhibitors.

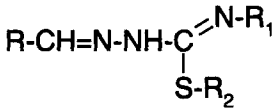
TABLE II Structural formulae of Thiosemicarbazide derivatives **46–100**



Compound	R	R_1
46	Trimethoxyphenyl	Methyl
47	Trimethoxyphenyl	Ethyl
48	Trimethoxyphenyl	*n*-Propyl
49	Trimethoxyphenyl	Isopropyl
50	Trimethoxyphenyl	Phenyl
51	Trimethoxyphenyl	Benzyl
52	Methylendioxyphenyl	Methyl
53	Methylendioxyphenyl	Ethyl
54	Methylendioxyphenyl	*n*-Propyl
55	Methylendioxyphenyl	Isopropyl
56	Methylendioxyphenyl	Phenyl
57	Methylendioxyphenyl	Benzyl
58	Dimethoxyethoxyphenyl	Methyl
59	Dimethoxyethoxyphenyl	Ethyl
60	Dimethoxyethoxyphenyl	*n*-Propyl
61	Dimethoxyethoxyphenyl	Isopropyl
62	Dimethoxyethoxyphenyl	Phenyl
63	Dimethoxyethoxyphenyl	Benzyl
64	Trimethoxyphenyl	Methyl
65	Trimethoxyphenyl	Ethyl
66	Trimethoxyphenyl	*n*-Propyl
67	Trimethoxyphenyl	Isopropyl
68	Trimethoxyphenyl	Phenyl
69	Trimethoxyphenyl	Benzyl
70	Methylendioxyphenyl	Methyl
71	Methylendioxyphenyl	Ethyl
72	Methylendioxyphenyl	*n*-Propyl
73	Methylendioxyphenyl	Isopropyl
74	Methylendioxyphenyl	Phenyl
75	Methylendioxyphenyl	Benzyl
76	Dimethoxyethoxyphenyl	Methyl
77	Dimethoxyethoxyphenyl	Ethyl
78	Dimethoxyethoxyphenyl	*n*-Propyl
79	Dimethoxyethoxyphenyl	Isopropyl
80	Dimethoxyethoxyphenyl	Phenyl
81	Dimethoxyethoxyphenyl	Benzyl



Compound	R	R_1
82	Trimethoxyphenyl	Methyl
83	Trimethoxyphenyl	Ethyl
84	Trimethoxyphenyl	Benzyl
85	Methylendioxyphenyl	Methyl
86	Methylendioxyphenyl	Ethyl
87	Methylendioxyphenyl	Benzyl
88	Dimethoxyethoxyphenyl	Methyl
89	Dimethoxyethoxyphenyl	Ethyl
90	Dimethoxyethoxyphenyl	Benzyl



Compound	R	R_1	R_2
91	Trimethoxyphenyl	Methyl	Methyl
92	Trimethoxyphenyl	Ethyl	Methyl
93	Trimethoxyphenyl	Ethyl	Ethyl
94	Trimethoxyphenyl	Benzyl	Methyl
95	Methylendioxyphenyl	Methyl	Methyl
96	Methylendioxyphenyl	Ethyl	Methyl
97	Methylendioxyphenyl	Benzyl	Methyl
98	Dimethoxyethoxyphenyl	Methyl	Methyl
99	Dimethoxyethoxyphenyl	Ethyl	Methyl
100	Dimethoxyethoxyphenyl	Benzyl	Methyl

METHODS

(a) Biological Evaluation

Rat liver mitochondria were isolated according to the method of Cambria *et al.*[17,18] Protein content of the mitochondrial preparation was determined according to the method of Lowry *et al.*,[36] in the presence of 0.01% sodium dodecyl sulfate, using bovine serum albumin as standard.

MAO activity was determined according to Morinan and Garratt by a fluorimetric method.[37] The enzyme activity was defined as nM of 4-hydroxyquinoline formed (mg^{-1} protein h^{-1}) and expressed as percentage (± S.E.) of inhibition of the respective controls. The assays were routinely performed in triplicate. A blank and a control were run together for each series tested. All experiments were performed under conditions in which the product formation was linear with the amount of enzyme and the time of incubation.

An apparent IC_{50} value for each highly active inhibitor (Table III) was estimated according

TABLE III Biological activities of Thiazoles **1–45** and Thiosemicarbazides **46–100**

Compound	activity – log IC_{50} μM
1	1.887
2	**2.217**
3	2.172
4	**2.258**
5	2.146
6	2.057
7	**2.241**
8	2.114
9	**2.242**
10	2.123
11	1.784
12	2.160
13	1.997
14	2.026
15	1.980
16	1.980
17	**2.239**
18	**2.255**
19	**2.238**
20	**2.277**
21	1.735
22	**2.278**
23	1.762
24	1.891
25	**2.414**
26	1.989
27	**2.252**
28	2.110
29	2.150
30	**2.272**
31	1.949
32	2.065
33	2.134
34	2.081
35	**2.259**
36	2.008
37	2.140
38	**2.210**
39	2.107
40[a]	**2.373**
41	1.844
42	**2.239**
43	**2.207**
44	**2.271**
45	2.100
46	2.120
47	2.199
48	2.171
49	**2.200**
50	2.193
51	2.189
52	2.194
53	**2.224**
54	**2.222**
55	**2.217**
56	**2.205**
57	**2.234**
58	1.976
59	**2.201**
60	2.052
61	**2.220**
62	2.184
63	2.195
64	**2.211**
65	**2.274**
66	2.129
67	**2.231**
68	**2.232**
69	**2.205**
70	2.103
71	**2.371**
72	**2.358**
73	**2.380**
74	**2.355**
75	**2.327**
76	**2.256**
77	**2.203**
78	**2.219**
79	**2.222**
80	**2.239**
81	**2.223**
82	1.836
83	1.917
84	2.008
85	1.978
86	2.011
87	2.103
88	1.803
89	1.873
90	2.100
91	**2.255**
92	**2.227**
93	**2.231**
94	**2.270**
95	**2.299**
96	**2.282**
97	2.105
98	**2.266**
99	**2.216**
100	N.I.

[a] See note at the end of the article.
[b] No inhibition at the limit of solubility.
N.I. = not inhibitor.

to Cambria *et al.*[19] IC_{50} for the remaining compounds was calculated from the equation: $IC_{50} = (A)/(\%)$ of inhibition therefore $-\log IC_{50} = \log(A) - \log(\%)$, where A is the enzyme activity.

(b) Molecular Modelling

The entire molecular modelling study was performed using the Catalyst 4.5 software package[3] installed on SGI desktop workstations (INDIGO2 R4400 and O2 R5000/R10000) operating under the IRIX 6.2 and 6.5 system.

Structural models for all the hydrazinothiazole and hydrazinothiosemicarbazide derivatives exhibiting an activity higher than 2.200 i.e. 12 thiazoles (underlined compounds, Table I) and 32 thiosemicarbazides (underlined compounds, Table II)[17,18] have been generated using the Catalyst molecule builder. After structure building, a potential energy minimization was done and the conformational models for the compounds were calculated using the default parameters (Maximum number of conformers: 250; Energy Range: 15.00 kcal/mol, BEST quality).

The Common Features Hypothesis[38] generation module was used to analyze the compounds and five different types of functions from the feature dictionary available in the CATALYST program[2] were selected. The feature selection was done according to the occurence of functional groups in the compounds of the training set. Features that do not correspond to these chemical functions were not used.

1. *HB acceptor*: any nitrogen, oxygen or sulfur atom is considered to be an acceptor atom with at least one available lone pair; basic amines are excluded because they are protonated at physiological pH.
2. *HB donor*: hydroxyls, thiols, NHs.
3. *Hydrophobic*: a contiguous set of atoms that are not adjacent to charged or electronegative atoms in a conformer that the atoms have surface accessibility, e.g. phenyl, cycloalkyl, isopropyl, methyl.
4. *Positive ionizable*: atoms or groups of atoms that are likely to be protonated at physiological pH, such as basic amines, amidines, guanidines.
5. *Ring aromatic*: 5- and 6-member aromatic rings; the feature defines two points, the ring centroid and a projected point normal to the ring plane. The projected point can map both above and below the ring

Pharmacophores containing a minimum number of three and a maximum number of ten pharmacophoric points (features) were requested.

Chemical Features Based Pharmacophore Hypotheses Starting from Thiazoles and Thiosemicarbazides

In a first step, multiple hypotheses were generated starting from different subsets of compounds, which can be divided into three groups:

1. Common feature hypotheses of thiazoles (**CFH1**).
2. Common feature hypotheses of thiosemicarbazides (**CFH2**).
3. Common feature hypotheses of thiazoles together with thiosemicarbazides (**CFH3**).

CFH1 The three most active thiazole derivatives (**20, 22, 25**, Table I) were used. The pharmacophore generation run returned two different types of five features hypotheses:

Hypo 1.1 consisting of three HB acceptors and two hydrophobic regions;
Hypo 1.2 including one HB acceptor, one HB donor and three hydrophobic functions (Figure 1). Feature definition and location constraints of the hypotheses are given in Table VI.

In a further common features generation run, 12 thiazole derivatives (activity over 2.200) produced only four features hypotheses, consisting either of three HB acceptors and of one hydrophobic (*Hypo 2.1*) or of two HB acceptors, of one hydrophobic and of one aromatic ring (*Hypo 2.2*) (Figure 2). Previously reported mutagenesis studies on MAO isoforms have identified Phe-208 in MAO-A and Ile-199 in MAO-B as key residues for substrate selectivity.[12,13]

CFH2 The five most active thiosemicarbazide derivatives (**71, 72, 73, 74, 75**, Table II) lead to

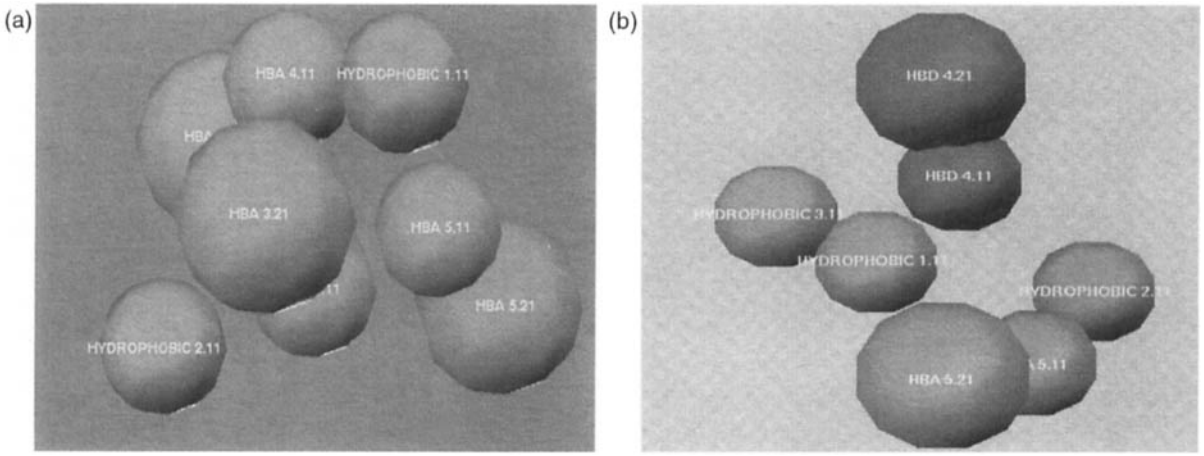


FIGURE 1 Five features hypotheses. (a) *Hypo 1.1.* Three HB acceptors and two hydrophobic regions. (b) *Hypo 1.2.* One HB acceptor, one HB donor and three hydrophobic functions. (See Color Plate I).

four features hypotheses including one HB donor, two HB acceptors and one hydrophobic feature (*Hypo 3.1*) or hypotheses similar to that for the thiazoles being *Hypo 3.2* and *Hypo 3.3* (Figure 3) related to *Hypo 1.1* and *Hypo 2.1*, respectively (Figures 1–3).

Attempts to generate hypotheses containing at least four features starting from all thiosemicarbazides with an activity higher than 2.200 (32 compounds) remained unsuccessfull. The maximum number of thiosemicarbazides for which such models could be generated was 14 (compound **49, 53, 54, 55, 56, 57, 59, 61, 68, 69, 74, 75, 80, 94**). Additional to the hypotheses families already obtained, two new different types of hypotheses were retained:

- three HB acceptors and one hydrophobic (*Hypo 3.3*) or
- one HB acceptor, one positive ionizable and two hydrophobic functions (*Hypo 3.4*) (Figure 4).

Feature definition and location constraints of the hypotheses are given in Table VI.

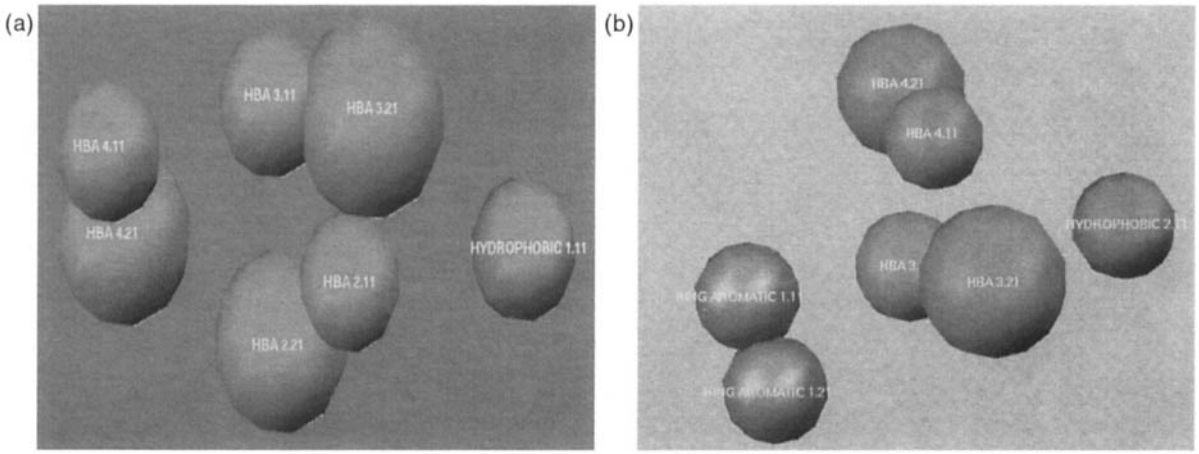


FIGURE 2 Four features hypotheses. (a) *Hypo 2.1.* Three HB acceptors and one hydrophobic ring. (b) *Hypo 2.2.* Two HB acceptors, one hydrophobic and one aromatic ring. (See Color Plate II).

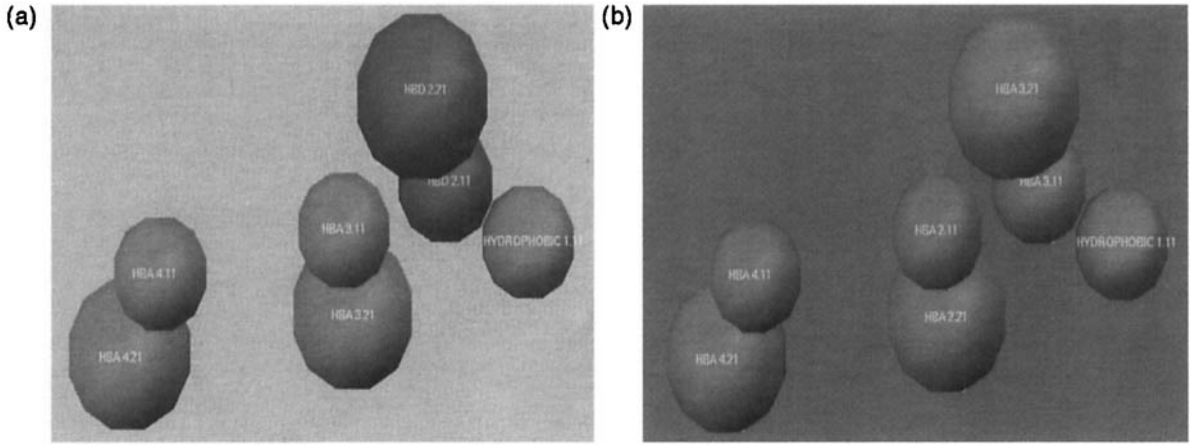


FIGURE 3 Four features hypotheses. (a) *Hypo 3.1.* One HB donor, 2 HB acceptors and 1 hydrophobic feature. (b) *Hypo 3.2.* Three HB acceptors and two hydrophobic regions (compare with *Hypo 1.1. See text for explanation*). (See Color Plate III).

CFH3 Compound **25** and compound **71** were used to disclose similarities between thiazoles and thiosemicarbazides. The hypothesis generation procedure produced different hypotheses consisting of the same features (see Figure 5 and compare separately *Hypo 2.2* or *Hypo 3.2* for thiazoles and thiosemicarbazides). In another run using compound **25** (thiazole), **49** and **71** (thiosemicarbazides) four features hypotheses containing the same functions were generated, e.g. *Hypo 4.1* and *Hypo 4.2* (Figure 6).

Features definition and location constraints of the hypotheses are given in Table VI and compounds matching the different hypotheses in Table IV.

Chemical Features Based Pharmacophore Hypotheses Starting from MAO-B Inhibitors Present in the Derwent World Drug Index

The Derwent world drug index database was searched for all MAO inhibitors. This query produced a hit list of 178 compounds. Further searches in literature (Medline Database) revealed that from this list 34 compounds act as selective MAO-B inhibitors. These molecules

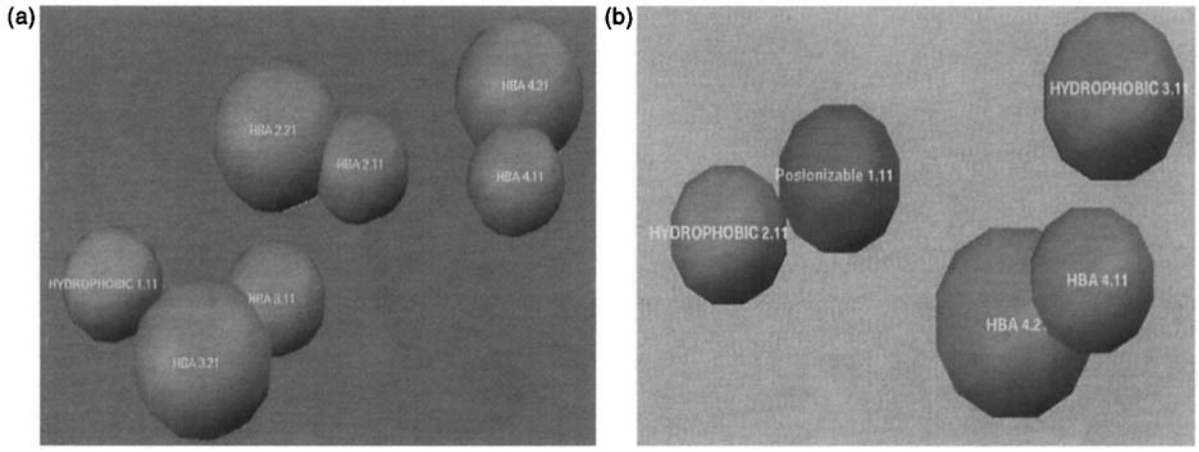


FIGURE 4 Four features hypotheses. (a) *Hypo 3.3.* Three HB acceptors and one hydrophobic sphere (compare with *Hypo 2.1. See text for explanation*). (b) *Hypo 3.4.* One HB acceptor, one positive ionizable and two hydrophobic functions. (See Color Plate IV).

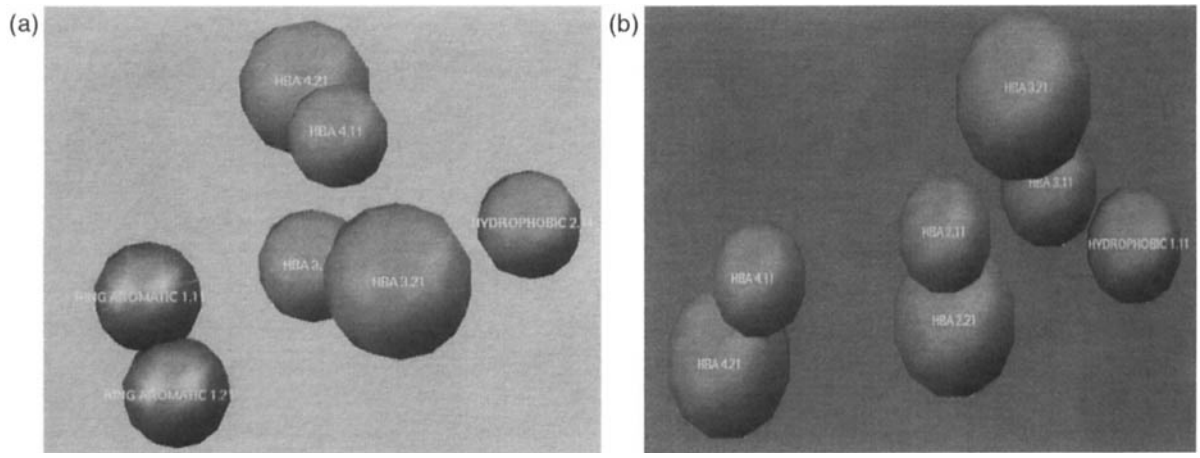


FIGURE 5 Five features hypotheses. (a) *Hypo 2.2.* Two HB acceptors, one hydrophobic and one aromatic ring. (b) *Hypo 3.2.* Hypothesis similar to the thiazole derivatives. (See Color Plate V).

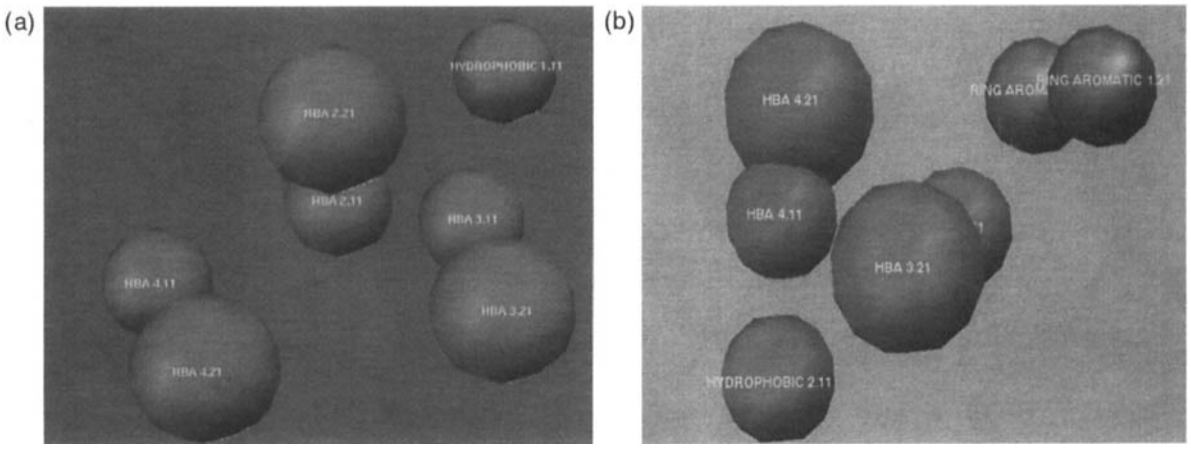


FIGURE 6 Four features hypotheses containing the same functions. (a) *Hypo 4.1.* Three HB acceptors groups and one hydrophobic feature. (b) *Hypo 4.2.* Two HB acceptors groups, one hydrophobic sphere and one aromatic ring. (See Color Plate VI).

were considered for further studies. Since the maximum number of compounds that can be used in a Common Features Search run is limited to 32, the entire group of MAO-B inhibitors was divided into two subsets, one containing compounds with one or two heteroatoms (mostly *N*) and the other with those containing more than two heteroatoms. For both of these groups, pharmacophore hypotheses were requested containing at least three features. In order to obtain such models, both groups had to be reduced to the following compounds:

Group 1 (Figure 7): Indanamine, AGN-1135, Pargyline, Propargylbenzylamine, TVP-101, Selegiline, TZ-650.

These compounds lead to hypotheses consisting of HB donor, positive ionizable and aromatic ring (*Hypo 5.1*) or HB donor, positive ionizable and hydrophobic (*Hypo 5.2*) (Figure 8).

Group 2 (Figure 9): MD-240929, MD-780236, MD-240233, MD-760548, MD-770222, MD-240931, MD-240098, MD-240926, MD-220662, MD-220661.

Pharmacophore generation using these 10 compounds returns one four-feature hypothesis:

TABLE IV Compounds matching the different hypotheses

Hypothesis	Thiazoles (18) Thiosemicarbazides (32)	MAO-inhibitors (178)	MAO-B inhibitors (34)
1.1	18/24	7	2
1.2	18/8	7	0
2.1	13/2	5	0
2.2	18/10	10	0
3.1	3/20	6	0
3.2	18/29	9	1
3.3	11/21	17	7
3.4	0/27	1	0
4.1	15/26	13	1
4.2	16/8	4	0
5.1	0/24	30	10
5.2	0/24	30	12
6.1	15/15	21	11

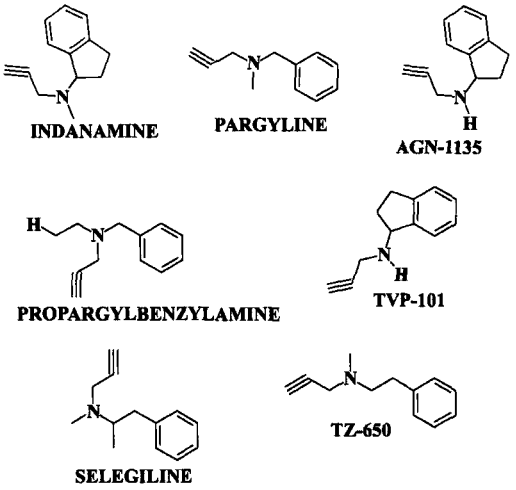


FIGURE 7 Group 1 compounds.

two HB acceptors, one hydrophobic and one ring aromatic (*Hypo 6.1*) (Figure 10). A similar hypothesis was also found with hydrazinothiazoles and hydrazinothiosemicarbazides (*Hypo 4.2*) (Figure 6).

The above mentioned hypotheses were used for a 3D database search (Derwent World Drug Index Database) and the fitting of thiazoles and thiosemicarbazides to these models was investigated.

All these queries returned hit lists containing over a thousand molecules belonging to different application groups (Table V) (see the section on Supporting Information Available at the end of the article).

In order to restrict the number of hits a 1D hypothesis including the property that the molecular

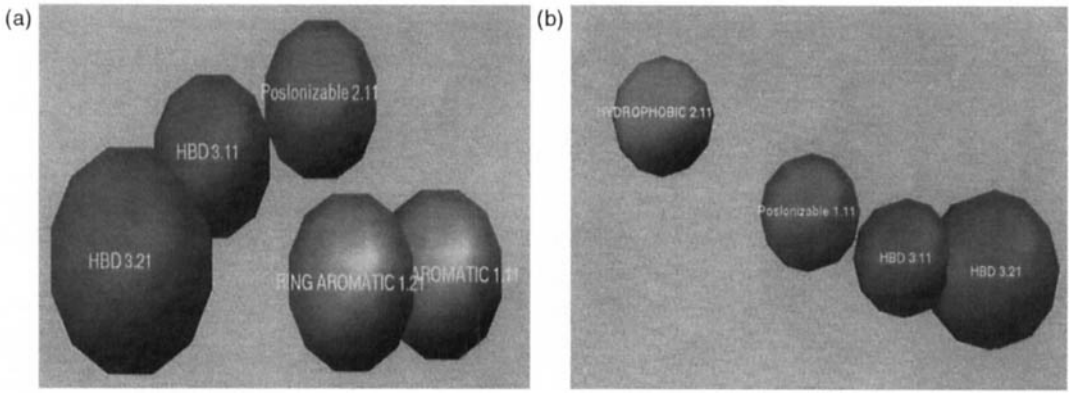


FIGURE 8 Three features hypotheses. (a) *Hypo 5.1.* One HB donor, one positive ionizable and one aromatic ring. (b) *Hypo 5.2.* One HB donor, one positive ionizable and one hydrophobic function. (See Color Plate VII).

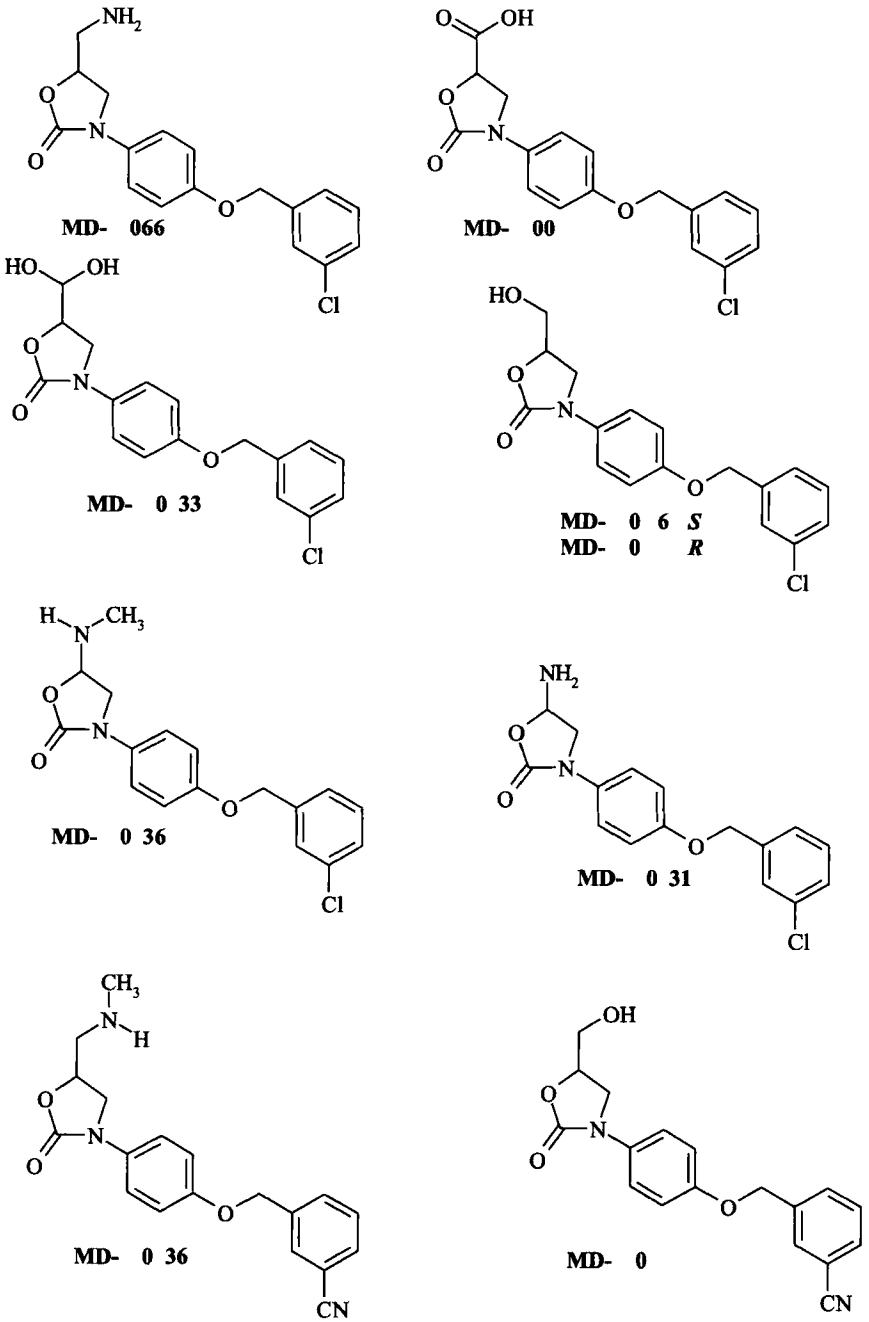


FIGURE 9 Group 2 compounds.

weight was less than 500 was used in order to retrieve only drug-like molecules. Moreover, this is justified by the fact that all known MAO-B inhibitors possess a molecular weight lower than 500.

No shape queries and shape – hypotheses queries were created because although the correct shape is important for fitting into the active site of a biological molecule, in this case it would

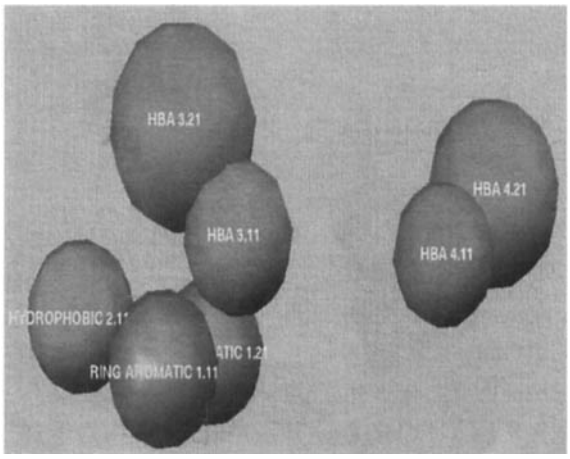


FIGURE 10 Four-feature hypothesis. *Hypo 6.1.* Two HB acceptors, one hydrophobic and one aromatic ring. (See Color Plate VIII).

TABLE V Some results from Derwent WDI Database search

Hypothesis	Derwent WDI database search	MW < 500
1.1	6667 hits	2974 hits
3.3	9973 hits	3433 hits
5.1	5439 hits	3798 hits

not be specific for MAO-B inhibitors but only for one fixed compound.

RESULTS AND DISCUSSION

Monoamine oxidase-A and -B have been sometimes recognized for being involved in neurodegenerative processes.[26–35] These mitochondrial flavoenzymes have yet to be crystallized and, therefore, our ability to design more efficient substrates or inhibitors still relies on structure-activity relationships to map the areas of the active sites that can accommodate pharmacophoric functionalities. A comparison of the generated CATALYST hypotheses shows that the common features hypotheses for both hydrazinothiazoles and hydrazinothiosemicarbazides[17,18] can be represented as four features model consisting of two or three HB acceptors and one hydrophobic function and/or one aromatic ring (e.g. *Hypo 3.3* or *Hypo 4.2*) (Figures 4 and 6). Trying to define a lowest common denominator pharmacophore hypothesis for all classes of compounds previously described will lead to failure, because the MAO-B inhibitors containing less than two heteroatoms will not fit to this model. However, all other classes of molecules will fit a common hypothesis derived from *Hypo 2.2* (Figure 2), *Hypo 4.2* (Figure 5) and *Hypo 6.1* (Figure 7) containing two H-bond acceptor functions and one aromatic ring. The lipophilic sphere in *Hypo 6.1* is located on the opposite site compared to *Hypo 2.2* and *Hypo 4.2* and therefore an overlay of all functions is not possible.

The known MAO-B inhibitors can be basically divided into two groups represented by two different chemical function models; a model consisting of HB donor, positive ionizable and aromatic ring or hydrophobic regions (*Hypo 5.1* or *5.2*) (Figure 8), and a model containing two HB acceptors, one hydrophobic and one aromatic ring were found (*Hypo 6.1*) (Figure 10).

Most of the hydrazinothiazole and hydrazinothiosemicarbazide derivatives (Figures 11–13) match the latter hypothesis, whereas only the hydrazinothiosemicarbazides fit to the three feature hypothesis consisting of HB donor, positive ionizable, aromatic or hydrophobic ring (*Hypo 5.1*, *Hypo 5.2*) (Figure 8).

From this an important factor for the interaction between MAO-B and inhibitors can be proposed.

A common structural feature of all the hypotheses is a hydrophobic site or an aromatic ring. Furthermore, an amino group plays an essential role either as HB donor, as HB acceptor or as positive ionizable site (basic site). Additional oxygen atoms may serve as HB acceptors supporting the hypothesis postulated by Mazouz *et al.*[25] of a specific interaction of the methoxyphenyl substructure with a nucleophilic site at the enzyme active site. This applies to both the thiazole and thiosemicarbazide chemotypes examined in this study. This interaction might be substantial for affinity and selectivity to MAO-B

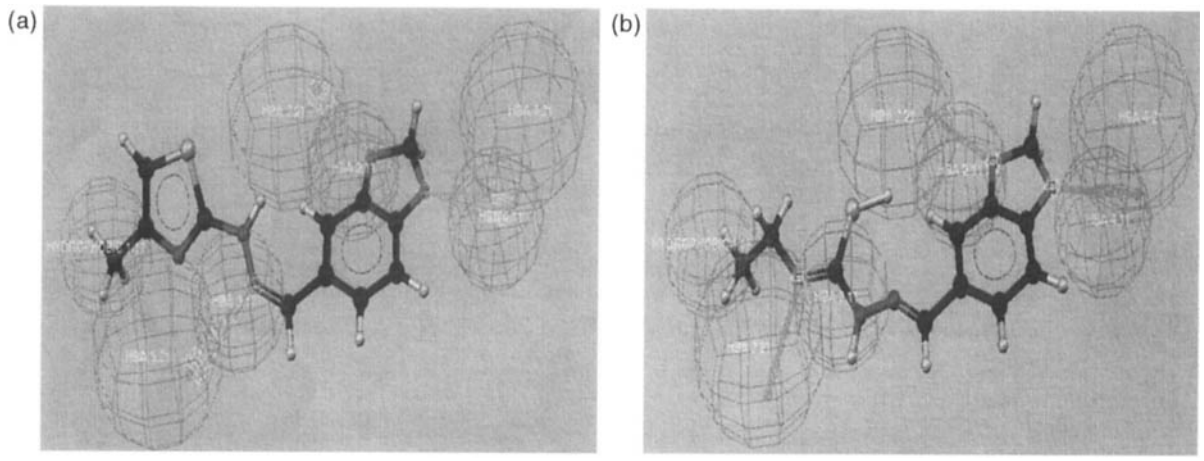


FIGURE 11 (a) Compound **25** fitting to *Hypo 3.3.* (b) Compound **71** fitting to *Hypo 3.3.* (See Color Plate IX).

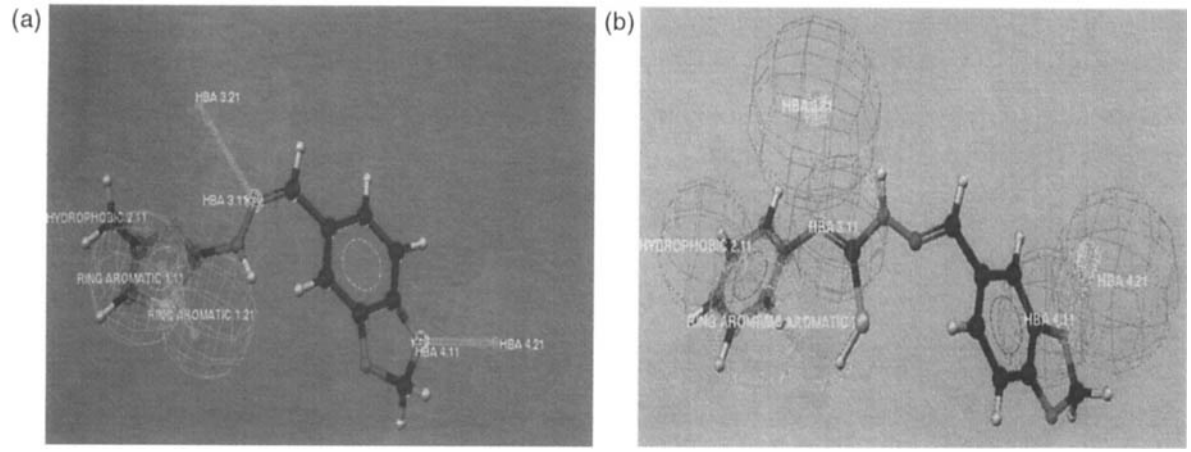


FIGURE 12 (a) Compound **25** fitting to *Hypo 6.1.* (b) Compound **74** fitting to *Hypo 6.1.* (See Color Plate X).

by forming a hydrogen bond that stabilizes the inhibitor in a hydrophobic pocket of the protein binding site by means of fixed anchoring points such as the C^{β}-H and C^{δ}-H electron-poor hydrogens of Ile-199 to form hydrogen bonds with the electron-rich OCH_3 substituent according to recent studies on the stabilization of biological molecules by such a type of hydrogen bonds.[13,39–41] Alternatively the C^{β}-H group may form a hydrogen bond to the O atom of the methoxy group and the C^{δ}-H group forms a hydrogen bond with the π electron clouds of the aromatic benzyl ring as recently reported for adenine packing in protein structures emphasizing the role of π electron rich clouds as in the aromatic ring for enzyme binding. The aromatic ring should also itself interact (suitable steric hindrance) with Ile-199 making the interaction stronger.[13,42]

The role of the heterocycle is not well established and the atom sequence seems to be more important than the ring itself as shown by the better activity of some thiosemicarbazides when compared to their cyclic thiazole analogs, provided that electronic properties are substantially conserved to have the interaction with the pattern of nucleophilic/basic sites present in the active site.[16] Similarly it was reported that the opening of the oxadiazolone ring leads to

TABLE VI Feature Definitions and Location Constraints[1] of Pharmacophore Hypotheses

Hypo	Feature 1	Feature 2	Feature 3	Feature 4	Feature 5
Hypo 1.1	HBA heavy atom 317.9 −279.7 70.5 170 HBA proj. point 422.5 −547.3 158 230	HBA heavy atom 58.7 90.3 −216.6 170 HBA proj. point −165.5 −105.3 −272 230	HBA heavy atom −38.3 −273.8 118.2 170 HBA proj. point 230 −89.5 18.7 90 230	Hydrophobic −457.5 −313.3 106 170	Hydrophobic 334.5 78.7 −106 170
Hypo 1.2	HBA heavy atom 204.5 −361.5 33.6 170 HBA proj. point −31.7 −385.3 222.3 230	HBD heavy atom 12 210.2 60.1 170 HBD proj. point −35.7 506.7 96.3 230	Hydrophobic −467.7 96.7 −37.7 170	Hydrophobic 368.3 −143.3 −121.7 170	Hydrophobic −203.7 −47.3 −29.7 170
Hypo 2.1	HBA heavy atom 137.8 −286.8 −139.4 170 HBA proj. point −42.4 −347.8 −375 230	HBA heavy atom −193.2 −78.9 247 170 HBA proj. point 95.6 −123.8 323 230	HBA heavy atom −663.1 −37.8 −129.5 170 HBA proj. point −582.4 −93.8 −413 230	Hydrophobic 651.6 −275.8 111 170	
Hypo 2.2	HBA heavy atom −193.3 −60.4 216.1 170 HBA proj. point 7.5 −13 440.9 230	HBA heavy atom 64.4 18.3 −338.3 170 HBA proj. point 39.5 39 −639.1 230	RA center atom −680.2 −178.1 37.2 170 RA proj. point −651.1 −413.2 221.2 170	Hydrophobic 541.5 −105 178.9 170	
Hypo 3.1	HBA heavy atom −15.7 −42.9 34.7 170 HBA proj. point 20.3 −278.4 −148.8 230	HBA heavy atom −668.7 −164.1 −41 170 HBA proj. point −793.7 −396.4 −188.8 230	HBD heavy atom 334.2 83.3 152.7 170 HBD proj. point 238.3 307.6 329.2 230	Hydrophobic 654.3 −76.4 −84.8 170	
Hypo 3.2	HBA heavy atom −15.8 −144.7 38.5 170 HBA proj. point 20.2 −380.2 −145 230	HBA heavy atom 334.1 −18.5 156.5 170 HBA proj. point 238.2 205.8 333 230	HBA heavy atom −668.8 −265.9 −37.2 170 HBA proj. point −793.8 −498.2 −185 230	Hydrophobic 654.2 −178.2 −81 170	
Hypo 3.3	HBA heavy atom 708.2 −106.4 127.6 170 HBA proj. point 861.2 −69.8 −132.4 230	HBA heavy atom −208.8 −194.9 156.3 170 HBA proj. point −496.8 −223.8 235.6 230	HBA heavy atom 241.4 −151.9 −128.2 170 HBA proj. point 29.2 −167.8 −344.4 230	Hydrophobic −664.8 −259.8 −116.4 170	

Hypo 3.4	HBA heavy atom 407.4 −223 −46.7 170 HBA proj. point 214.7 −323.2 −257.8 230	Positive Ionizable −257.2 −4.2 −45.8 170	Hydrophobic −579.3 −125.2 −311.8 170	Hydrophobic 402.7 140.8 444.2 170
Hypo 4.1	HBA heavy atom −395.9 −508.1 418.3 170 HBA proj. point −304.2 −775.2 314.2 230	HBA heavy atom 248.5 −307.4 −312.4 170 HBA proj. point 307.8 −599.2 −363.8 230	HBA heavy atom −27.2 −246 −5 170 HBA proj. point 129.8 −73.2 186.2 230	Hydrophobic 335.8 206.8 −425.8 170
Hypo 4.2	HBA heavy atom −270 −106.4 263.5 170 HBA proj. point −221.8 180.7 204.2 230	HBA heavy atom 120.3 −160.7 −115.9 170 HBA proj. point 76.2 −273.3 160.2 230	RA center atom 274.8 221.6 −454.4 170 RA proj. point 542.1 193.4 −321 170	Hydrophobic −271.8 −529.3 336.2 170
Hypo 5.1	HBD heavy atom −375.8 94.1 −78.4 170 HBD proj. point −592.4 −105.3 −25.8 230	RA center atom 241.5 −129.5 25.6 170 RA proj. point −10 −136.5 188.9 170	Positive Ionizable −73.8 210.1 −188.4 170	
Hypo 5.2	HBD heavy atom 93.8 −250.1 −347.7 170 HBD proj. point 93.7 −365.5 −628.4 230	Positive Ionizable −0.2 −40.1 −95.7 170	Hydrophobic −22.3 350.5 301.6 170	
Hypo 6.1	HBA heavy atom 22.3 84.3 −319.3 170 HBA proj. point 270.5 195.4 −446.5 230	HBA heavy atom 76.3 −55.7 434.7 170 HBA proj. point 244.5 −20.6 683.5 230	RA center atom −71.3 −182.7 −544.7 170 RA proj. point 100 −298.8 −327.5 170	Hydrophobic 164.5 −220.6 −750.5 170

[1] Feature definitions: x y z coordinates and tolerance sphere (pm).
HBA = H Bond Acceptor, HBD = H Bond Donor, RA = Ring Aromatic.

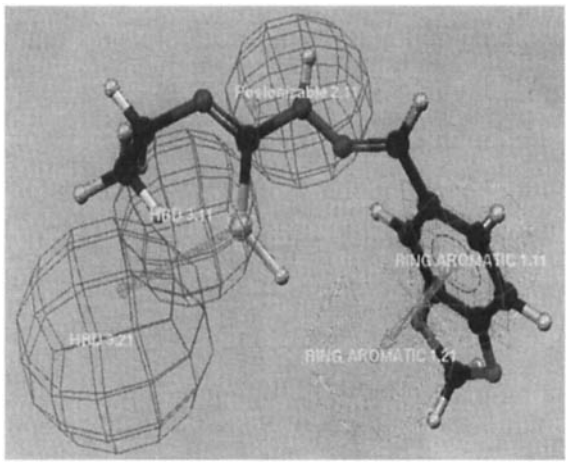


FIGURE 13 Compound **71** fitting to *Hypo 5.1*. (See Color Plate XI).

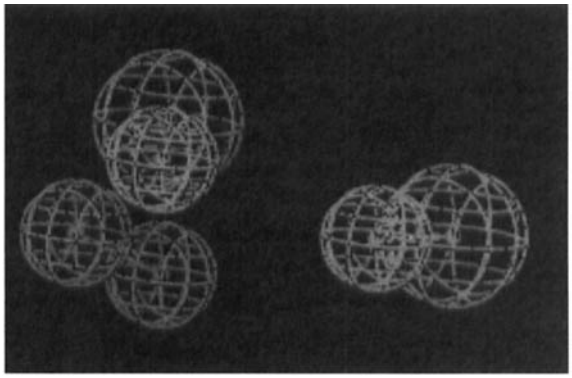
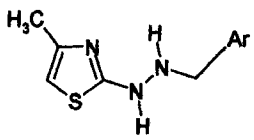


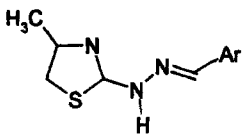
FIGURE 14 Fit values calculated to the lowest common denominator hypothesis derived from *Hypo 6.1* (2 HBA, 1 Aromatic ring). See text for explanation. (See Color Plate XII).

acylhydrazone derivatives which retain MAO-B inhibitory activity.[16,43] By chance (see note[a]) support for the role of the heterocyclic thiazole ring as determinant of the MAO-B selectivity arises by comparing the biological profiles of compound **25** and compound **40**, which contains the "unconstrained" thiazolidine ring. The thiazole ring might act as a *selector* between the two isoforms, by correctly orienting the pharmacophoric side chain, or by creating a specific interaction with some sub-structural elements in the MAO-B active site which may be more specific than that of MAO-A as previously reported by Palmer *et al.*[44]

[a] **Note**: During the preparation of this manuscript, a X-ray crystallography study [*unpublished results*] made clear that the previously reported structure of compound **40**[17] is not correct (**Formula A**), i.e. a thiazolidine ring actually is the molecule "scaffold" (**Formula B**). Compounds **31–45**, synthesis of which is similar to that of compound **40**, might present the same drawback and were not considered in this study. Further X-ray studies are currently in progress. It is interesting to note that compound **40** (**Formula A**) and the actual "thiazole" compound **25**, the side chain of which is similar, are active in the same range. This data is consistent with previously reported findings on the importance of the charge on the side chain sp^2 nitrogen instead of the heterocycle itself for MAO-B inhibition.[16,19,42] Moreover compound **40** shows some inhibitory activity against MAO-A whereas compound **25** shows specific activity against MAO-B and completely fits hypo *Hypo 1.1* and *1.2* (Figure 1). The "unconstrained" thiazolidine ring of compound **40** could allow the methyl group on the C4 carbon to fit the small cavity present only in the MAO-A isoform catalytic site whereas the rigid thiazole ring does not allow this to occur. None of the compounds structurally related to compound **40** fits to the 5 features generated pharmacophoric hypothesis.



Formula A



Formula B

SUPPORTING INFORMATION AVAILABLE

Tables of the Derwent World Drug hits and their fit values calculated to the lowest common denominator hypothesis derived from *Hypo 6.1*: (two HBA, one Aromatic Ring) as represented in Figure 14 are available free on request to the corresponding Author.

Hypo 6.1 (Figure 10) actually consists of the following features: two H-bond acceptors, one aromatic ring, one hydrophobic feature (the aromatic ring features consists of two spheres.)

Hypo 6.1 is closely related to *Hypo 2.2* and *Hypo 4.2*, therefore it is a common denominator, however the hydrophobic feature in *Hypo 6.1* does not occupy the same region as in *Hypo 2.2* and *Hypo 4.2* whereas the other features are well superimposed.

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