Eur J Med Chem (1997) 32, 721–730

Reversible inhibition of type B monoamine oxidase. Theoretical study of model diazo heterocylic compounds

J Wouters[1]*, F Ooms[1], S Jegham[2], JJ Koenig[3], P George[2], F Durant[1]

[1]*Laboratoire de Chimie Moléculaire Structurale, Facultés N-D de-la-Paix, 61, rue de Bruxelles, B-5000 Namur, Belgium;*
[2]*CNS Research Department, Synthélabo Recherche, 10, rue des Carrières, BP 248, 92504 Rueil-Malmaison;*
[3]*Structural Chemistry and Molecular Modeling Section, Synthélabo Recherche, 10, rue des Carrières, BP 248, 92504 Rueil-Malmaison, France*

(Received 20 January 1997; accepted 3 April 1997)

Summary — Different families of heterocycles containing 2 to 4 nitrogen atoms (oxadiazolones, tetrazoles and oxadiazinone derivatives, so-called diazoheterocyclics) are currently used as lead compounds for the design of reversible and selective monoamine oxidase B (MAO-B) inhibitors. In order to clarify the mechanism of interaction of these molecules with the enzyme, we adopted a theoretical approach (ab initio calculations) and studied several structural and electronic properties of prototype molecules of the aryl diazo heterocyclic chemical series. This work provides a theoretical basis for structure–inhibition relationships in chemical series with potential IMAO-B properties.

reversible MAO-B inhibition / model of interaction / ab initio calculation

Introduction

In the symptomatic treatment of Parkinson's disease a useful approach consists in restoring an appropriate concentration of dopamine (DA) in the synaptic cleft of DA neurons of the central nervous system. Oral levodopa substitution continues to be the most effective and well-tolerated drug treatment for early Parkinson's disease, but leads to failure as a result of the development of drug-induced dyskinesia, motor response oscillations, psychiatric complications and the progressive emergence of poorly responsive gait and balance problems.

An alternative strategy involves the administration of an inhibitor of type B monoamine oxidase (MAO-B) in order to inhibit the metabolism of dopamine and consequently increase the DA concentration in the synaptic cleft of the remaining DA neurons of the nigrostriatal pathway. Clinical use of L-deprenyl, an irreversible inhibitor of MAO-B, confirms this hypothesis and opens new perspectives in the treatment of Parkinson's diseases. However, only a few selective and potent inhibitors of MAO-B are available and there is a considerable interest in understanding their mechanism of interaction with the enzyme in order to design new molecules [1–2].

The first generation of inhibitors of MAO-B were irreversible, mechanism-based compounds such as propargylamine deprenyl (fig 1a). A lot of effort has been dedicated during the past years to develop more potent compounds, in particular among irreversible propargylamines AGN 1135 [3], fluoroalkylamines (MDL 72145 and 72974) [4] and aminomethylaryl-oxazolidinones [5–8] chemical series. Those studies led to a better understanding of the molecular mechanism catalyzed by the monoamine oxidase and these chemical series are of great interest as pharmacological tools.

Another class of compounds has been developed, belonging to the benzamide series: Ro 16-6491 and lazabemide (Ro 19-6327) (fig 1b). These two IMAO-B's are considered as reversible inhibitors [9, 10]. More recently, an additional set of molecules was discovered as IMAO-B's and belong to various aryl diazoheterocyclic families including oxadiazolones, tetrazoles and oxadiazinones (fig 1c). These new inhibitors of MAO-B are reversible, very potent and selective for the type B of monoamine oxidase [11, 12]. They contain the 'diazo' N–N moiety (scheme 1) which is present among other MAO inhibitors and that can also be found in another family of newly synthetized competitive MAO-B inhibitors, the inde-

*Correspondence and reprints

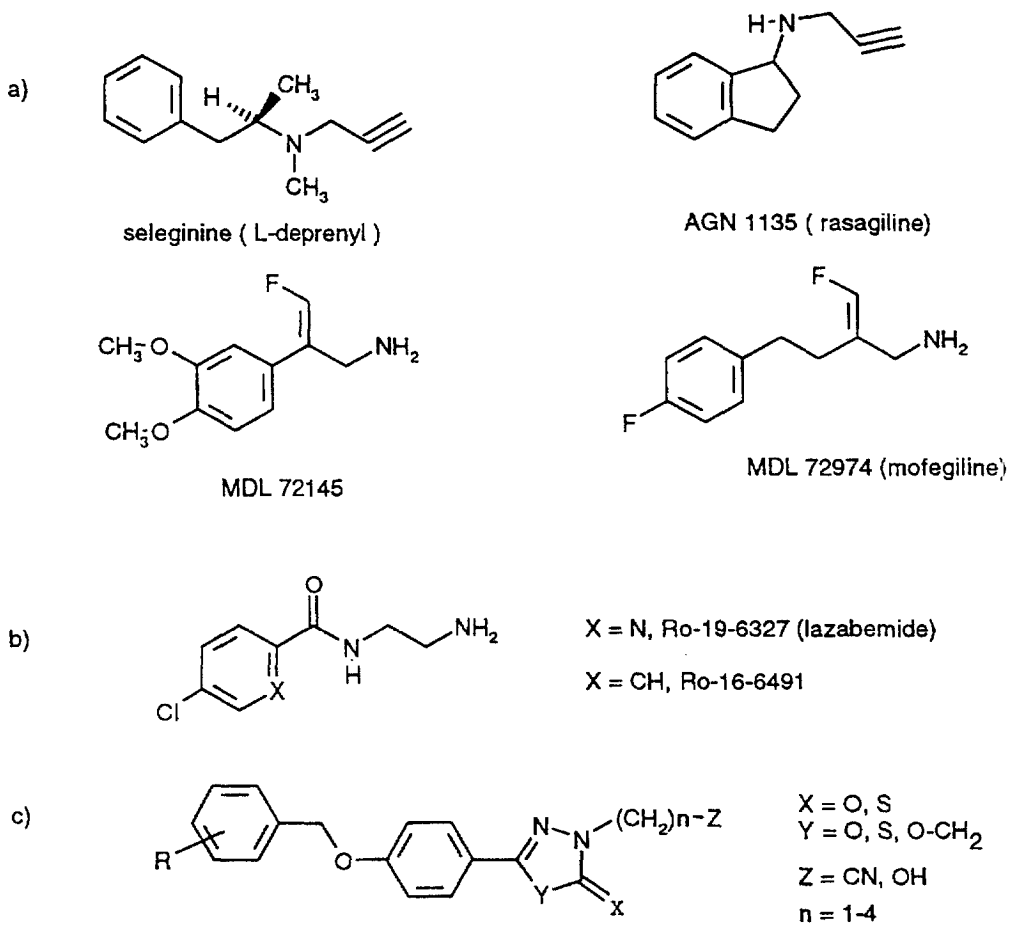
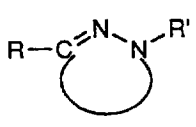


Fig 1. Examples of structures of irreversible (a) and reversible (b and c) inhibitors of type B monoamine oxidase.

nopyridazines [13]. A detailed description of the structure–activity relationships of these original molecules can be found in the papers of Milcent et al [12] and Testa et al [13] respectively.

Several features emerged from those studies that are relevant for enzyme–inhibitor interaction of potent, selective IMAO-B's. They can be briefly summarized as follows (fig 2):

(i) Lipophilicity plays a crucial role for the activity of these inhibitors. It has been demonstrated that the presence of a benzyloxy group in the *para* position of



Scheme 1. Common diazo N–N moiety.

the aryloxadiazolones and aryloxadiazinones is essential for their activity. The introduction of both electron acceptor or donor substituents on the terminal phenyl ring leads to less active molecules. Those inhibitors which contain an unsubstituted terminal benzyloxy function present the best inhibition activity. This underlines both electronic and steric constraints for this part of the molecule.

(ii) The exact role of the heterocycle is not well established although it seems that five-membered heterocycles (oxadiazolone, oxadiazolethione, thiadiazolone or thiadiazolethione) usually present a better affinity for MAO-B than six-membered rings (oxa- and thiadiazinones). The inhibitory potency also seems to be related to the electron density at the sp^2 atom of YC=X group (fig 1c), heterocycles D < B < C < A and in the C=X group, heterocycles C, D < A, B, F (figs 2 and 3).

(iii) As was similarly observed for the substrates of MAO-B (benzylamine, phenylethylamine), the length of the lateral chain of the aryl diazo derivatives should not be longer than two carbon atoms. This indicates that the groups being present on the ethyl chain (Z = OH, OMe, CN, in fig 2) could interact with the same binding site as the amine of the substrate, which could explain the competitive profile of these compounds.

The mode of interaction of the previously mentioned aryl diazo heterocyclic IMAO-B's with the enzyme must be investigated, as the role of the heterocyclic moiety.

In the present work we analyse the role of various heterocycles (oxadiazolones, tetrazoles and oxadiazinone derivatives) that were used as lead compounds for reversible and selective MAO-B inhibition in order to contribute to a better understanding of the mechanism of interaction between these molecules. We adopted a theoretical approach (ab initio calculation) and studied several structural and electronic properties of various prototype molecules in some aryl diazoheterocyclic series. This report provides a theoretical basis for structure–inhibition relationships in chemical series with potential IMAO-B properties.

Results and discussion

In order to investigate the influence of the heterocycle present in potential MAO-B inhibitors on the parameters supposed to be important for the affinity to the enzyme, we selected several prototype compounds (A–F) which were studied through a quantum chemical approach to measure electronic properties (net atomic charges, molecular electrostatic potential, energy and topology of molecular orbitals). Based on the results published in the literature for substituted analogs [11–13], it was possible to propose the affinity order for this set of molecules and it is reasonable to expect that the inhibitory potency of the chosen fragments should decrease as follows: A ~ E > C > B > D ~ F. Those fragments proved to be useful in understanding the influence of the heterocycle (part (ii) in fig 2) of different MAO-B inhibitors.

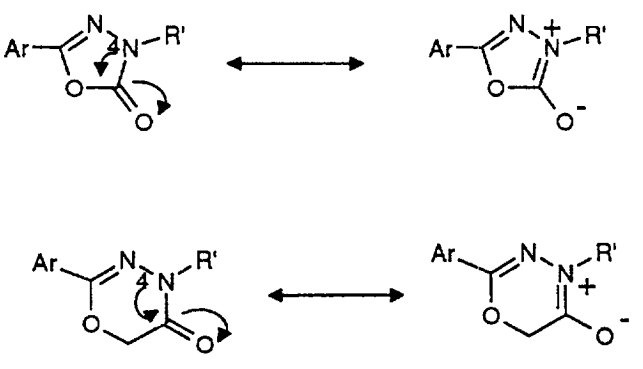
The internal geometries of all these compounds correspond to minimal-energy conformations and were obtained by geometry optimization within the SCF–MO–LCAO formalism using the 3-21G (or 3-21G*) basis set (see methods). The minimal-energy structures are completely planar with the methoxyphenyl ring coplanar to the different heterocycles studied (data not shown). This is convenient as the molecules lie in the *xz* plane and the π density can be calculated along the *z* axis.

Net atomic charges

Total atomic charges (e) and π (p_z–p_z) overlap (%) for fragments A to F are presented in figure 3 and result from a Mulliken analysis [14]. The charge density on the common methoxy phenyl rings (not included in figure 3) is the same for all the fragments A–F indicating that this part of the molecules is influenced in a similar way whatever the nature of the heterocycle (oxadiazolone, oxadiazothione, tetrazole, oxadiazinone, etc).

The electronic delocalization scheme significantly differs among the different molecules, the tetrazole ring (E) being completely delocalized in contrast with the oxadiazinone (F). Introduction of a sulfur atom (B, C, D) in the oxadiazolone (A) cycle only slightly affects the distribution of π electrons (fig 3b). In terms of atomic charges, however, the replacement of an oxygen atom by a sulfur atom induces a major modification of the electronic structure of the molecule and can lead to the inversion of the sign of the charge carried by some heteroatoms (fig 4a). As a consequence, the electrostatic potential that is induced by those fragments must also be different. This point will be discussed in more detail later and may be of interest in characterizing the several MAO-B inhibitor models.

Mazouz and colleagues [12] postulate a specific interaction of the phenyl heterocycle part of their molecules and a nucleophilic site at the active site of the enzyme. This hypothesis tends to explain the better affinity of oxadiazolones, with respect to the corresponding oxadiazinones, in terms of a δ+ excess of positive charge generated by electronic delocalization on atom N1 (scheme 2), more important in the case of oxadiazolones. Stabilization of this pseudo-aromatic δ+ entity by a nucleophilic site in the protein active site should explain the observed differences in affinities.



Scheme 2. Hypothesis of Mazouz et al.

(i) **Hydrophobic pocket**
(ii) Interaction site with the **heterocycle**
(iii) Stabilization of the lateral chain by a **catalytic residue**

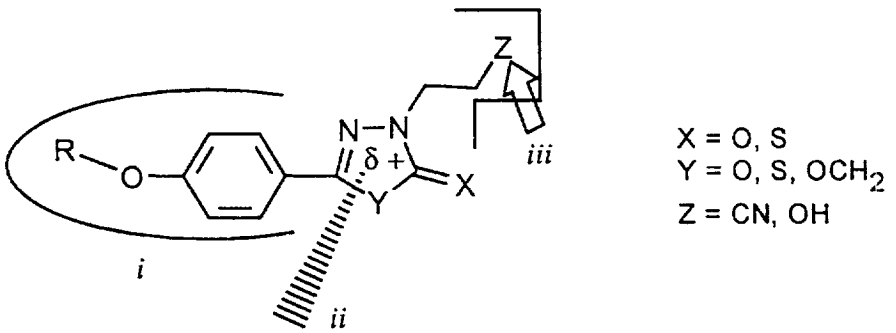


Fig 2. Main structure–activity relationship features among aryl diazo heterocyclic derivatives.

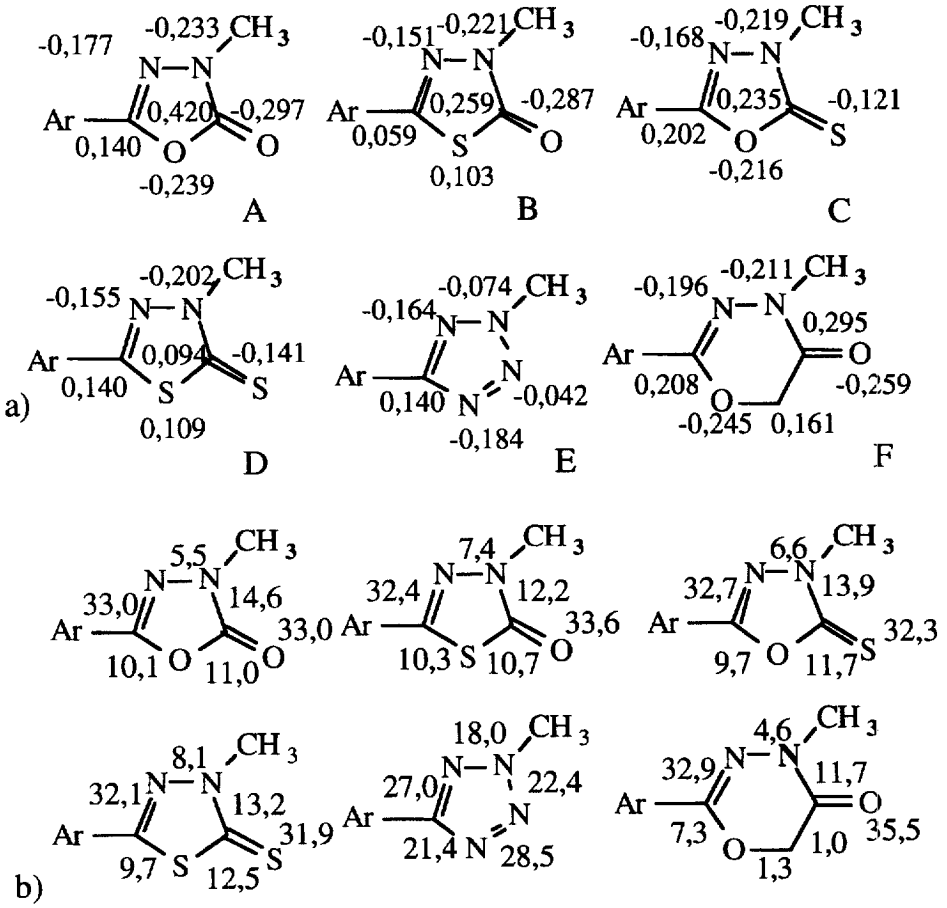
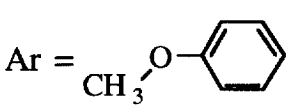


Fig 3. Total atomic charges (e, (a)) and π (π_z–π_z) overlap (%, (b)) calculated on A to F.



However, no correlation is possible between the calculated total atomic density at some heteroatom levels (eg, on N1) and the inhibitory potency of the compounds. As a consequence, additional electronic descriptors were investigated as the topology of frontier molecular orbitals and the molecular electrostatic potential.

Energy and topology of molecular orbitals

Examination of the topology of the highest molecular orbital occupied by electrons, the HOMO (fig 4) confirms what has already been observed regarding the different electronic distribution among the aryl heterocycles present in reversible MAO-B inhibitors studied here.

In contrast to MAO-A reversible inhibitors, for which the π type HOMO always seems to be located on a phenyl ring rich in electrons as observed in different families of compounds including aryl oxazolidinones and β carbolines [15–17], it is not possible to find a unique pattern among reversible MAO-B inhibitors (fig 4). For befloxatone, a very potent reversible and selective MAO-A inhibitor belonging to the aryloxazolidinone family [18], the energy of the HOMO (located on the oxyphenyl moiety) is –6.18 eV (for comparison, E_{HOMO} of hydroquinone, calculated with the same method, is –6.08 eV). It has been proposed that this compound acts as electron donor in a charge transfer with the flavin cofactor of MAO [19–21]. Based on the sole energy of the HOMO criteria (fig 4a), some MAO-B inhibitors could be good electron donors ($E_{HOMO} > -6.0$ eV) versus a common electron acceptor (the flavin cofactor of the enzyme). This has been observed experimentally by absorption spectroscopy (data not shown). This interaction would however involve the heterocycle instead of the oxyphenyl moiety of the molecules, as can be expected from the topology of the HOMO (fig 4b).

The electron donor properties (topology and energy of the HOMO) of the model molecules we investigated is not related to their affinity for MAO-B. The reversible inhibition of MAO-B can not be explained in terms of a specific, reversible interaction (charge transfer) with the flavin cofactor of the protein, in contrast with what is proposed for MAO-A inhibitors. Such an interaction with the cofactor is not excluded but certainly does not represent the major stabilizing contribution of the inhibitor in the active site of the enzyme. This also tends to demonstrate that, although both MAO-A and B share some structural properties, including amino acid sequences [22] and secondary structure patterns [23], their inhibition mechanisms could be different.

Molecular electrostatic potential

It became clear, after the examination of total atomic charges and π overlap percentage of the model molecules A–F, that a specific interaction of the aryl heterocycle of MAO-B inhibitors with a nucleophilic site of the enzyme is unprobable. Based on the molecular electrostatic potential (MEP) calculated around the several model compounds A–F (fig 5), we propose that those parts of the MAO-B inhibitors, enriched in electrons, interact with specific hydrogen donors (electrophilic sites) in the active site of the protein. It is clear from figure 5 that the several heterocycles that were included in this study (oxadiazolone, oxadiazothione, tertazole, oxadiazinone, etc) generate important attractive (versus a proton) potential wells that could play a crucial role in anchoring the MAO-B inhibitors at the active site of the protein. The identification of a common pattern of three nucleophilic sites for MAO-B inhibitors and the geometrical characterization of this pharmacophoric element are illustrated in table I. The importance of those attractive potential sites is quantified by a parameter *P* which is in fairly good correspondance with the order of affinity for MAO-B. Particularly the decrease in activity observed for the heterocycles containing a sulfur atom can be explained by their lower nucleophilic/basicity property.

The superimposition of the molecular structures presented in figure 6 allows the oxyphenyl part, which is common to all the molecules studied, to be in the same region of space. For this geometry, the attractive potential wells generated by the several heteroatoms of the heterocycles define a unique pharmacophoric pattern and can be considered as privileged anchoring parts to the active site of the protein. Identification of those interaction sites with appropriate hydrogen donor residues (Ser, Thr, Tyr, Lys, His, etc) or with the peptide backbone of the protein is an important step in the understanding of the role played by the phenylheterocycle moiety of several MAO-B inhibitors. This also demonstrates that, in contrast with reversible MAO-A inhibitors [16–21], a charge transfer component is not the major stabilization contribution of the MAO-B inhibitors we studied. It is however interesting to note that stabilization of MAO-A inhibitors by hydrogen bonds was also proposed to improve the interaction of those compounds with monoamine oxidase [19–21]. For aryloxazolidinones (toloxatone, befloxatone) those sites correspond to P1 and P2 in table I. This interaction seems however less crucial in the case of reversible MAO-A inhibitors (for which the charge transfer component is important) than for MAO-B inhibitors.

The pharmacophoric elements defined in our study are useful for the generation of original leads.

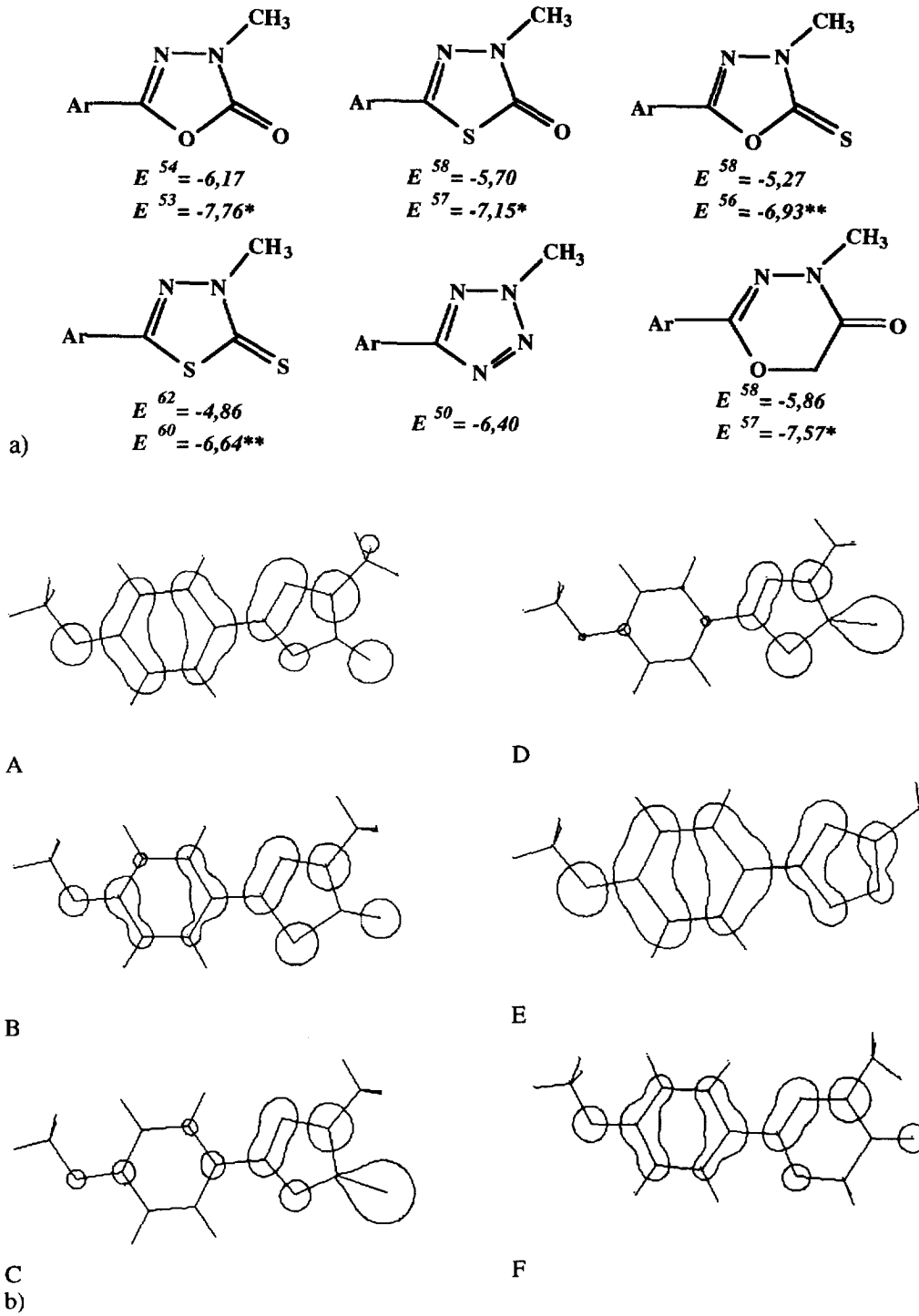
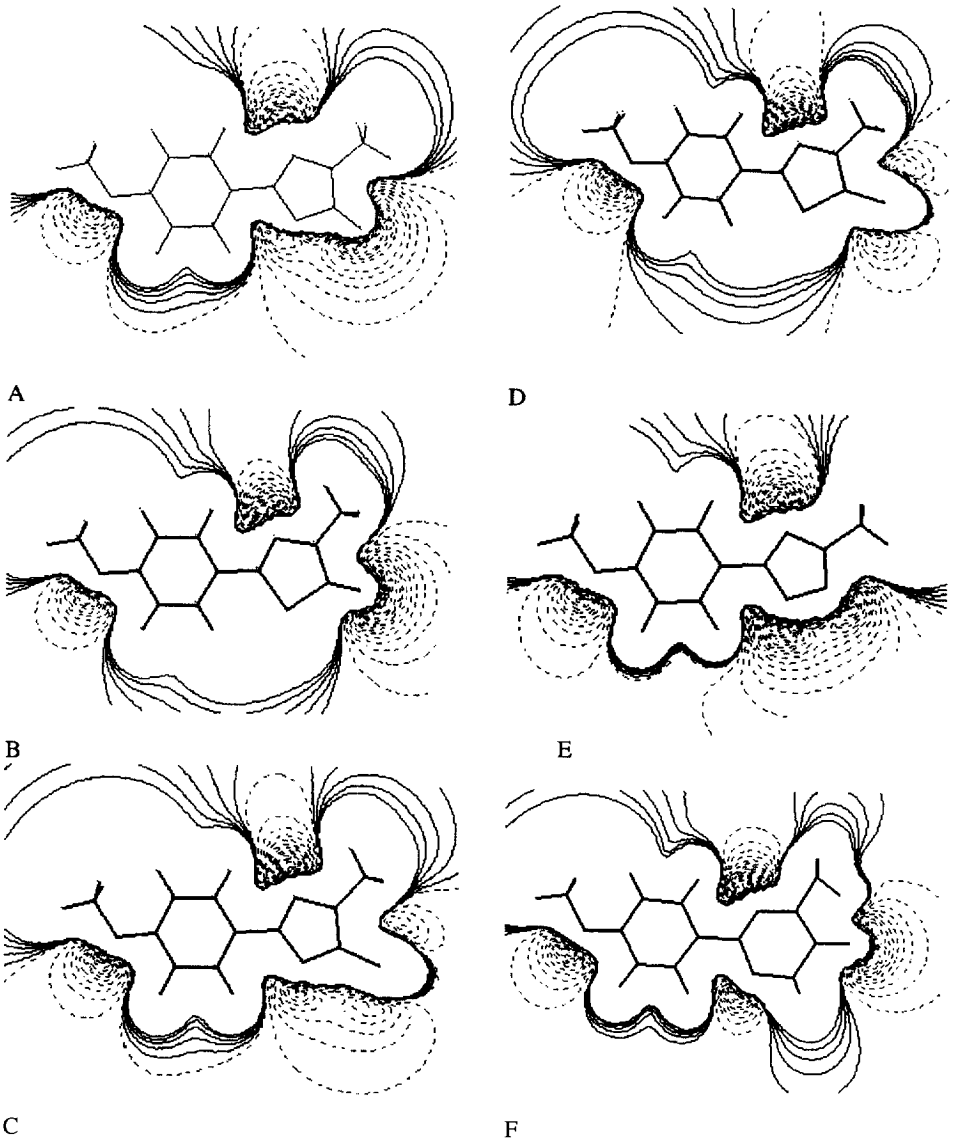


Fig 4. (a) Energy (eV) of the highest occupied molecular orbital (HOMO) for different aryl-heterocycle fragments (A–F). The energy of the first π type molecular orbital localized on the oxyphenyl ring is also presented. *: HOMO-1 and **: HOMO-2. (b) Topology (isodensity contour) of the HOMO in the same A–F fragments of MAO-B inhibitors. The electronic density is presented at 0.005 e/Å^3. More details are given in the methods section.

a



b

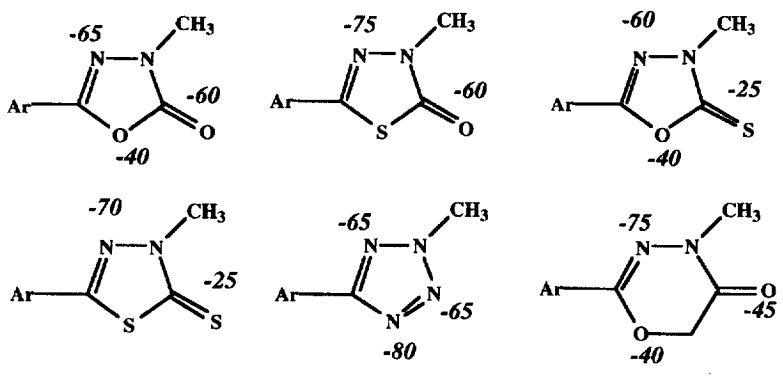
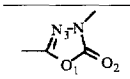
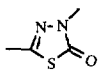
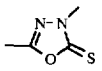
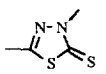
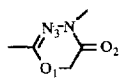
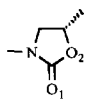
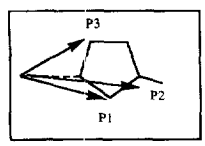


Fig 5. Molecular electrostatic potential (MEP) calculated (ab initio, 3-21G(*) basis set) around the several model compounds A–F. (a) MEP calculated in the *xz* plane of the different molecules with repulsive potential zones presented in solid lines (isocontours of the molecular electrostatic potential representation from 1 to 5 kcal/mol) and attractive regions in dashed lines (from –80 to 0 kcal/mol with a contour-to-contour interval of 5 kcal/mol).
(b) Representation of the attractive potential wells (given in kcal/mol) generated by the heteroatoms of fragments A–F.

Table I. Topology of the attractive regions of the molecular electrostatic potential (nucleophilic/basic sites) generated around the arylheterocycles A–F. The MEP map of 5-(*R*)-methyl 3-[4-methoxyphenyl] oxazolidin-2-one is also represented for comparison. P_1, P_2 and P_3 define attractive MEP wells generated at a distance d_1, d_2 and d_3 of the centroid of the adjacent phenyl ring.

	d_1 Å	P_1 kcal/mol	d_2 Å	P_2 kcal/mol	d_3 Å	P_3 kcal/mol
	3.746	–40	6.024	–60	3.750	–65
	4.125	–	6.549	–60	3.708	–65
	3.858	–40	6.558	–25	3.699	–60
	4.133	–	6.910	–25	3.708	–70
	3.767	–80	4.915	–65	3.788	–65
	3.735	–40	6.927	–45	3.691	–75
	3.995	–65	4.963	–50		

Fragment I (fig 7) was defined from the elements deduced in table I. This fragment was submitted to a systematic search of the Version 5 Cambridge Structure Database. The search fragment consists of a total of 109 molecules. Among those molecules interesting structures emerge. They are presented in figure 7.

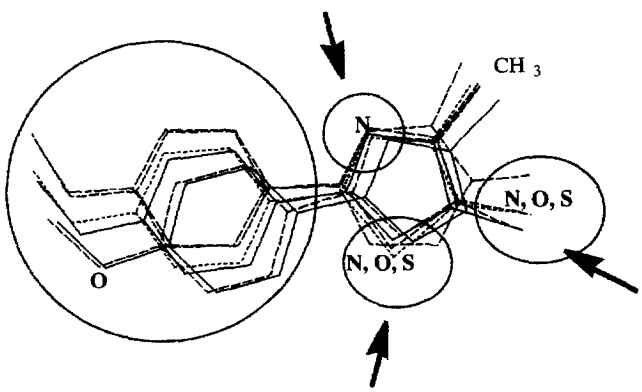


Fig 6. Superimposition of the molecular structures of the model compounds A–F based on the topology of the attractive potential wells (indicated by an arrow) generated by the heteroatoms of the heterocycles. All molecules lie in the same plane.

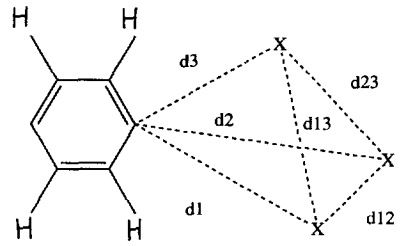
The electrostatic anchoring points proposed for the heterocycles are however not sufficient to explain the very high affinity of the corresponding MAO-B inhibitors (I_{50} in the nanomolar range when well substituted) and it is therefore clear that the other parts of the molecule ((i) and (iii) in fig 2) play the most important role in the affinity for the enzyme.

Conclusions

Taking into account the results we presented in this work, it is possible to propose a mode of reversible interaction of certain MAO-B inhibitors with the enzyme, based on the electronic characterization of several prototype compounds (fig 8).

In this model of interaction elaborated on the pharmacophoric elements deduced from this study and including structure activity results from the literature [11–13], it is important to underline the crucial role played by the terminal benzyloxy parts of the compounds. In a more general way, the lipophilicity of those MAO-B inhibitors seems to be the most important parameter governing the affinity for MAO-B.

The nature of the heterocycle (oxadiazolone, tertazole, oxadiazinone, etc) is not essential. However,



X : N, O or S (in the plane of the phényl ring within ±10°

d1 = 2.3 - 3.0 Å	d12 = 1.25 - 3.60 Å
d2 = 3.3 - 5.7 Å	d23 = 2.15 - 3.70 Å
d3 = 2.3 - 2.7 Å	d13 = 2.15 - 2.70 Å

Fragment 1

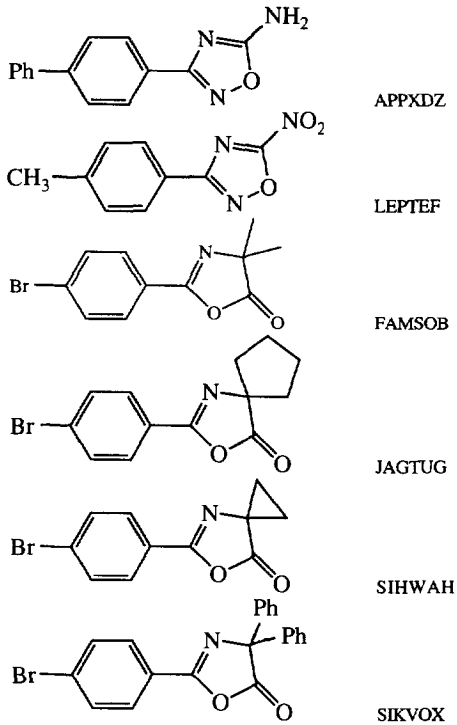


Fig 7. Results of the CSD (Cambridge Structure Database) statistical search. The search fragment (1) corresponds to the pharmacophoric elements deduced in table I. The most interesting molecules retrieved from the database (corresponding to a refcode) are presented.

their electronic properties must be conserved as this part of the inhibitor has to fit with the pattern of nucleophilic/basic sites presented in this study (fig 6). This point is clearly demonstrated by recent results of the literature. Opening of the oxadiazolone heterocycle leads to acylhydrazone derivatives (scheme 3) which retain MAO-B inhibitory activity [24]. The

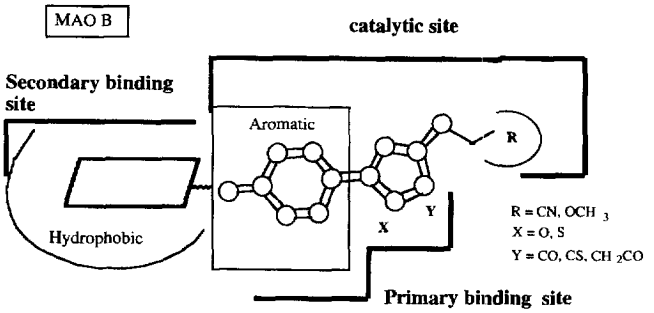
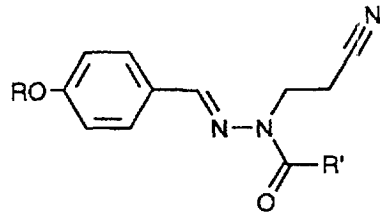


Fig 8. Proposition of interactions between MAO-B inhibitors at the active site of the protein.

presence of a 4-benzyloxyphenyl entity is however essential for affinity and selectivity for MAO-B. This may well confirm the fact that what is important for MAO-B inibition is the possibility to stabilize (*via* hydrogen bonds) the inhibitor in a hydrophobic pocket of the binding site of the protein by means of fixed anchoring points (see pattern in table I and fig 6).

The presence of an appropriate lateral functionalized (CN, OR) ethyl chain is also essential for interaction with MAO-B. It is reasonable to propose that this chain reaches a catalytic Cys residue as do inactivators [3–7] and substrates of this enzyme. In contrast with the substrates, the reversible inhibitors do not react with the protein (no amine function) and can only lead to reversible interactions (H bonds) with this catalytic cystein. This may also explain the behavior of these molecules.

The proximity of the isoalloxazine ring (planar) of the flavin cofactor of MAO may explain the preferred planar conformation of the aryl heterocyclic part of the compounds we considered (steric constrain). It is also interesting to note that our hypothesis of a charge transfer component being less important for MAO-B inhibition than MAO-A inhibition is in good agreement with the substrate specificities of the two types of monoamine oxidase. Indeed, substrates of MAO-A



Scheme 3. Acyclic acylhydrazone derivatives.

(serotonine (5-HT), (nor)adrenaline), because of their chemical structure (catechol, hydroxyindole), are better electron donor groups in the perspective of a charge transfer interaction than MAO-B substrates (benzylamine, phenylethylamine) which are more hydrophobic and characterized by an aromatic ring more poor in electrons.

The present study allows us to underline different points to be taken into account for the inhibitor–enzyme interactions: a planar support presenting a conserved pattern of H-bonding acceptor sites, substituted by a functionalized (CN, OR) ethyl lateral chain, able to reach the catalytic site of MAO-B without reacting with it (reversibility and competitivity), and a benzyloxy chain, conferring lipophilicity to the molecule so that it interacts with the hydrophobic binding site of the protein.

Experimental protocols

The molecular orbital topology, ie, pattern of the highest occupied molecular orbital (HOMO), and the electronic properties of the model compounds, ie, overlap populations, atomic charges and molecular electrostatic orbitals (MEP) have been obtained at the non-empirical Restricted Hartree–Fock (RHF) LCAO–MO–SCF (linear combination of atomic orbitals–molecular orbitals–self consistent field) level of electronic theory [25]. At such a level, one considers the independent motion of a single electron in the electrostatic field of fixed nuclei and averaged Coulomb and exchange fields due to other electrons. This level of the theory results in the traditional molecular orbital (MO) language. Within this framework, calculations have been performed at the 3-21G [26] (or 3-21G* when the molecule contains a sulfur atom [27]) degree of sophistication in the LCAO expansion of the molecular orbitals. For qualitative interpretations, this scheme has the great advantage of relating molecular properties to simple atomic parameters and allow for a conceptual approach common to both theoreticians and experimentalists.

The internal geometries of all the compounds (A–F) correspond to minimal-energy conformations and were obtained by geometry optimization within the SCF–MO–LCAO formalism using the 3-21G(*) basis set. The minimal energy structures are completely planar with the methoxy phenyl ring coplanar to the different heterocycles studied. This is convenient as the molecules lie in the xz plane and the density can be calculated along the z axis.

The generation of the electron charge density two-dimensional iso-contour maps was performed with the MOPLOT (Molecular Orbital Plot) subprogram [28] available within the MOTECC (Modern Techniques in Computational Chemistry) package [29]. All computations were carried out using the GAUSSIAN92 programs [30] adapted to a IBM Risc 6000 computer system. The 2D iso-contour maps were drawn with an in-house device-independent contouring program CPS (Contouring Plotting System) [31] developed in Fortran with the IBM graPHIGS software [29]. A fragment corresponding to the pharmacophoric elements deduced from our study was submitted to a systematic search of the Version 5 Cambridge Structure Database [32]. The corresponding hits (109) were retrieved from the database.

Acknowledgments

JW thanks the National Foundation for Scientific Research (FNRS Belgium) for his Senior Research Assistant position. The authors acknowledge IBM Belgium and the Facultés Notre Dame de la Paix for the use of the Namur Scientific Computing Facilities.

References

1 Cesura A, Da Prada M (1992) *Progress in Drug Research* 38, 171–297
2 Strolin Benedetti M, Dostert P (1992) In: *Advances in Drug Research* Vol 23 (Testa B, ed) Academic Press, London, 62–125
3 Yu P, Davis B, Boulton A (1992) *J Med Chem* 35, 3705–3713
4 McDonald IA, Bey P, Zreika M, Palfreyman MG (1991) *Drug of the Future* 16, 428–431
5 Ding C, Lu X, Nishimura K, Silverman R (1993) *J Med Chem* 36, 1711–1715
6 Ding C, Lu X, Nishimura K, Silverman R (1993) *J Med Chem* 36, 3606–3610
7 Gates K, Silverman R (1989) *J Am Chem Soc* 111, 8891–8895
8 Gates K, Silverman R (1990) *J Am Chem Soc* 112, 9364–9372
9 Cesura A, Galva M, Imhof R, Da Prada M (1987) *J Neurochem* 48, 170–176
10 Da Prada M, Kettler R, Keller H, Bonetti E, Imhof R (1986) In: *Advances in Neurology* (Yahr M, Bergmann K, eds) Raven Press, New York, Vol 45, 175–178
11 Koenig JJ, Lebreton L, Masson M, PCT Int Appl WO 9108201 (1991) *Chem Abstr* 115, 183315s
12 Mazouz F, Gueddari S, Burstein C, Mansuy D, Milcent R (1993) *J Med Chem* 36, 1157–1167, and references cited
13 Kneubuhler S, Carta V, Altomare C, Carotti A, Testa B (1993) *Helv Chim Acta* 76, 1956–1963
14 Mulliken RS (1955) *J Chem Phys* 23, 1833–1840
15 Moureau F, Wouters J, Vercauteren DP, Evrard G, Durant F, Ducrey F, Koenig JJ, Jarreau FX (1992) *Eur J Med Chem* 27, 939–948
16 Moureau F, Wouters J, Vercauteren DP, Evrard G, Durant F, Ducrey F, Koenig JJ, Jarreau FX (1994) *Eur J Med Chem* 29, 269–277
17 Moureau F, Wouters J, Depas M, Vercauteren DP, Evrard G, Durant F, Ducrey F, Koenig JJ, Jarreau FX (1995) *Eur J Med Chem* 30, 823–838
18 Jarreau FX, Rovei V, Koenig JJ, Scooffs A, Eur Pat Appl EP 424,244 (1991) *Chem Abstr* 115, 136085g
19 Wouters J, Moureau F, Dory M, Evrard G, Durant F, Ducrey F, Koenig JJ, Jarreau FX (1993) In: *Trends in QSAR and Molecular Modelling 92* (Wermuth C, ed) ESCOM, Leiden, 291–293
20 Wouters J (1995) PhD Thesis, Facultés Universitaires Notre Dame de la Paix, Namur, Belgium
21 Wouters J, Moureau F, Vercauteren DP, Evrard G, Durant F, Koenig JJ, Ducrey F, Jarreau FX (1994) *J Neural Transm [Suppl]* 41, 313–319
22 Weyler W, Hsu Y, Breakefield X (1990) *Pharmac Ther* 47, 391–417
23 Wouters J, Ramsay R, Goormaghtigh E, Ruysschaert JM, Brasseur R, Durant F (1995) *Biochem Biophys Res Commun* 208, 773–778
24 Bernard S, Paillat C, Oddos T, Seman M, Milcent R (1995) *Eur J Med Chem* 30, 471–482
25 Pople JA (1977) In: *Application of Electronic Structure Theory* (Schaefer HF, ed) Modern Theoretical Chemistry, Plenum, New York, Vol 4, 95–170
26 Hehre WJ, Steward RF, Pople JA (1969) *J Chem Phys* 51, 2657–2664
27 Collins JB, Scheyer PVR, Binkley JS, Pople JA (1976) *J Chem Phys* 64, 5142–5151
28 Hinde R, Luken W, Chin S (1988) IBM Kingston Technical Report KGN-141, New York
29 Chin S, Vercauteren D, Luken W, Re M, Scateni R, Tagliavini R, Vanderveken D, Baudoux G (1989) Modern Techniques in Computational Chemistry: MOTECC 89 (Clementi E, ed) ESCOM, Leiden, 499–546
30 Frisch M, Trucks G, Schlegel H, Gill P, Johnson B, Wong M, Foresman J, Robb M, Head-Gordon M, Replogle E, Gomperts R, Andres J, Raghavachari K, Binkley J, Gonzalez C, Martin R, Fox D, Defrees D, Baker J, Stewart J, Pople J (1993) Gaussian92, Inc, Pittsburgh, PA
31 Baudoux G, Vercauteren D (1989) CPS: A contour plotting system, Facultés Universitaires Notre Dame de la Paix, Namur, Belgium
32 Allen F, Kennard O (1993) *Chem Design Autom News* 8, 31–37