Pergamon

Bioorganic & Medicinal Chemistry, Vol. 3, No. 11, pp. 1537–1545, 1995

0968-0896/95 \$9.50 + .00

0968-0896(95)00144-1

Structure–Affinity Relationships of Baclofen and 3-Heteroaromatic Analogues

Bernard Pirard,*[a] Pierre-Alain Carrupt,[b] Bernard Testa,[b] Ruey-Shiuan Tsai,[b] Pascal Berthelot,[c] Claude Vaccher,[c] Michel Debaert[c] and François Durant[a]

[a] *Laboratoire de Chimie Moléculaire Structurale, Facultés Universitaires Notre-Dame de la Paix, rue de Bruxelles, 61, B-5000 Namur, Belgium*

[b] *Institut de Chimie Thérapeutique, Section de Pharmacie, Université de Lausanne, CH-1015 Lausanne, Switzerland*

[c] *Laboratoire de Pharmacie Chimique, UFR de Pharmacie, rue du Professeur Laguesse, 3, F-59006 Lille Cedex, France*

Abstract—Substituting a furan, a thiophene, a benzo[*b*]furan, a benzo[*b*]thiophene, or a quinoline ring for the *p*-chlorophenyl moiety of baclofen has led to $GABA_B$ ligands with different affinities depending on the nature of the heteroaromatic ring, and on the nature and position of its substituent. As steric effects cannot account for all the affinity variations, we have studied the lipophilic and electronic properties of baclofen and selected 3-heteroaromatic analogues, gaining insight into the structural features necessary for $GABA_B$ affinity. Centrifugal partition chromatography (CPC) has been used to measure octan-1-ol water distribution coefficients, while *ab initio* molecular orbital (MO) calculations were performed to study electronic properties.

Introduction

Within the central and peripheral nervous systems, γ-aminobutyric acid (GABA) can bind to at least two different kinds of receptors, $GABA_A$ and $GABA_B$. $GABA_A$ receptors are involved in fast synaptic transmission, while $GABA_B$ receptors act in a modulatory fashion.[1,2] $GABA_B$ agonists have therapeutic potential in certain respiratory diseases such as asthma, while antagonists could be useful in the treatment of cognitive disorders, absence epilepsy and possibly in depression.[2–4]

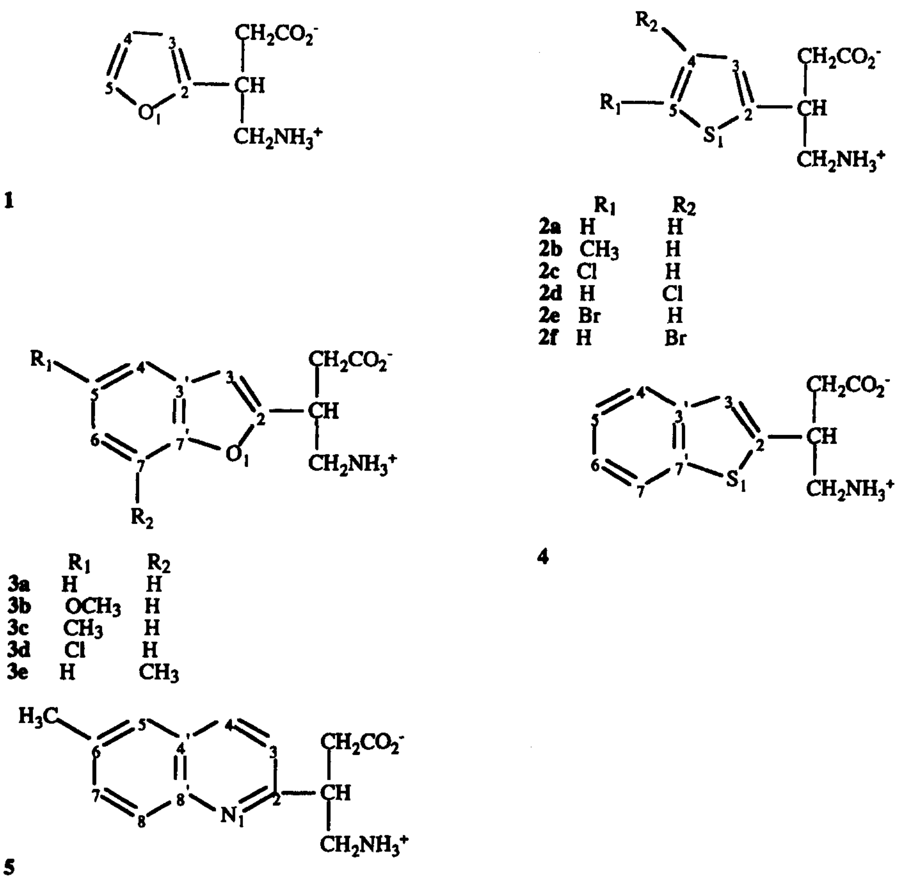
To date, *R*-(–)-γ-amino-β-(*p*-chlorophenyl)-butyric acid (baclofen) and γ-aminopropylphosphinic acid have been considered as lead compounds for $GABA_B$ ligands. Systematic variations of their structures led to the discovery of new specific $GABA_B$ ligands.[5] The *p*-chlorophenyl moiety in baclofen has been replaced by a furan, thiophene, benzo[*b*]furan,[6,7] benzo[*b*]thiophene, or a quinoline ring (Fig. 1). Two structural features are crucial for $GABA_B$ affinity: the nature of the heteroaromatic ring and the nature and position of its substituent.[6–9] The racemic thienyl analogues substituted in 5 (compounds **2b**, **2c** and **2e**, Fig. 1) and the racemic benzo[*b*]furan analogue substituted in 7 by a methyl group (compound **3e**, Fig. 1) show IC_{50} values in the micromolar range (Table 1), while the racemic furan, benzo[*b*]thiophene, and quinoline analogues (compounds **1**, **4** and **5**, Fig. 1) are devoid of $GABA_B$ affinity (Table 1). On the other hand, some benzo[*b*]-furan analogues have been described as $GABA_B$ antagonists in both the central nervous system (CNS) and the periphery.[10,11] Thienyl analogues are agonists in the periphery,[12] while no data is available for the CNS.

The aim of this work was to understand the influence of both the heteroaromatic moiety (nature) and its substituent (nature and position) on $GABA_B$ affinity. Previously, conformational analysis has enabled some of us to identify six features necessary for $GABA_B$ affinity of 3-heteroaromatic baclofen analogues:[13] (1) a carboxylate group; (2) a primary ammonium group; (3) a mean distance between the ionized moieties of 4.6 Å; (4) an aromatic ring (phenyl, thienyl, benzo[*b*]furan) bound to Cβ; (5) a lipophilic substituent in position *para* (phenyl) or 5 (thienyl, benzo[*b*]furan), a region sensitive to steric bulk; and (6) another lipophilic group in position 7 (benzo[*b*]furan). For fused heterorings, an electron-rich heteroatom (oxygen) may be mandatory to stabilize the γ-aminobutyric (GAB) chain conformation. However, this pharmacophoric pattern cannot help to explain the very weak $GABA_B$ affinity ($IC_{50} >$ 100 μM, Table 1) of compound **5**. Moreover, the stereoelectronic requirements for the substituent in 5 (compounds **2** and **3**) or in 7 (compound **3e**) remain unclear. These facts led us to investigate both the lipophilicity and the electronic properties of 3-heteroaromatic baclofen analogues. As a result, we have refined the $GABA_B$ pharmacophoric pattern for 3-heteroaromatic baclofen analogues.

Results

Lipophilicity

The distribution of solutes between water and octan-1-ol, as expressed by the partition coefficient (log P) or the distribution coefficient (i.e. apparent partition coefficient, log D), is often taken as an index of lipophilicity.



	R_1	R_2
2a	H	H
2b	CH_3	H
2c	Cl	H
2d	H	Cl
2e	Br	H
2f	H	Br

	R_1	R_2
3a	H	H
3b	OCH_3	H
3c	CH_3	H
3d	Cl	H
3e	H	CH_3

Figure 1. Planar structure formulae with numbering of relevant furan, thienyl, benzo[*b*]furan, benzo[*b*]thiophene, and quinoline analogues of baclofen. The atomic numbering scheme adopted for the rings is also given.

Table 1. Compilation of $GABA_B$ affinities,[7–9] measured and calculated octan-1-ol water distribution coefficients, volumes and polarity parameters of baclofen and its 3-heteroaromatic analogues

Compounds[a]	IC_{50}[b] (μM)	IC_{50}[c] (μM)	log D[d]	log $D^{calc,e}$	V^f (Å^3)	Λ^g
baclofen	0.33		−0.97(1)	−0.74	180.5	6.9
1	> 100		−2.38(2)	−2.28	144.1	7.2
2a	9.72		−2.07(2)	−1.76	156.0	7.2
2b	1.34	6.4	−1.46(1)	−1.24	173.2	7.1
2c	0.61		−1.07(1)	−1.03	170.6	6.7
2d	45.3		−1.22(1)	−1.03	170.8	6.8
2e	1.86		−0.90(1)	−0.83	178.1	6.7
2f	39.1		−1.06(2)	−0.83	177.9	6.9
3a[h]			−0.88(1)	−1.00	191.5	7.1
3b	22.2	190	−0.89(1)	−0.93	216.3	7.9
3c		108	−0.28(0)	−0.48	208.6	7.1
3d		17	−0.05(0)	−0.27	206.1	6.8
3e		5.4	−0.34(0)	−0.48	207.8	7.1
4		> 10	−0.53(0)	−0.47	203.0	7.1
5		> 100	−0.42(1)	−1.03	223.4	7.7

[a] Compounds are presented according to the numbering given in Figure 1.

[b] IC_{50} values were determined by displacement of *R*-(−)-^{3}[H] baclofen.[7,8]

[c] IC_{50} values were determined by displacement of ^{3}[H] GABA.[9]

[d] Each log D value is the mean of three measurements. Each value in parentheses is the maximum deviation relative to the average log D value.

[e] Calculated log D according to the fragmental approach developed by Rekker.[33]

[f] Molecular volume computed according to Gavezotti.[34]

[g] Polarity parameter according to El Tayar *et al.*.[22]

[h] For compound **3a**, IC_{50} value which was determined by displacement of racemic ^{3}[H] baclofen,[6] amounts to 18 μM. This value has to be compared with IC_{50} = 0.2 and 5.6 μM for baclofen and **3b**, respectively.[6]

As the lipophilic properties of a zwitterionic species can be difficult to predict from its chemical structure,[14] we measured the log D of baclofen and the compounds under study (Fig. 1) by the recently developed CPC technique.[15–18] CPC combines the advantages of the shake-flask method (ability to obtain genuine partition coefficients, possibility of using a variety of solvent systems) with those of reversed-phase HPLC (rapidity, reproducibility, decreased interference by impurities).

The results are given in Table 1. For all compounds under study except 5, a strong correlation between measured log D and calculated log D^{calc} (see Experimental for more details about the calculations) is observed:

$$\log D = 1.18\ (\pm 0.24) \log D^{calc} + 0.12\ (\pm 0.18)$$
$$n = 14,\ r^2 = 0.951,\ q^2 = 0.918,\ SD = 0.150,\ F = 231 \quad (1),$$

where n is the number of compounds, r^2 the correlation coefficient, q^2 the cross-validated correlation coefficient, SD the standard deviation, and F the Fischer test of statistical significance. Values in parentheses are the 95% confidence limits. As conformational changes can alter lipophilicity,[19–21] the deviation observed for compound 5 (log D – log D^{calc} = 0.61, Table 1) might arise from an intramolecular N-H---N(sp^2) hydrogen bond.

This hypothesis is in close agreement with the results of *ab initio* MO STO-3G conformational calculations. For compound 5, the folded conformation (N---X = 2.94 Å, Table 2) is more stable (8.7 kcal mol^{-1}) than the extended one (N---X = 3.90 Å). Moreover, this energy difference has to be compared with the lower one (5.8 kcal mol^{-1}) computed between folded (N---X = 2.88 Å, Table 2) and extended (N---X = 3.94 Å) conformations of compound 3c whose log D and log D^{calc} are not significantly different (log D – log D^{calc} = 0.20 as compared to 0.61 for 5, Table 1). The larger basicity of quinoline nitrogen as expressed by electrostatic potential derived (EPD) charges (Fig. 2) accounts for these energy differences.

The decomposition of log D into a cavity/volume term (V, expressing mainly hydrophobic and dispersive solute-solvent interactions) and polarity term (Λ, reflecting electrostatic solute–solvent interactions)[22,23] led us to identical conclusions. For most compounds, the variations of log D arise from variations in volume term as expressed by the following correlation:

$$\log D = 0.032\ (\pm 0.006)\ V - 6.82\ (\pm 1.30)$$
$$n = 13,\ r^2 = 0.920,\ q^2 = 0.887,\ SD = 0.199,\ F = 127 \quad (2).$$

However, two compounds (3b and 5) show Λ values which are significantly different than those derived for the 13 others (Λ = 7.9 and 7.7, respectively, as to be compared with 6.7 ≤ Λ ≤ 7.2 for the others, Table 1). For compound 3b, the discrepancy arises from the presence of a polar substituent (OCH_3). On the other hand, an intramolecular N-H---N(sp^2) bond, as suggested above, might account for the Λ value of compound 5.

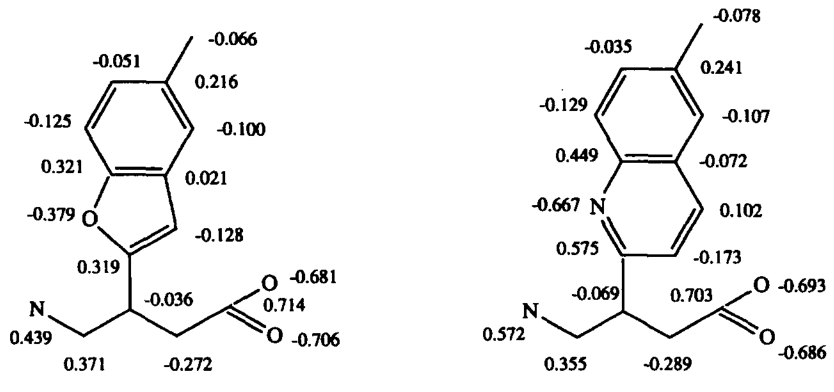
Topologies of the highest occupied molecular orbital located on the aromatic moiety (HOMOΠs)

The aromatic moiety of 3-heteroaromatic baclofen analogues is likely to be involved in Π–Π interactions with aromatic residues at the receptor site. Within this scope, the topology of frontier molecular orbitals

Table 2. Conformational analysis of compounds 3c and 5

	Folded conformations		Extended conformations	
Compounds	Relative energy (kcal mol^{-1})	N---X (Å)[a]	Relative energy (kcal mol^{-1})	N---X (Å)[a]
3c	0.0	2.88	5.8	3.94
5	0.0	2.94	8.7	3.90

[a] N---X: distance between the nitrogen atom of the ammonium group and the ring heteroatom, oxygen (3c) or nitrogen (5).



a) b)

Figure 2. *Ab initio* STO-3G atomic charges (electron) derived from the surrounding electrostatic potential for compounds (a) 3c and (b) 5. The contributions of the hydrogen atoms were summed into those of the carrier atoms. The folded conformation was considered for both compounds.

provide an invaluable source of information for structure–affinity relationships. As previously demonstrated by Weinstein and colleagues,[24] the localization of HOMO density matches the sites at which the highest percentile contribution to the polarization is calculated. The polarization encodes for Π–Π interactions which may significantly affect the affinity of a ligand for its receptor.[25,26] Previously, the analysis of the localization of HOMO has provided an invaluable insight in the study of binding of indolealkylamines on LSD/5-HT receptors.[24] Recently, a detailed analysis of the topologies of the frontier molecular orbitals has led to the proposal of an interaction model between a monoamine oxidase A inhibitor (A-MAOI) and substituted lumiflavin.[27] The relative orientation of both A-MAOI and substituted lumiflavin allows a maximum matching fit between their frontier molecular orbitals.

Using the *ab initio* MO STO-3G (STO-3G* when chlorine or sulfur are present) method, we have computed the topology of the HOMOΠs for baclofen and selected 3-heteroaromatic analogues (compounds **2a–d**, **3b–e**, **4** and **5**). Compound **1** was not considered (see Discussion).

The results are presented in Figures 3 and 4. The HOMOΠs show different topologies according to the nature of the ring, and the nature and position of its substituent. The effect of the nature of the substituent is more pronounced for benzofuran (Fig. 4a) than for thienyl analogues (Fig. 3b–e).

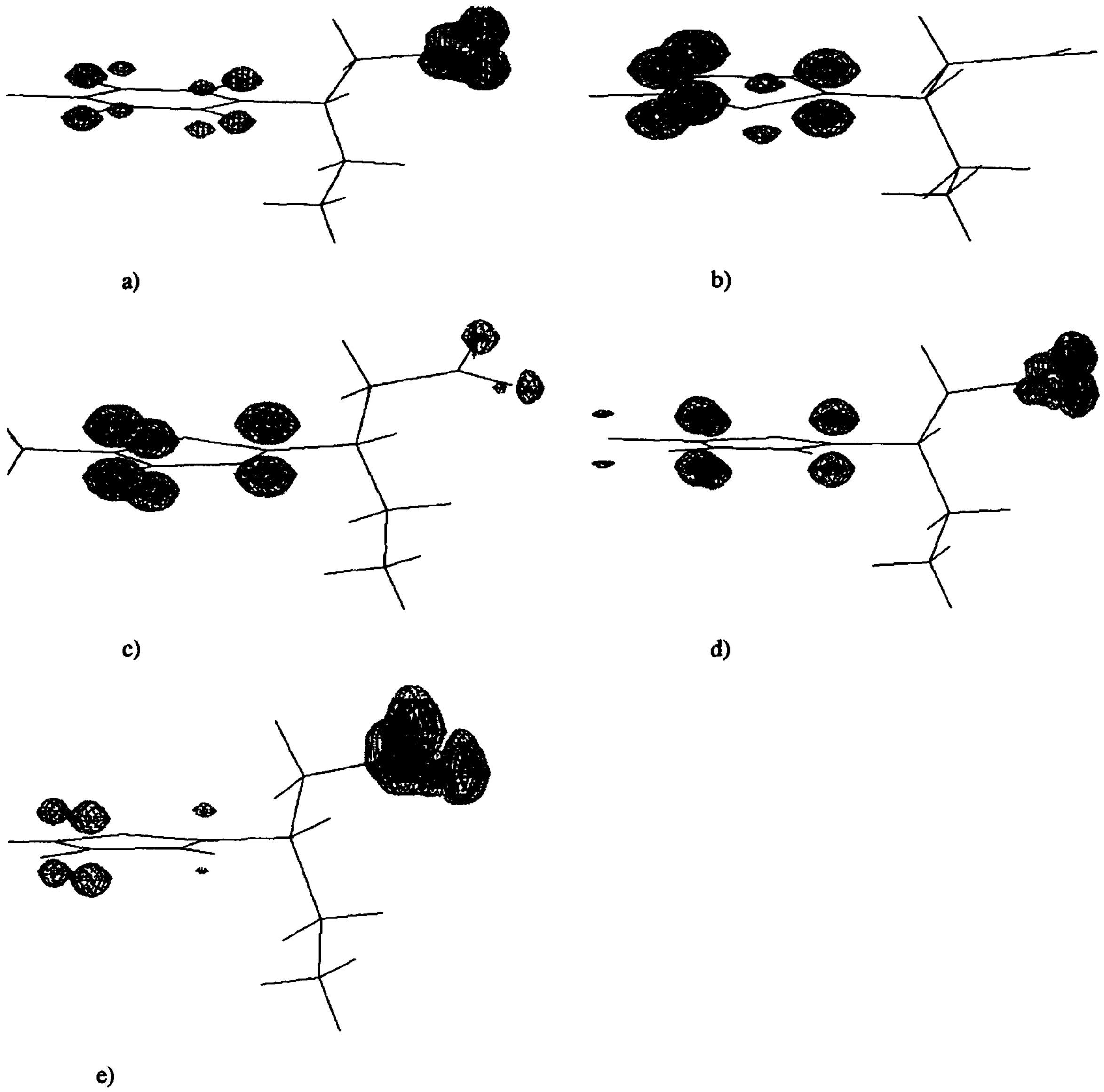


Figure 3. *Ab initio* STO-3G or STO-3G* *iso*-electron density (electron Å^{-3}) surfaces for (a) baclofen; (b–e) compounds **2a–d**. Black surfaces indicate densities of 0.020 electron Å^{-3}; grey surfaces densities of 0.010 electron Å^{-3}.

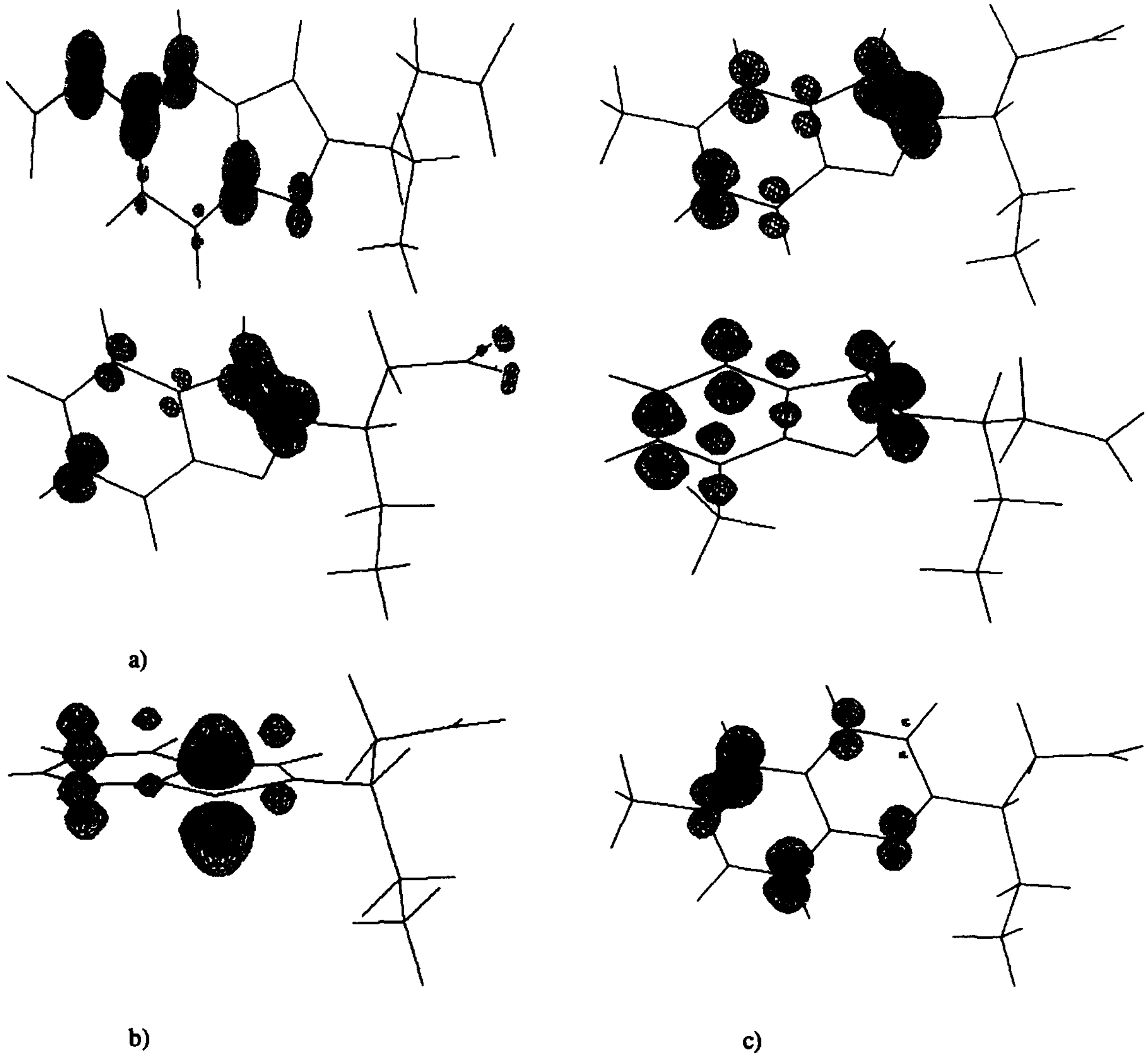


Figure 4. *Ab initio* STO-3G or STO-3G* *iso*-electron density (electron Å^{-3}) surfaces for (a) compounds **3b–e**; (b) compound **4**; and (c) compound **5**. Black surfaces indicate densities of 0.020 electron Å^{-3}; grey surfaces densities of 0.010 electron Å^{-3}.

Discussion

The nature of the aromatic moiety, and the nature and position of its substituent strongly affect the GABA$_B$ affinity of 3-heteroaromatic baclofen analogues. Steric effects[13] can account for some observations. However, other parameters have to be taken into account to explain all the variations of affinity within this class of compounds. Within this scope, both the lipophilicity (log D) and the topology of the HOMOΠs for baclofen and selected 3-heteroaromatic analogues have been studied.

For all compounds under study, log D and affinity ($pIC_{50} = -\log IC_{50}$ or A, Fig. 5 for definition) are poorly correlated. The structural diversity of the ring systems may explain this bad correlation. As a consequence, small rings (furan, phenyl and thiophene) and fused heterorings (benzo[*b*]furan, benzo[*b*]thiophene and quinoline) have been considered separately.

For thienyl analogues, the introduction of a lipophilic substituent in position 5 leads to an increase in GABA$_B$ affinity (pIC$_{50}$), as expressed by the following slight correlation with the bromo derivative (**2e**) as the most deviant:

$$pIC_{50} = 0.94\ (\pm\ 0.38)\ \log D + 7.08\ (\pm\ 0.38)$$
$$n = 5,\ r^2 = 0.670,\ SD = 0.40,\ F = 6.09 \qquad (3).$$

This correlation is in agreement with the variation of GABA$_B$ affinity previously reported for baclofen analogues: *p*-Cl > *p*-F >> unsubstituted.[28] However, thienyl analogues with a larger substituent in 5 (ethyl or propyl group) are devoid of GABA$_B$ affinity.[29] This substituent probably interacts with a region of the receptor sensitive to steric bulk, effect probably responsible for the deviation of compound **2e** in equation 3. This hypothesis is in agreement with our previous molecular modelling study.[13] Steric effects may also account for the lower affinity of compounds **2d** and **2f** (IC$_{50}$ = 45.3 and 39.1 μM as compared to 0.61 and 1.86 μM for their positional isomers, compounds **2c** and **2e**, Table 1). This latter hypothesis can also help to explain the similar decrease in affinity for the *meta* isomer of baclofen (IC_{50} = 20 μM[7] as compared to 0.33 μM for baclofen).

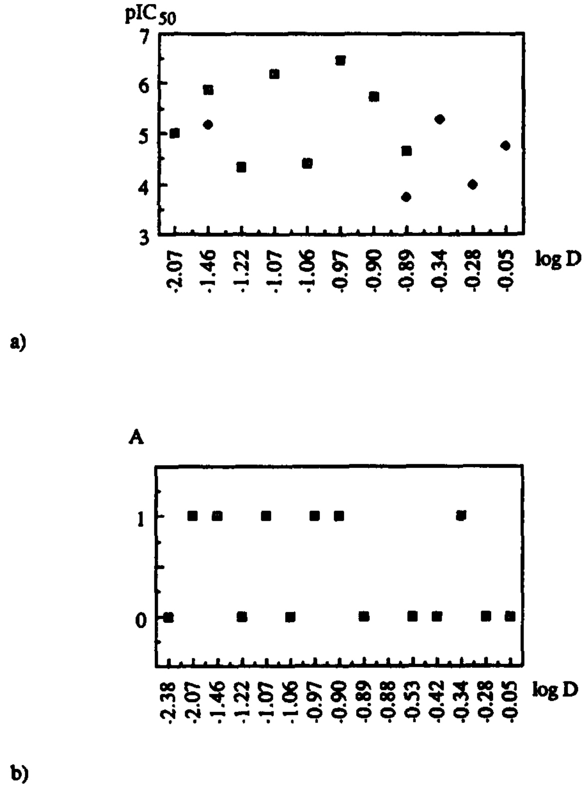


Figure 5. Scatter plots of (a) the pIC_{50} ($pIC_{50} = -\log IC_{50}$) versus log D for baclofen, compounds **2a–f**, **3b–e** (squares and stars correspond respectively to the first and second columns of Table 1); (b) the affinity parameter (A) versus log D for all compounds under study except compound **3a**. The value of A was fixed to 1 if IC_{50} was lower than 10 μM, while it was fixed to 0 when IC_{50} was greater than 10 μM. The IC_{50} are given in the first and second column of Table 1.

Substituting a furyl for a thienyl ring leads to a decrease in both lipophilicity and affinity (log D = –2.07 and IC_{50} = 9.72 μM for compound **2a** as compared to log D = –2.38 and IC_{50} > 100 μM for compound **1**, Table 1). However, in contrast to the thienyl analogue **2a**, the introduction of a methyl group in position 5 of compound **1** does not improve the $GABA_B$ affinity (IC_{50} > 100 μM for both furyl analogues[7]). In other words, the isosteric replacement of a thienyl by a furyl ring leads to a complete loss of $GABA_B$ affinity. The variations in the cavity term (V) among compounds **1**, **2a** and **2b** (Table 1) cannot help to explain these biological data: from the V values given in Table 1, one may reasonably expect a volume of 161 $Å^3$ (contribution of a methyl = ± 17 $Å^3$, Table 1) for the 5-methyl-2-furyl analogue (as compared to 156.0 $Å^3$ for compound **2a**, Table 1). On the other hand, the different molecular electrostatic potential (MEP) patterns computed for furyl and thienyl analogues may account for their different $GABA_B$ affinities: a MEP minimum (–30 kcal mol^{-1}) is observed in the furyl plane, while the thienyl plane does not show any attractive area.[5] This MEP minimum may prevent furyl analogues favourably interacting with the receptor. It is also noteworthy that the identical Λ values (expressing mainly, in the octan-1-ol water system, identical hydrogen bond acceptor basicities)[22] for **1** and **2a** (Λ = 7.2, Table 1) cannot help to predict these different MEP patterns. Indeed, the most negative potentials which were previously correlated with the hydrogen bond acceptor parameters in a variety of oxygen- and nitrogen-containing molecules,[30] are generated by the same moiety for both compounds **1** and **2a**: the carboxylate group.[5]

Substituting a fused heteroring (benzo[*b*]furan, benzo[*b*]thiophene or quinoline) for a phenyl or thienyl ring leads to an increase in lipophilicity (Table 1). However, this increase is not correlated to any variation in affinity (Fig. 5). In addition, for benzofuran analogues substituted in 5, lipophilicity and affinity do not seem to be correlated. This latter observation contrasts with the interpretation of equation 3. However, the topologies of the HOMOΠs show larger variations among compounds **3b–d** than among baclofen and compounds **3a–d**. Therefore, this electronic effect may account for the discrepancies between benzofuran and thienyl analogues substituted in 5. Moreover, this electronic effect may also explain the very weak $GABA_B$ affinity of compounds **4** and **5**: their HOMOΠs show very different topologies than the one computed for compound **3d**, one of the most potent among the benzofuran analogues (Fig. 4). Indeed, the most important contributions to the HOMOΠs of nonpotent (**4** and **5**) and potent (**3d**) compounds are situated on different atomic centres. On the other hand, the introduction of a methyl group in position 7 of the benzo[*b*]furan ring leads to an increase in $GABA_B$ affinity (Table 1). Although this substituent slightly alters the topology of the HOMOΠ, it may favourably interact with a lipophilic pocket at the receptor site. As a result, the affinity of compound **3e** slightly increases (Table 1).

Lipophilicity measurements and *ab initio* MO STO-3G or STO-3G* computations of electronic properties have enabled us to suggest structure–affinity relationships for baclofen analogues. Both the position (position 5 is favourable, position 4 unfavourable) and the size of the lipophilic substituent are critical for $GABA_B$ affinity of phenyl and thienyl analogues. In other words, the region of the receptor which interacts with the aromatic moiety seems to be sensitive to steric bulk. On the other hand, a different MEP pattern may account for the very weak $GABA_B$ affinity of furyl analogues. For fused heterorings, both an electron-rich heteroatom (stabilization of the GAB chain conformation) and a well-defined HOMOΠ topology (Π–Π interactions) may be mandatory for $GABA_B$ affinity. The latter can be increased by the introduction of a lipophilic substituent in position 7 of the benzo[*b*]furan ring.

Experimental

Measurement of log D

The measurement of CPC retention times (R_t) for the compounds under study has enabled us to determine their log D in octan-1-ol/aqueous buffered systems with pH 7.4. This pH value is close to the isoelectric pH (pI = 6.63 for the most water soluble analogue, compound **1**) where log D reaches a maximum.[14,23] This pI value has been obtained after potentiometric titrations of **1** as previously reported by Avdeef.[31]

After a rough estimation of log D values (log D^{est}) using the ClogP software,[32] we have chosen a 0.01 M 3-morpholinopropanesulphonic acid (MPS) aqueous solution as the mobile phase and octan-1-ol saturated with MPS as the stationary phase. In this case, log D can be obtained from the following equation: $\log D = \log (R_t - t_0)\, U - \log (V_t - U t_0)$; where R_t is the retention time for a solute, t_0 is the dead time measured for an unretained solute (potassium dichromate in this case), U is the flow rate of the mobile phase, and V_t the total capacity of the three columns (385 mL in this case). The design principle of the instrument has been described.[15–18] When the three columns were full, the centrifuge was rotated at a speed of 800 rpm. Then the mobile phase was propelled into the columns. Depending upon the log D^{est} values, the flow rate varied from 0.5 to 3.1 mL min^{-1} (0.5 mL min^{-1} for log $D^{est} \leq -1.9$, 1.6 mL min^{-1} for $-1.9 \leq$ log $D^{est} \leq -1.5$ and 3.1 mL min^{-1} for $-1.3 \leq$ log $D^{est} \leq -0.8$).

Measurements were performed in triplicate on horizontal flow-through centrifugal partition chromatograph made up of three columns and a coil planet type centrifuge (Pharma-Tech Research Corporation, Baltimore, Maryland, U.S.A.). A Kontron model 420 HPLC pump (Kontron Instrument, Zürich) was used to propel the mobile phase and a Kontron model 432-UV visible detector coupled with a Hewlett Packard 3392A (Hewlett Packard, Avondale, Pennsylvania, U.S.A.) integrator to detect the solutes. The solutes were detected at wavelengths varying from 220 to 320 nm according to the nature of the aromatic ring. A flowmeter (Phase Separations, Queensferry, U.K.) was used for precise measurements of flow rates.

Analysis of log D values

In order to put forward potential intramolecular effects affecting log D values of compounds under study, we have estimated their log D using the fragmental approach developed by Rekker.[33] Within this scope, the analysis of molecular volumes (V) and polarity parameters (Λ) also provides an invaluable source of information. The values of V were computed according to Gavezotti,[34] while the Λ parameters were derived as previously described.[22,23] The program MOLSV[35] running on a Silicon Graphics Personal Iris Workstation 4D25 was used for this purpose. Statistical analyses were performed by the QSAR module of SYBYL[36] and the REGMUL2 program.[37]

Quantum theoretical molecular orbitals calculations

The Restricted Hartree Fock Linear Combinations of Atomic Orbitals–Molecular Orbitals–Self Consistent Field (RHF LCAO–MO–SCF) method was used to optimize the molecular geometries, to compute the energies of conformers (compounds **3c** and **5**), the atomic charges (compounds **3c** and **5**), and the topologies of the HOMOΠs (baclofen, compounds **2a–d**, **3b–e**, **4**, and **5**).

For most compounds under study, the crystal structure is unknown. As a result, their 3-D structures were built using both mean geometrical data from the Cambridge Structural Database (CSD)[38] for the aromatic rings, and crystallographic data observed for the GAB chain of other 3-heteroaromatic baclofen analogues.[8,13,39,40] For the hydrogen atoms, only the torsion angles were retained from the X-ray data. The interatomic distances were fixed at 1.09, 1.08 and 1.02 Å depending on the nature and hybridization state of the carrier atom, while the bond angles were fixed at 109.5° and 120.0° according to the hybridization of the carrier atom. Then the geometries of the aromatic rings were optimized using the *ab initio* MO method. Our experience has shown that for this purpose the 3-21G or 3-21G* (when sulfur or chlorine atoms are present) basis[41] is more suitable.[42] At this point, we should also mention that no geometry optimization of the GAB chain was performed in order to avoid the formation of an intramolecular hydrogen bond between the anionic and cationic moieties.[43] Moreover, as the HOMOs are located on the carboxylate groups, the HOMOΠs correspond to the HOMO-3.

Starting from these geometries, we have computed the energies of extended and folded conformations for compounds **3c** and **5** (Table 2) using the *ab initio* MO method. These computations were performed at the STO-3G level of sophistication of the LCAO expansion as introduced by Pople.[44,45] For both compounds, the atomic charges were derived from the MEP. The EPD charges are less basis set dependent than the Mulliken ones and are more suitable to represent the effects of the surrounding atoms.[46–48] These charges were obtained using the Merz-Kollman/Singh scheme as implemented in the GAUSSIAN92 program.[49]

Starting from the *ab initio* MO STO-3G or STO-3G* (when chlorine or sulfur are present) wave functions of baclofen, compounds **2a–d**, **3b–e**, **4** and **5**, the topologies of the HOMOΠs were computed in a parallelepiped surrounding each analogue. The increment was fixed to 0.100 Å in planes parallel to the aromatic planes, to 0.500 Å in planes perpendicular to the aromatic planes.

All these computations were performed by the GAUSSIAN92 program.[50] In each case, the convergence criteria on the density matrix were fixed to 10^{-7} au. Our experience has shown that within the STO-3G or STO-3G* basis set, the computed total energy has at least seven significant digits; it corresponds to a numerical error of about 0.1 kcal mol^{-1}. The EPD charges have four significant digits.

All *ab initio* MO computations were performed on the RISC6000 computer system of the Scientific Computing Facility (SCF) center of the University of Namur.

Molecular graphics

The *iso*-density surfaces were generated by the CMS3D program.[51]

Acknowledgements

The authors would like to thank Dr P. Renard (ADIR, Neuilly/France) for his gift of the compounds and his fruitful scientific collaboration. They would also like to thank the National Belgium Foundation for Scientific Research (FNRS), IBM Belgium and the Facultés Notre-Dame de la Paix (FNDP) for the use of the Namur SCF. Dr B. Pirard is also pleased to acknowledge the ADIR society for financial support. Dr P.-A. Carrupt and Prof. B. Testa acknowledge their support by the Swiss National Science Foundation.

References

1. Krogsgaard-Larsen, P.; Frølund, B.; Jørgensen, F. S.; Schousboe, A. *J. Med. Chem.* **1994**, *37*, 2489.

2. Bowery, N. G. *Ann. Rev. Pharmacol. Toxicol.* **1993**, *33*, 109.

3. Chapman, R. W.; Hey, J. A.; Rizzo, C. A.; Bolser, D. C. *Trends Pharmacol. Sci.* **1993**, *14*, 26.

4. Bittiger, H.; Froestl, W.; Mickel, S. J.; Olpe, H. R. *Trends Pharmacol. Sci.* **1993**, *14*, 391.

5. Pirard, B. PhD Thesis, Facultés Universitaires Notre-Dame de la Paix, Namur, Belgium, 1994.

6. Berthelot, P.; Vaccher, C.; Musadad, A.; Flouquet, N.; Debaert, M.; Luyckx, M. *J. Med. Chem.* **1987**, *30*, 743.

7. Berthelot, P.; Vaccher, C.; Flouquet, N.; Debaert, M.; Luyckx, M.; Brunet C. *J. Med. Chem.* **1991**, **34**, 2557.

8. Berthelot, P.; Vaccher, C.; Flouquet, N.; Luyckx, M.; Brunet, C.; Boulanger, T.; Frippiat, J.-P.; Vercauteren, D. P.; Debaert, M.; Evrard, G.; Durant, F. *Eur. J. Med. Chem.* **1991**, *26*, 395.

9. Renard, P., personal communication.

10. Hammond, D. L.; Moy, M. L. *Eur. J. Pharmacol.* **1992**, *229*, 227.

11. Kerr, D. I. B.; Ong, J.; Johnston, G. A. R.; Berthelot, P.; Debaert, M.; Vaccher, C. *Eur. J. Pharmacol.* **1989**, *164*, 361.

12. Ong, J.; Kerr, D. I. B.; Berthelot, P.; Vaccher, C.; Flouquet, N.; Debaert, M. *Eur. J. Pharmacol.* **1992**, *221*, 145.

13. Pirard, B.; Paquet, B.; Evrard, G.; Berthelot, P.; Vaccher, C.; Ansard, M'H.; Debaert, M.; Durant, F. *Eur. J. Med. Chem.* (in press).

14. Tsai, R.-S.; Testa, B.; El Tayar, N.; Carrupt P.-A. *J. Chem. Soc. Perkin Trans 2* **1991**, 1797.

15. Tsai, R.-S.; Carrupt, P.-A.; Testa, B. *Countercurrent Chromatography*, American Chemical Society Monograph (in press).

16. El Tayar, N.; Tsai, R.-S.; Vallat, P.; Altomare, C.; Testa, B. *J. Chromatogr.* **1991**, *556*, 181.

17. Vallat, P.; El Tayar, N.; Testa, B. *J. Chromatogr.* **1990**, *504*, 411.

18. Berthod, A.; Armstrong, D. W. *J. Liq. Chromatogr.* **1988**, *11*, 547.

19. Tsai, R.-S.; Carrupt, P.-A.; Testa, B.; Gaillard, P.; El Tayar, N. *J. Med. Chem.* **1993**, *36*, 196.

20. Carrupt, P.-A.; Testa, B.; Bechalany, A.; El Tayar, N.; Descas, P.; Perrissoud, D. *J. Med. Chem.* **1991**, *34*, 1272.

21. Walther, B.; Carrupt, P.-A.; El Tayar, N.; Testa, B. *Helv. Chim. Acta* **1989**, *72*, 507.

22. El Tayar, N.; Testa, B.; Carrupt, P.-A. *J. Phys. Chem.* **1992**, *96*, 1455.

23. El Tayar, N.; Tsai, R.-S.; Carrupt, P.-A.; Testa, B. *J. Chem. Soc. Perkin Trans 2* **1992**, 79.

24. Weinstein, H.; Chou, D.; Kang, S.; Johnson, C. L.; Green, J. P. *Int. J. Quantum Chem. Quantum Biology Symposium* **1976**, *3*, 135.

25. Andrews, P. *Trends Pharmacol. Sci.* **1986**, *7*, 148.

26. Blaney, J. M.; Dixon, J. S. *Perspectives in Drug Discovery and Design* **1993**, *1*, 301.

27. Moureau, F.; Wouters, J.; Vercauteren, D. P.; Collin, S.; Evrard, G.; Durant, F.; Ducrey, F.; Koenig, J. J.; Jarreau, F. X. *Eur. J. Med. Chem.* **1994**, *29*, 269.

28. Bowery, N. G. *Trends Pharmacol. Sci.* **1982**, *3*, 403.

29. Debaert, M., personal communication.

30. Politzer, P.; Murray, J. S. In *Reviews in Computational Chemistry*; Lipkowitz, K. B.; Boyd, D. B., Eds; VCH: Weinheim, 1991; Vol. 2, pp. 273–312.

31. Avdeef, A. *Quant. Struct–Act. Relat.* **1992**, *11*, 510.

32. Hansch, C.; Leo, A. MEDCHEM Project version 3.54, Pomona College; Claremont, California.

33. Rekker, R. F.; Mannhold R. *Calculation of Drug Lipophilicity. The Hydrophobic Fragmental Approach* VCH: Weinheim, 1992.

34. Gavezzotti, A. *J. Am. Chem. Soc.* **1983**, *105*, 5220.

35. MOLSV, Quantum Chem. Progr. Exchange, 509.

36. SYBYL 6.0, Tripos Assoc.; St. Louis, Missouri.

37. Collin, S.; Vercauteren, D. P. REGMUL2, Facultés Universitaires Notre-Dame de la Paix; Namur, Belgium, 1986.

38. Allen, F. H.; Kennard, O. *Chemical Design Automation News* **1993**, *8*, 31.

39. Pirard, B.; Evrard, G.; Norberg, B.; Berthelot, P.; Vaccher, C.; Debaert, M.; Vercauteren, D. P. ; Durant, F. *J. Cryst. Spec. Res.* **1993**, *23*, 843.

40. Pirard, B.; Evrard, G.; Norberg, B.; Berthelot, P.; Vaccher, C.; Debaert, M.; Durant, F. *Bull. Soc. Chim. Belg.* **1993**, *102*, 639.

41. Binkley, J. S.; Pople, J. A.; Hehre, W. J. *J. Am. Chem. Soc.* **1980**, *102*, 939.

42. Moureau, F. PhD Thesis, Facultés Universitaires Notre-Dame de la Paix, Namur, Belgium, 1992.

43. Sapse, A. M.; Strauss, R. *Int. J. Quantum Chem.* **1990**, *37*, 197.

44. Hehre, W. J.; Stewart, R. F.; Pople, J. A. *J. Chem. Phys.* **1969**, *51*, 2657.

45. Pople, J. A. In *Applications of Electronic Structure Theory, Modern Theoretical Chemistry Series*; Schaefer, H. F., Ed; Plenum Press: New York, 1977; Vol. 4, pp. 1–27.

46. Williams, D. E. In *Reviews in Computational Chemistry*; Lipkowitz, K. B.; Boyd, D. B., Eds; VCH: Weinheim, 1991; Vol. 2, pp. 219-271.

47. Singh, U. C.; Kollman, P. A. *J. Comp. Chem.* **1984**, *5*, 129.

48. Cox, S. R.; Williams, D. E. *J. Comp. Chem.* **1981**, *2*, 304.

49. Foresman, J. B.; Frisch, A. *Exploring Chemistry with Electronic Structure Methods: A Guide to Using Gaussian*; GAUSSIAN Inc., Pittsburgh, Pennsylvania, 1993.

50. Frisch, M. J.; Trucks, G. W.; Heard-Gordon, M.; Gill, P. M W.; Wong, M. W.; Foresman, J. B.; Johnson, B. G.; Schlegel, H. B.; Robb, A. M.; Replogle, E. S.; Gomperts, R.; Anders, J. L.; Raghavachari, K.; Binkley, J. S.; Gonzales, C.; Martin, R. L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople, J. A. GAUSSIAN92, GAUSSIAN Inc.: Pittsburgh, PA, 1992.

51. Baudoux, G.; Dory, M.; Vercauteren, D. P. CMS3D. Contour Monitoring System for Three-Dimensional Data, Rel. 1, Facultés Universitaires Notre-Dame de la Paix; Namur, Belgium, 1993.

(*Received in U.S.A.* 15 *May* 1995; *accepted* 23 *August* 1995)