Pergamon

Bioorganic & Medicinal Chemistry, Vol. 3, No. 11, pp. 1547–1561, 1995
Copyright © 1995 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0968-0896/95 $9.50 + .00

0968-0896(95)00135-2

A Behaviorally Selective Class of Thiophene-containing Benzodiazepine Receptor Ligands

Laura T. Schove,[a]*† Shu-Wen Chen,[a] Mark Beatty,[b] Patricia A. Maguire,[a] M. Frances Davies[a] and Gilda H. Loew[a]

[a]*Molecular Research Institute, 845 Page Mill Road, Palo Alto, CA 94304, U.S.A.*
[b]*School of Pharmacy and Pharmacology, University of Bath, Bath BA2 7AY, U.K.*

Abstract—In a continued effort to probe the role of the aromatic rings in classical 1,4-benzodiazepine (BDZ) ligand pharmacology, a series of new thiophene-containing benzodiazepine receptor (BDZR) ligands were synthesized. As a first step in determining the binding profile and selectivity to BDZR functional subtypes, the affinities in two central nervous system (CNS) regions, cerebellum, in which a single 'Type I' BDZR could be labeled; and spinal cord, in which we have previously demonstrated some receptor heterogeneity, were determined. These compounds were also assessed for their compliance with a recently developed three dimensional pharmacophore for recognition and activation of the 'Type I' BDZR, using the techniques of computational chemistry. The computations showed all ligands synthesized fulfilled the minimum requirements for recognition, further validating the current pharmacophore. Using the criteria for activation, the new ligands were all predicted to be agonists at the cerebellar 'Type I' BDZR. Since the compounds showed reasonable affinity, the behavioral profile of one of them at five *in vivo* endpoints was determined. This compound demonstrated more behavioral selectivity than the typical 1,4-BDZ ligand. While they fulfilled the requirements for agonist activity at the 'Type I' BDZR, these ligands showed significantly greater delocalization in the electron density distribution in the lowest unoccupied molecular orbital (LUMO), so that either aromatic ring could serve as an electron accepting site, not just the one comparable to the more classical BDZR agonist, flunitrazepam. It is possible that the ability of the second ring in the tested compound (**5a**) to also function as an electron acceptor can affect the recognition and activation of other receptor types leading to the more discriminate behavioral profile of this thiophene analog compared to flunitrazepam.

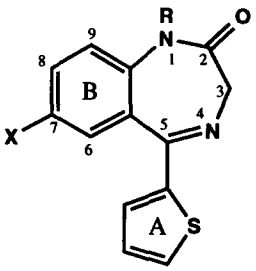
Introduction

The benzodiazepines constitute a class of psychoactive drugs which are widely prescribed. A large number of ligands from diverse chemical families have been shown to bind with high affinity to the $GABA_A$/BDZ receptor(s).[1] BDZR ligands modulate the activity of $GABA_A$ on this chloride ion channel receptor.[1a,c] The BDZR ligands have been categorized as agonists, antagonists, and inverse agonists, depending on whether they enhance, have no effect, or diminish the effect of GABA on this channel. This categorization has also been used to describe the BDZR ligands activity in diverse pharmacological endpoints. Ligands labeled agonists at a given behavioral endpoint are those with similar *in vivo* activities to the prototypical 1,4-BDZ drugs, such as flunitrazepam, exhibiting anxiolytic, anticonvulsant, hyperphagic, muscle relaxant, and sedative/hypnotic activity. Inverse agonists cause opposite behavioral effects, such as anxiogenesis, proconvulsant, anorectic and procoginitive actions. Antagonists are able to inhibit the action of both agonists and inverse agonists.[2]

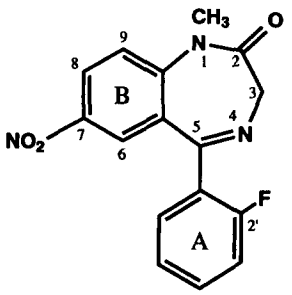
In early studies of the benzodiazepines, Sternbach and coworkers[3] described the fundamental structure–activity relationship (SAR) of the classical BDZR ligands. They found the presence of the seven-membered imino-lactam ring to be essential, with substitution favored in the 1, 3, 7, and 2' positions (labeled for flunitrazepam in Fig. 1). The importance of the imine nitrogen and carbonyl oxygen was also established and indicated potential proton accepting interactions with the BDZR(s). Following this early study, many laboratories have performed quantitative structure–activity relationship (QSAR) studies.[4] Specifically, Haefely and coworkers[5] have demonstrated the importance of the rotatable phenyl ring (ring A, Fig. 1 for flunitrazepam) in position 5 of the seven-membered lactam ring. Replacing the ring with a methyl group resulted in a complete loss of affinity as measured by inhibition of [^{3}H]diazepam binding in rat whole brain. However, saturation or replacement with a 2-pyridine ring maintains significant affinity for the BDZRs, although approximately four-fold lower than the classical BDZR ligand flunitrazepam. Cook and colleagues have recently reported that replacement of this ring with a thiophene ring in an analog with a 7-Cl substituent has little or no effect on affinity in whole brain binding[6] compared to typical 1,4-BDZ ligands.

Both proton accepting sites and aromatic rings are common features of most pharmacophore models of

†Current address: Immunopharmaceutics, Inc., 11011 Via Frontera, San Diego, CA 92127, U.S.A.



X = H, R = H **5a**
X = H, R = CH_3 **5b**
X = CH_3, R = H **5c**
X = CH_3, R = CH_3 **5d**



Flunitrazepam

Figure 1. Thiophene ligands synthesized and studied, along with fluntirazepam, a classical BDZR agonist.

BDZR ligands reported in the literature. In a model by Codding and Muir, one proton accepting site and one moiety involved in a π–π stacking complex with the receptor was invoked.[7] In another study, Fryer *et al.*[8] proposed that the type of activity elicited by the BDZR ligands could be determined by the distance between a proton-accepting group and a π aromatic ring. Tebib and Bourguignon[9] have developed a pharmacophore using crystallographic data and molecular mechanics calculations and found six 'critical zones' of the BDZR ligands: four which modify recognition and two others which modify activation, including two electron-rich zones corresponding to proton accepting centers.

In previous work in our laboratory, a model was developed which includes the minimal requirements for recognition and activation of non-selective ligands based on a single functional BDZR model.[10] *In vivo* convulsant profiles and receptor affinities measured by inhibition of [^{3}H]Ro15-1788 in rat whole brain were used to characterize 15 compounds, including three 'classical' benzodiazepine agonists; flunitrazepam, diazepam, and prazepam; which were all found to have significant affinity and anticonvulsant activity, as measured by the ability to prevent clonic convulsions.

While most of the pharmacophores developed for the BDZ receptor ligands were based on the assumption of a single receptor site, there is now robust evidence for more than one functional BDZR.[4d,5] The $GABA_A$/BDZ receptor system is now known to be composed of various combinations of five membrane spanning subunits (α, β, γ, δ, and others) with different subunit combinations resulting in the possibility of many functionally distinct receptor subtypes.[1a,2a–d] To date, little is known about the specific subunit composition, the number of functionally distinct central benzodiazepine receptors in different central nervous system brain regions, and their correlation to physiological effects. However, among the many possible BDZ/$GABA_A$ receptor subtypes, the central cerebellar ('Type I') BDZR is the most promising one for characterization, due to the fact that cerebellum tissue can be used to determine receptor affinities and activation at this 'Type I' site. In addition, there are also 'Type I' selective ligands, xolpidem and alpidem and the less selective ICI 190,622 and 2-oxoquazepam which can be useful in determining molecular requirements for recognition. Because of the radiolabeled ligand Ro15-1788 labels only one receptor subtype in this tissue, it can be used to probe the pharmacology of this receptor.[11]

The goal of the current study is to continue to investigate the role of the rotatable A ring (Fig. 1) in 1,4-BDZ ligands. Specifically, we wished to further investigate whether changing the phenyl ring to a thiophene ring in a series of thiophene compounds (**5a–d**) with no polar A or B ring substituents leads to any qualitative differences in binding or behavior, as compared to the classical 1,4-BDZR ligand, flunitrazepam (Flu). To this end, we have synthesized these four new sulfur-containing BDZR ligands and have measured the binding affinity in two CNS regions, cerebellum and spinal cord, as a first step in probing binding of these ligands to more than one BDZR subtype. Previous work has determined cerebellar membrane to have one functional BDZR, whereas the spinal cord appears to have three additional receptor subtypes.[12] In a parallel effort, we have also calculated the conformational, electronic, and physical properties of these analogs to determine to what extent they satisfied our current three dimensional pharmacophore requirements for recognition and activation of the 'Type I' BDZ receptor. Finally, since reasonable affinity was obtained for these ligands, a complete pharmacological profile at five *in vivo* endpoints was determined for ligand **5a**. These results showed a surprising increase in behavioral selectivity for the thiophene ligand as compared to the more classical BDZR ligand, flunitrazepam.

Chemistry and Pharmacology

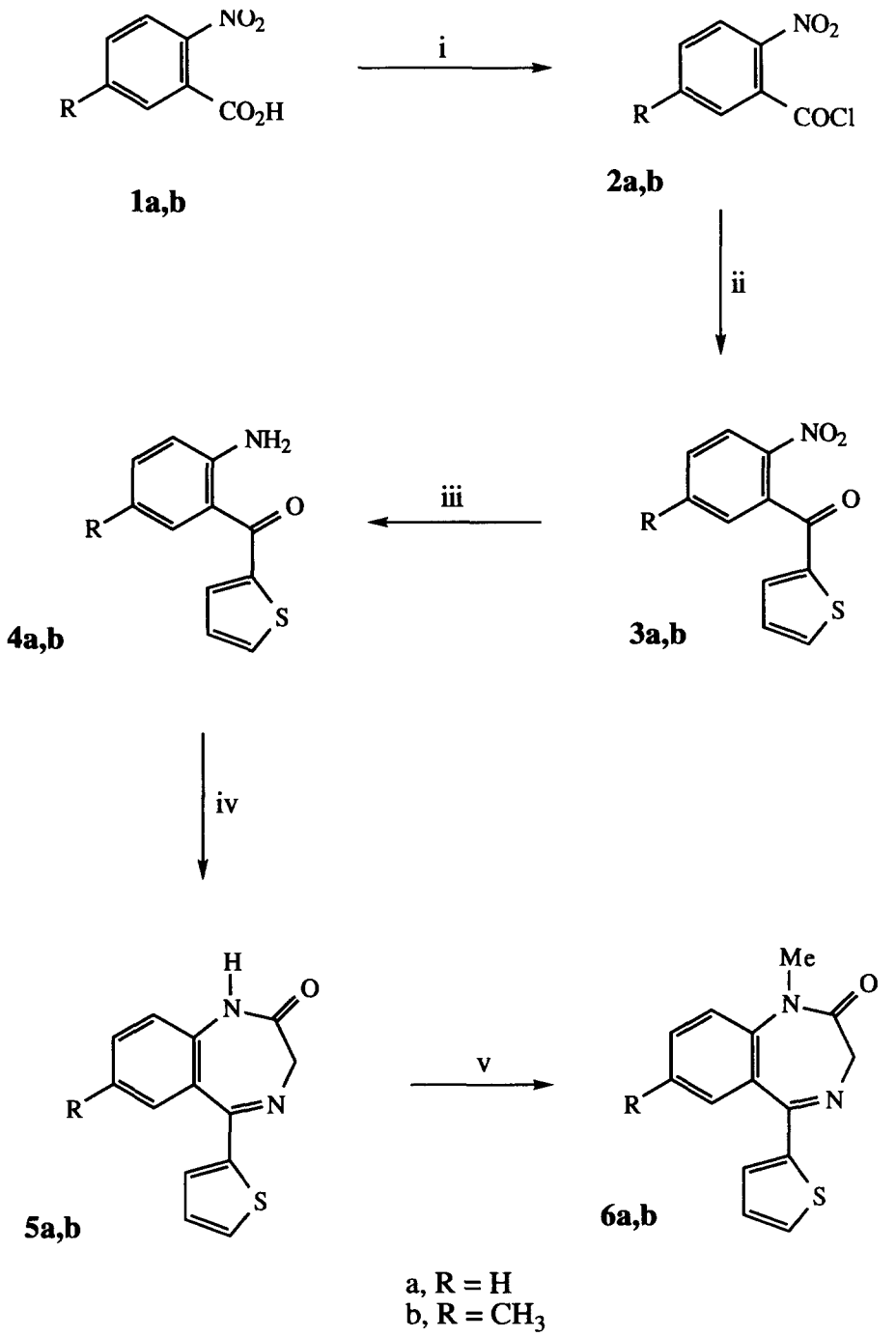
Chemistry

Target compounds **5a,b** and **c,d** were prepared from the appropriate 2-nitrobenzoic acids **1a,b** as depicted in Schemes 1 and 2. Reaction of the acids **1a,b** with thionyl chloride gave the acid chlorides **2a,b**. Friedel–Crafts

acylation of **2a,b** with thiophene gave the ketones **3a,b**; which were reduced with a 15 wt% aq. titanium trichloride solution containing 15 wt% hydrogen chloride to afford the amines **4a,b**. Condensation of amines **4a,b** with glycine ethyl ester hydrochloride gave the target compounds **5a,b**. Methylation of **5a,b** using sodium methoxide and methyl iodide afforded the target compounds **5c,d**. The spectra and physical data for these compounds are presented in Table 1.

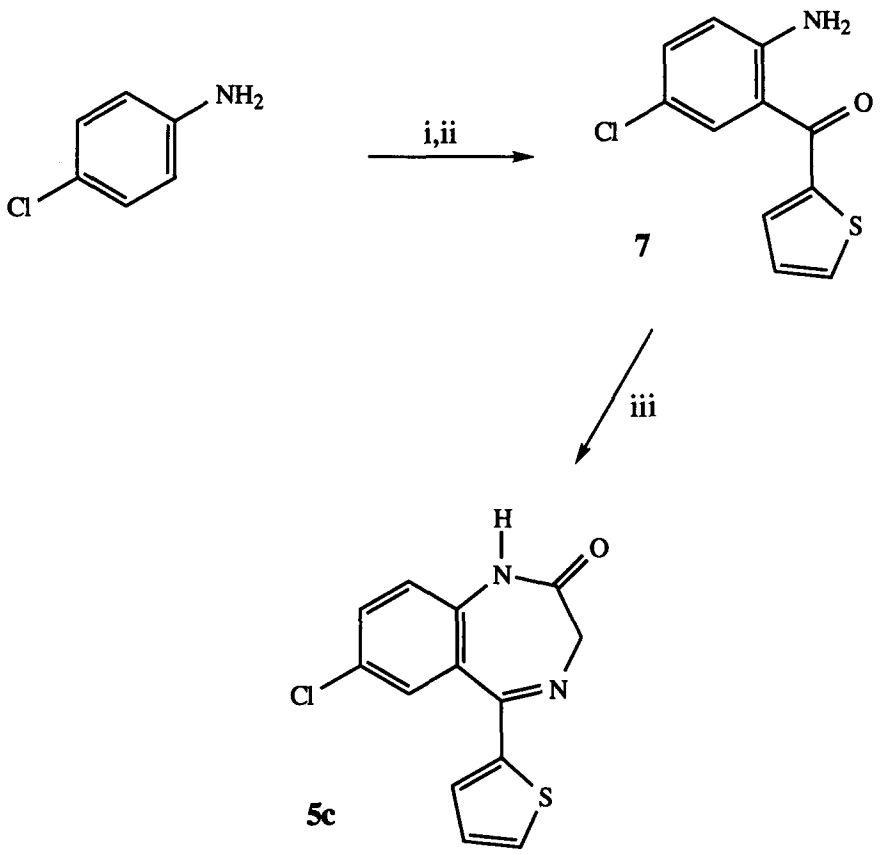
Binding studies

Frozen rat cerebella or spinal cord (Pel Freeze, Rogers, AR, U.S.A.) were homogenized in 50 mM Tris–HCl, pH 7.7 at 0 °C and centrifuged at 20,000 *g* for 10 min.[12] The



i, $SOCl_2$, reflux; ii, thiophene, $AlCl_3$; iii, 15% aq $TiCl_3$, toluene; iv, Gly-OEt·HCl, pyridine, reflux; v, a. NaOMe, MeOH, b. MeI, DMF, 5 °C.

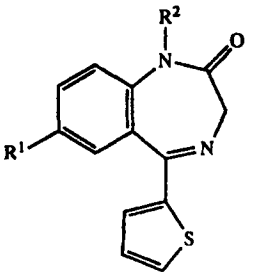
Scheme 1.



i, 2-Thiophenecarbonyl chloride, $ZnCl_2$, 180 °C; ii, 75% H_2SO_4, reflux; iii, Gly-OEt·HCl, pyridine, reflux.

Scheme 2.

Table 1. Spectral and physical data for thienyl benzodiazepines



Compound	R^1	R^2	^1H NMR signals[a]	mp, °C[b]	Overall % yield	MS (M^+)	formula[c]
5a	H	H	3.5–5.0, *br m*, 2H; 7.0–7.5, *m*, 7H; 9.0, *s*, 1 H	191–192.5	18[d]	243 (FAB)	$C_{13}H_{10}N_2OS$
5b	CH_3	H	2.39, *s*, 3H; 3.5–5.0, *br m*, 2H; 7.0–7.5, *m*, 6H; 8.98, *s*, 1 H	198–200	14[d]	257 (FAB)	$C_{14}H_{12}N_2OS$
5c	H	CH_3	3.39, *s*, 3H; 3.78, *d*, J = 10.8 Hz, 7.0–7.7, *m*, 7H	110–112	12[e]	256 (low EI)	$C_{14}H_{12}N_2OS$
5d	CH_3	CH_3	2.40, *s*, 3H; 3.36, *s*, 3H; 3.77, *d*, J = 10.8 Hz, 1H; 4.68, *d*, J = 10.8 Hz, 1H; 87.0–7.6, *m*, 6H	glass	9[e]	270 (low EI)	$C_{15}H_{14}N_2OS$

[a]Spectra were recorded in $CDCl_3$ with TMS as reference.
[b]All compounds were recrystallized from EtOH.
[c]All compounds were analyzed for C, H, and N and all results were within 0.40 of theoretical values.
[d]Four-step synthesis.
[e]Five-step synthesis.

pellet was rehomogenized and centrifuged twice, frozen, thawed and washed an additional two times. The binding assay method was performed as previously described.[12,13] Briefly, membranes (1 mL, 4.5 mg wet weight of cerebellar tissue, and 10 mg wet weight of spinal cord) were incubated in triplicate with 0.5 nM [^{3}H]Ro15-1788 and eight concentrations of competing ligand in total volume of 2 mL for 90 min. Nonspecific binding was determined in the presence of 1 μM Ro15-1788. The reaction was terminated by filtration (Whatman GF/B) followed by three 5 ml washes with ice-cold buffer. Radioactivity retained on the filters was determined by liquid scintillation with ECOLITE(+) (ICN) after 12 h at room temperature. K_i values from cerebellum data were calculated using the Cheng–Prusoff equation.[14] For spinal cord assays, only the IC_{50} was calculated because of extensive receptor heterogeneity in that tissue.[13]

Behavioral studies

Ligand **5a** was tested in five *in vivo* endpoints with full dose–response curves, using the procedures previously reported[15] and used for flunitrazepam. The behavioral results were analyzed with one-way ANOVA using the Statview® program, the treatment effect was separated using Dunnett-t's test. Ligand **5a** was also characterized for antagonism, determined as the ability of 10 mg kg^{-1} of ligand **5a** to reverse the effect of 1–2 mg kg^{-1} of Flu in the endpoints in which ligand **5a** was inactive. Flunitrazepam was given 15 min before ligand **5a**, the behavior tests were conducted 15 min after the animal was given ligand **5a**.

Computational methods

The initial structures of the ligands were generated interactively using the ChemNote program as implemented in the Quanta 3.3 package (Polygen Corp., Waltham, MA, U.S.A.). The structures were then minimized with the CHARMm force field 2.2[16] using the steepest descent method until a convergency of 0.001 Å on the rms force. CHARMm minimized structures were used as starting points for the semiempirical quantum mechanics AM1 method using the PRECISE key as implemented in the MOPAC 5.0 package.[17] Minima in the conformations of the rotatable aromatic rings and the other substituents were obtained by nested rotations in 60 degree increments using AM1. The AM1 optimized geometries were used to calculate all thermodynamic and electronic properties reported.

Electronic properties considered to be modulators of receptor recognition and/or activation were calculated using the AM1 hamiltonian for all the compounds in their lowest energy conformations. All the calculations were carried out using the MOPAC 5.0 package. The properties calculated include dipole moments, calculated as the expectation value of the corresponding operator; mean molecular polarizabilities, evaluated by an additive scheme using the parameters proposed by Fraga;[18] and proton affinities of the carbonyl oxygen and imine nitrogen as measures of proton accepting ability.

Subsequent analysis was carried out using the in-house *Grapha* toolkit.[19] *Grapha* makes use of the MOPAC output to calculate useful molecular descriptors or to generate grids that can be displayed on the solvent accessible (or Connolly) surface using the ATHAY software embedded in *InsightII* (Biosym Corp., San Diego, CA, U.S.A.).

Properties related to the hydrophobic interactions. These type of interactions were evaluated using an atom-based, conformational-dependent hydrophobic index developed in this laboratory and described elsewhere.[20] The procedure provides a list of atomic contributions based on the donation of each atom to the total van der Waals area and its charge derived from the Mulliken population analysis. The total hydrophobic index, calculated as the sum of all atomic contributions is directly comparable to the logarithm of the octanol/water partition coefficients (log *P*). The index is used in two ways: to identify the most lipophilic sites in the ligands and to obtain a distribution of the lipophilicity on the solvent-accessible surface of the ligands. This latter calculation was carried out using a program developed in our laboratory called *Grapha* that utilizes the same distance dependence of hydrophobicity proposed by Audry *et al.*[21] The program calculates the hydrophobicity at each point on a preselected grid and also at points on the van der Waals and solvent accessible surfaces by implementation of a 'Molecular Lipophilicity Potential' procedure as described by Brickmann *et al.*[22] Using the ATHAY routine in *InsightII*, a color scale corresponding to a range of hydrophobic values from the highest value of 0.2 (red) to the lowest of –0.4 (blue) with intermediate values linearly interpolated between blue and red was established and used for all ligands. By keeping the scale the same, comparisons of the ligands can be made systematically.

HOMO/LUMO distribution. The energies of and electronic density distributions in the highest molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) were used as indicators of the ability of the ligand to act as an electron acceptor or donor respectively in an electron transfer interaction with the receptor. Frontier electron densities on each atom were calculated from the sum of the squares of the orbital coefficients for HOMO and LUMO, providing a measure of the extent of delocalization of the electron accepting and donating centers. These densities were multiplied by two to represent the maximum values corresponding to double occupancy of both HOMO and LUMO and projected onto the solvent accessible surface using the *Grapha* tool-kit and ATHAY/*InsightII* with the color scheme corresponding to the range of calculated densities from 0.00 represented by blue to a maximum value of 0.15 represented by red for all ligands.

Free energies of solvation. In order to ascertain the importance of solvation/desolvation in the process of binding, the free energies of solvation in aqueous solution of the different ligands were calculated using the AM1–SM2 parameterization of the AMSOL 3.0 program,[23] with their geometries frozen in the optimized forms obtained *in vacuo*.

Results and Discussion

As shown in Table 2, compounds **5a–d** display moderate affinity for the BDZRs in cerebellum and spinal cord, markedly lower than the classical BDZR ligand flunitrazepam.[13] The K_i reported for the cerebellar receptor represents the affinity for the 'Type I' BDZR, whereas the IC_{50} in spinal cord represents a composite affinity of at least three binding sites in that tissue, as previously determined in our laboratory.[12] In addition, each ligand fully displaced [^{3}H]Ro15-1788 in the spinal cord membranes, suggesting that in addition to the cerebellar 'Type I' BDZR, ligands **5a–d** also bind to all three binding sites in the spinal cord. There are no significant differences in binding to cerebellum and spinal cord among the five ligands (**5a–d**) and no inference can be made about the selectivity of these ligands for BDZRs in these regions.

Having found significant binding of all the compounds to the 'Type I' cerebellar BDZR, the question remained as to whether this finding is consistent with the current pharmacophore for recognition of this receptor (Fig. 2), that we have recently developed.[24] To address this question, we have determined to what extent they fulfill the minimum requirements for recognition of this receptor. We see from Figure 2 that the minimum requirements for recognition include the presence of at least two of three proton accepting centers and the most lipophilic aromatic ring of the ligand in a specific spatial relationship to interact with complimentary moieties in the receptor. The geometric requirements for recognition have been defined in terms of these complementary receptor sites namely three proton donating receptor sites, RP1, RP2, RP3, and a lipophilic aromatic region interacting with the most lipophilic ring of the ligand as determined by the calculated atom-based hydrophobic index.

Table 2. Binding affinities in cerebellum and spinal cord

Ligand	K_i cerebellum (nM)	IC_{50} spinal cord (nM)
Flunitrazepam[a]	1.5	2.4
5a	177	350
5b	97	110
5c	105	210
5d	87	120

[a]From Ref. 13.

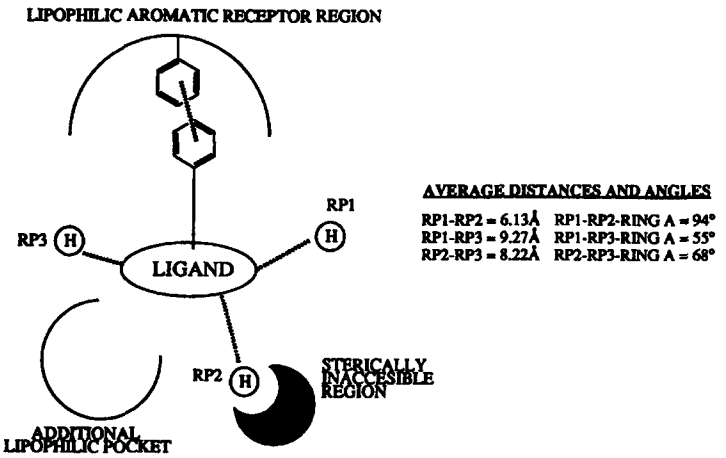


Figure 2. Summary of the current pharmacophore developed for recognition and activation of the cerebellar or 'Type I' BDZR.

Table 3. Heats of protonation and hydrophobicity for the ligands studied

Ligand	ΔH_f (N) kcal mol^{-1}	ΔH_f (O) kcal mol^{-1}	ΔH_f (NO_2) kcal mol^{-1}	Hydrophobic Index Total	Ring A	Ring B
Flu	149.5	167.3	174.2	0.5	1.6	1.9
5a	150.3	164.9	–	2.6	1.8	2.3
5b	151.1	166.3	–	2.9	1.6	2.3
5c	150.8	165.0	–	2.9	1.7	2.4
5d	150.5	165.4	–	3.2	1.6	2.4

Given in Table 3 are the calculated electronic and physical properties already identified as relevant to ligand recognition of the 'Type I' BDZR. Specifically, these are the proton accepting capabilities of the three most favorable proton accepting moieties found in each analog and the calculated values of total hydrophobicity and local values associated with rings A and B. Ligands **5a–d** have only two possible proton accepting centers, the imine nitrogen and carbonyl oxygen, while flunitrazepam has additional sites (NO_2 and F). The hydrophobic index shows that ligands **5a–d** are more hydrophobic than flunitrazepam and that ring A is the most hydrophilic both in the new ligands and in flunitrazepam, in which it was the ring used for recognition in our current pharmacophore model. Figure 3 shows the hydrophobic field displayed on the solvent accessible surface for ligands **5a** and flunitrazepam. This figure shows that, by this criterion as well, the lipophilic ring A is maintained in both ligands, even though there is a thiophene ring substituted for the phenyl ring in ligand **5a**. Both ligands also contain hydrophilic regions corresponding to the imine nitrogen and carbonyl oxygen of the benzodiazepine ring, which are proposed to interact with RP1 and RP2 in our current pharmacophore for recognition. The only significant difference seen in Figure 3 is the hydrophilic region in flunitrazepam seen around the 7-substituted nitro group, which is proposed to interact with RP3 in the pharmacophore. There is no corresponding region for ligand **5a**, indicating no possible interaction with RP3 for this class of ligands.

Given in Table 4 are the distances and angles between the corresponding proton donating receptor points and the lipophilic ring of the receptor proposed as determinants of recognition. These values can be compared with the range of values proposed as criteria for the 'Type I' pharmacophore, also given in this table.

As can be seen from Tables 3 and 4, all of the analogs studied fulfill the minimum criteria for the pharmacophore for recognition through RP1 and RP2 and the lipophilic ring A. However, ligands **5a–d** have only two of the three proton accepting sites together with the lipophilic ring in the correct orientation for interaction with the receptor. The third proton accepting site is not present in these ligands. By contrast, the third proton accepting site in flunitrazepam is positioned to interact with RP3, a supplemental recognition site in the current pharmacophore. This difference between the two families is illustrated in Figure 4, showing the superimposition of flunitrazepam and the thiophene ligand **5a**.

Candidate electronic properties considered as additional modulators of receptor recognition were dipole moment and mean molecular polarizabilities, measures of the extent of electrostatic and van der Waals interactions with the receptor, respectively. In addition, free energies of solvation were also calculated as indicators of the entropic contributions to receptor affinities. The results, given in Table 5, show that the dipole moment is larger in ligands **5a–d** than in flunitrazepam. However, the values calculated for these new ligands are in the range previously found for both high and low affinity ligands used for development and validation of the 'Type I' pharmacophore.[24] This result indicates that the magnitude of the dipole moment does not appear to modulate the 'Type I' receptor recognition observed for this class of compounds. The magnitude of the negative free energy of solvation, however, did show significant increase in the thiophene ligands, indicating a larger free energy of desolvation penalty for these analogs. If the free energy of binding of both types of ligands to the receptor were the same, then the greater the absolute value of the negative free energy of solvation the lower the affinity of the ligands would be. Since the relationship found is in this direction (i.e. the lower affinity compounds have the larger absolute values of negative free energies) it can be inferred that the greater desolvation energy of the thiophene compounds could be one factor in their lower receptor affinities. In a recent study,[25] however, we have reported that for a different series of BDZR ligands,

Table 4. Distance and angle criteria according to the pharmacophore for the 'type I' BDZR for the ligands studied

Ligand	RP1–RP2 (Å)	RP1–RP2–RING A (°)	RP1–RP3 (Å)	RP1–RP3–RING A (°)
CRITERIA	**5.4-7.7**	**81.117**	**8.6-11.0**	**45-65**
Flunitrazepam	5.6	116.5	10.0	59.4
5a	5.4	115.6	–	–
5b	5.5	120.0	–	–
5c	5.5	117.7	–	–
5d	5.4	118.3	–	–

Table 5. Dipole moment, free energies of solvation, and polarizabilities for the ligands studied.

Ligand	Dipole Debye	ΔG_{sol} kcal mol^{-1}	Polarizability Total	Ring A	Ring B
Flu	3.1	–11.3	42.8	13.9	13.8
5a	4.8	–19.5	39.5	14.5	13.3
5b	5.1	–19.1	42.4	14.5	13.9
5c	4.8	–24.4	42.3	14.5	13.3
5d	5.1	–17.7	45.1	14.5	14.3

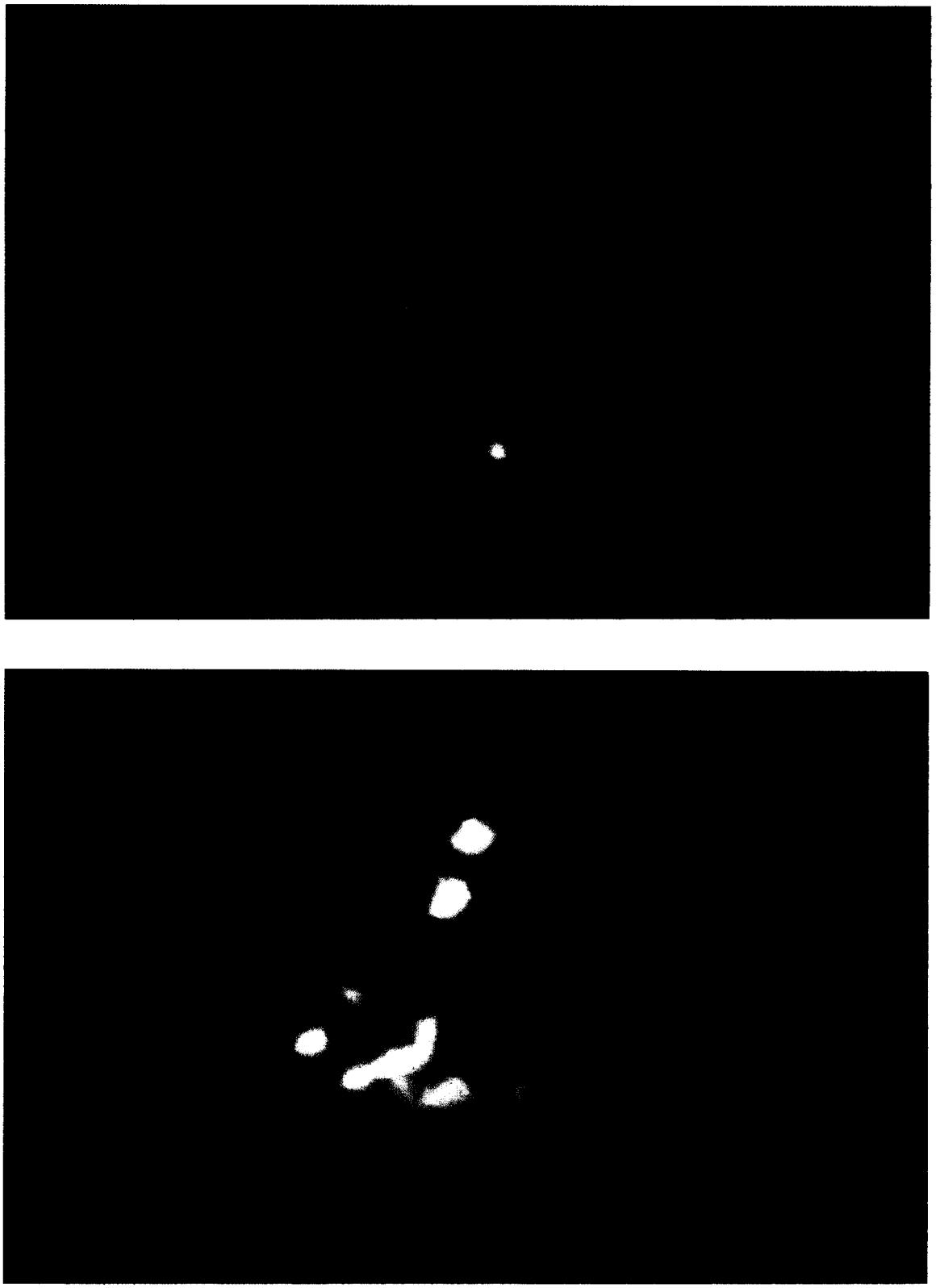


Figure 3. Hydrophobic field displayed on the Connolly surface for flunitrazepam and ligand **5a**. The scale is blue–red with blue indicating the hydrophilic regions of the ligands. Values ranged from –0.4 (blue) to 0.2 (red).

pyrazoloquininolinone compounds, the free energy of solvation differences did not appear to be a discriminant of relative affinities. Thus the importance of the free energy of solvation as a determinant of recognition appears to be different for different classes of 'Type I' BDZR ligands.

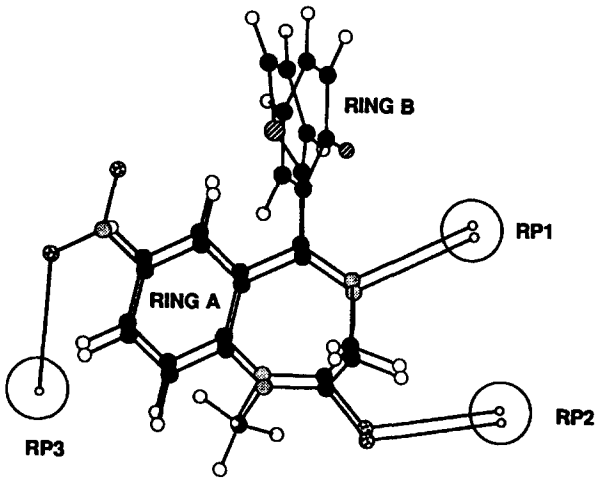


Figure 4. Overlay of ligand **5a** and flunitrazepam so that the minimum requirements for recognition as determined by the current 'Type I' pharmacophore are met.

Since these thiophene ligands displayed reasonable affinity for BDZRs, a complete pharmacological profile for one of them, the parent ligand **5a** was determined. The results obtained for this compound and, for comparison, with flunitrazepam, are shown in Figure 5 and Table 6.

Behavioral effects of ligand 5a. As shown in Figure 5, in the anxiolytic test, both ligand **5a** [F (4, 25) = 3.649; P = 0.0179] and flunitrazepam significantly increased the time spent in the open arms but had no effect on number of entries into the open or closed arms, or central platform, or time spent in the closed arms (data not shown) as measured in elevated plus maze. Ligand **5a** shows no apparent dose response at this endpoint, reaching maximum effect at a dose of 1 mg kg^{-1}. As shown in Figure 5, compared to Flu, ligand **5a** had no activity in sedation [F (4, 25) = 0.989; P = 0.4316], hypothermia [F (4, 25) = 1.739; P = 0.173], hyperphagia [F (4, 25) = 1.156; P = 0.3536] and had very little effect protecting rats from PTZ induced seizures. Ligand **5a** was further assessed by testing this compound for antagonist activity at the three endpoints for which it showed no activity; sedation, hypothermia, and hyperphagia. Table 6 shows that 10 mg kg^{-1} of ligand **5a** was unable to reverse any effect induced by low doses of Flu (1–2 mg kg^{-1}). However, ligand **5a** did increase the effect of Flu in hypothermia, suggesting the compound either has weak agonist activity or no effect at all.

A surprising result found from the behavior studies was the unusual profile for ligand **5a**. Namely, this ligand appears to have robust activity at only one of the five endpoints measured, the anxiolytic endpoint, where its activity is comparable to flunitrazepam. These results indicate that either ligand **5a** binds with high affinity to BDZR subtypes (other than cerebellum or spinal cord) that modulate anxiolytic behavior; or that it has a greater efficacy than flunitrazepam in activating the BDZR that modulates anxiolytic activity. For the other four endpoints; hyperphagia, sedation, anticonvulsion, and hypothermia; flunitrazepam is a potent agonist, while ligand **5a** has weak agonist activity or no effect.

The pharmacological profile obtained for ligand **5a** indicates that thus far it is an agonist or has no effect at all on receptors to which it binds, although with very different relative potencies. We, therefore, compared this result with our prediction for activity at the 'Type I' BDZR using the activation criteria embedded in our current 'Type I' pharmacophore.[24] This pharmacophore indicates that activation occurs through an electron transfer mechanism, with the position of the electron accepting aromatic ring acting as a discriminant for agonism, inverse agonism, and antagonism. Specifically, the requirement for activation given in our current pharmacophore, deduced from comparisons of agonist, antagonist, and inverse agonist properties, is the presence of an electron accepting aromatic ring in a specific geometric arrangement with respect to the components of recognition. Since receptor points RP1 and the lipophilic ring are proposed as universal requirements for recognition of the cerebellar BDZ receptor by agonists, antagonists and inverse agonists,

Table 6. Tests for antagonism of ligand 5a

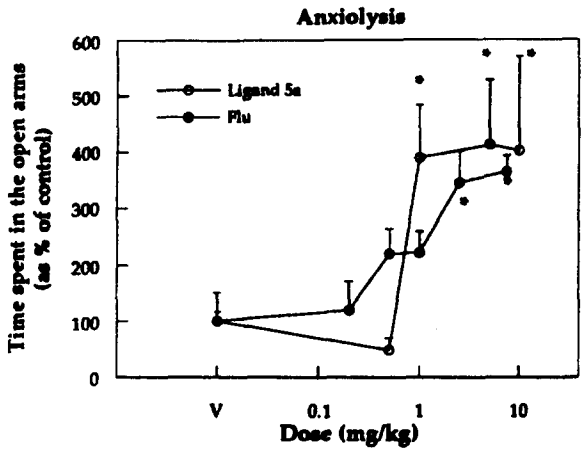
	V + V	Flu + V	V + Ligand **5a**	Flu + Ligand 5a
Sedation:				
Locomotor activity	135 ± 24	62.5[a] ± 13.7	145.7 ± 20.7	59.3[a] ± 12.8
Hypothermia:				
Change in temp.(°C)				
Temp (30)–Temp (0)	0.47 ± .09	–0.77 ± 0.30	0.10 ± 0.40	–1.40[a] ± 0.20
Temp (45)–Temp (0)	0.37 ± 0.18	–0.90 ± 0.33	0.43 ± 0.37	–1.73[a] ± 0.31
Hyperphagia:				
Food intake (g)	3.3 ± 0.52	5.8[a] ± 0.72	3.2 ± 0.43	5.2[a] ± 0.92

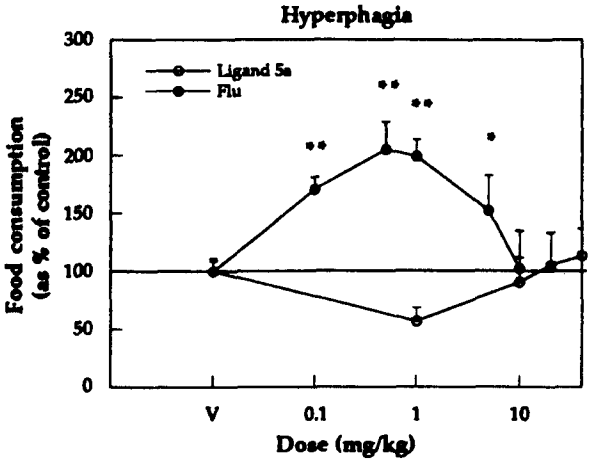
Ligand **5a** had no effect, neither did it reverse any effect of flunitrazepam (Flu) in sedation endpoint measured as locomotor activity; hypothermia endpoint measured as decreased in rectal temperature both at 30 or 45 min after first injection; hyperphagia endpoint measured as food intake after 16 h food deprivation.

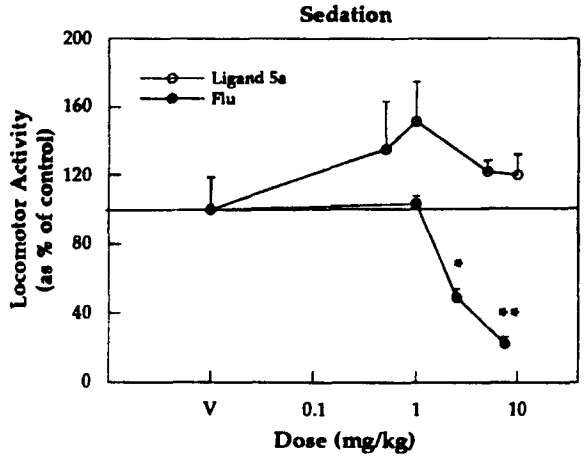
V + V: vehicle treated group; Flu + V: animals were given 2 mg kg^{-1} (1 mg kg^{-1} for hyperphagia) of Flu, then vehicle; V + ligand **5a**: animals were given vehicle then 10 mg kg^{-1} of ligand **5a**; Flu + ligand **5a**: animal treated with both FLU and ligand **5a**. Values are expressed as mean ± SEM.

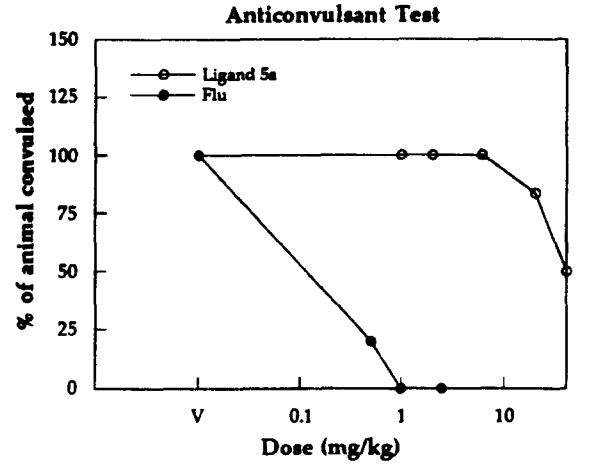
The experiments were conducted using six to 10 rats in a group, data were analysed using one-way ANOVA, Dunnett-t test was used to detect the treatment effect.

[a]Significantly different from V + V group.









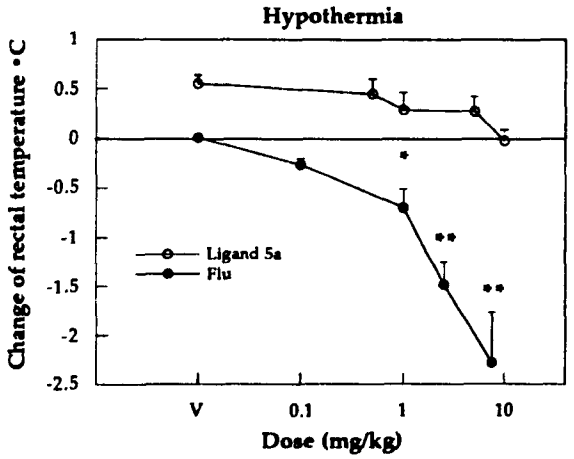


Figure 5. The effect of ligand **5a** in five behavioral endpoints and comparison with flunitrazepam. Data of Flu were previously reported,[15] Flu is a full agonist as it produced anxiolytic, sedative, hypothermic, hyperphagic and anticonvulsive effects. Ligand **5a** was as effective as Flu in the anxiolytic endpoint but had little or no effect on other endpoints. See Procedure section for protocol. Data were analyzed using one-way ANOVA, Dunnett-*t* test was used to detect the treatment effect. The perameter presented here is the mean ± SEM (n = 6–10). $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different from vehicle treated animals.

the discriminant for activation can be defined as the angle between the aromatic ring implicated as an electron acceptor site in LUMO, RP1 and the lipophilic aromatic ring. This angle was measured from the center of the electron accepting ring to RP1 to a point in the center of the lipophilic ring. The value of this angle calculated for the compounds used in the pharmacophore development was found to be < 50° for agonists and > 70° for inverse agonist activity. This angle is not defined in the antagonists, since the electron accepting and lipophilic ring are the same ring.

Table 7 gives the energies of the HOMO and LUMO and the virtual electron density distribution on each aromatic ring in LUMO for the BDZR ligands. Energies of the LUMOs are small negative quantities. Although negative energies for unoccupied orbitals are not physically realistic and a consequence of using semi-empirical methods, comparisons of the relative order of the LUMO energies for zolpidem and alpidem from *ab initio* calculations[26] with the results here indicated the relative magnitudes of these values can be adequately used as a measure of electron accepting abilities. Thus, these ligands can function as efficient electron acceptors.

Table 7. HOMO and LUMO energies and distribution in LUMO for the ligands studied

Ligand	HOMO	LUMO	Ring A	Ring B
Flunitrazepam	-9.9	-1.4	0.74	0.01
5a	-9.3	-0.6	0.30	0.40
5b	-9.1	-0.5	0.31	0.39
5c	-9.2	-0.6	0.42	0.29
5d	-9.1	-0.5	0.39	0.31

As summarized in Table 7, ligands **5a–d** have the electron densities in LUMO equally distributed over rings A and B, which is not the case for Flu where the aromatic ring with the localized electron density in LUMO is ring B, distinct from the most lipophilic ring A. Thus, for ligands **5a–d** the distribution of the electron density in LUMO is more delocalized.

In order to evaluate this property in a complimentary way, we compared the LUMO electron density contours projected onto the solvent accessible surface for flunitrazepam and ligand **5a** (Fig. 6). This figure illustrates the difference in LUMO distribution on the aromatic rings for these two classes of compounds. Namely, flunitrazepam has a very localized LUMO distribution, all concentrated on ring B. Ligand **5a**, however, due to the sulfur atom in ring A, shows a delocalized distribution over both aromatic rings in the compound, again showing the electron density in LUMO is more delocalized for ligands **5a–d**. Thus the two different methods of comparing the LUMO electron density distribution lead to the conclusion that either ring A or B in the new analogs can function as an electron acceptor.

Table 8 gives the angle between the aromatic ring B used as the electron acceptor site in LUMO, RP1, and the lipophilic aromatic ring A. This table predicts all ligands to show agonist activity at the 'Type I' BDZ receptors, consistent with the activity profile. Although it is not proven, if the reasonable assumption is made that these ligands exert their *in vivo* effects, at least in part, through 'Type I' BDZRs (the most common type in brain), these comparisons help validate our current pharmacophore for activation and implicate ring B in activation of this receptor. It is possible that the ability of ring A to function as an alternative electron acceptor can affect the recognition and activation of other BDZR subtypes, leading to the more discriminate behavioral profile of the thiophene analog compared to flunitrazepam.

Table 8. Activation angle between the lipophilic ring, RP1, and LUMO containing ring

Ligand	Lipo Ring B-RP1-LUMO Ring A (°)
Flunitrazepam	47.3
5a	48.9
5b	49.2
5c	46.6
5d	47.6

Taken together, the receptor binding profiles, the behavior profiles, and the computational studies have important implications for the BDZR ligands modes of recognition and activation. The binding studies show that both ligand **5a** and flunitrazepam bind to at least two BDZR subtypes, with the 'Type I' receptor common to both ligands. The behavior studies found flunitrazepam to be an agonist at all five *in vivo* endpoints studied, while ligand **5a** was a strong anxiolytic agent with weak anticonvulsant and hypothermic agonist effects and no effect at sedation or hyperphagia, including the inability to antagonize the activity of Flu at these endpoints, even at a high dose. These results, taken together, indicate that flunitrazepam does not exert its sedative or hyperphagic effects through the 'Type I' receptor in cerebellum, but that both the 'Type I' and spinal cord receptors could be involved in the anxiolytic, anticonvulsant, and hypothermia effects. Thus, these studies have provided the basis for an emerging relationship between recognition of specific subtypes and behavioral endpoints. In addition, while the binding studies thus far made do not provide knowledge of other BDZR subtypes that these ligands might recognize and/or activate, the combined studies made here indirectly allow the inference of different subtype selectivity between the two types of analogs. Specifically, ligand **5a** does not bind to those BDZR subtypes through which flunitrazepam acts as a sedative or exerts its hyperphagic effects, since it has no activity and cannot antagonize the activity of Flu at these endpoints. The observation that ligand **5a** is a strong agonist at the anxiolytic endpoint and much weaker at anticonvulsant and hypothermia can be explained in three ways. First, it is possible that ligand **5a** has different efficacies at each of these

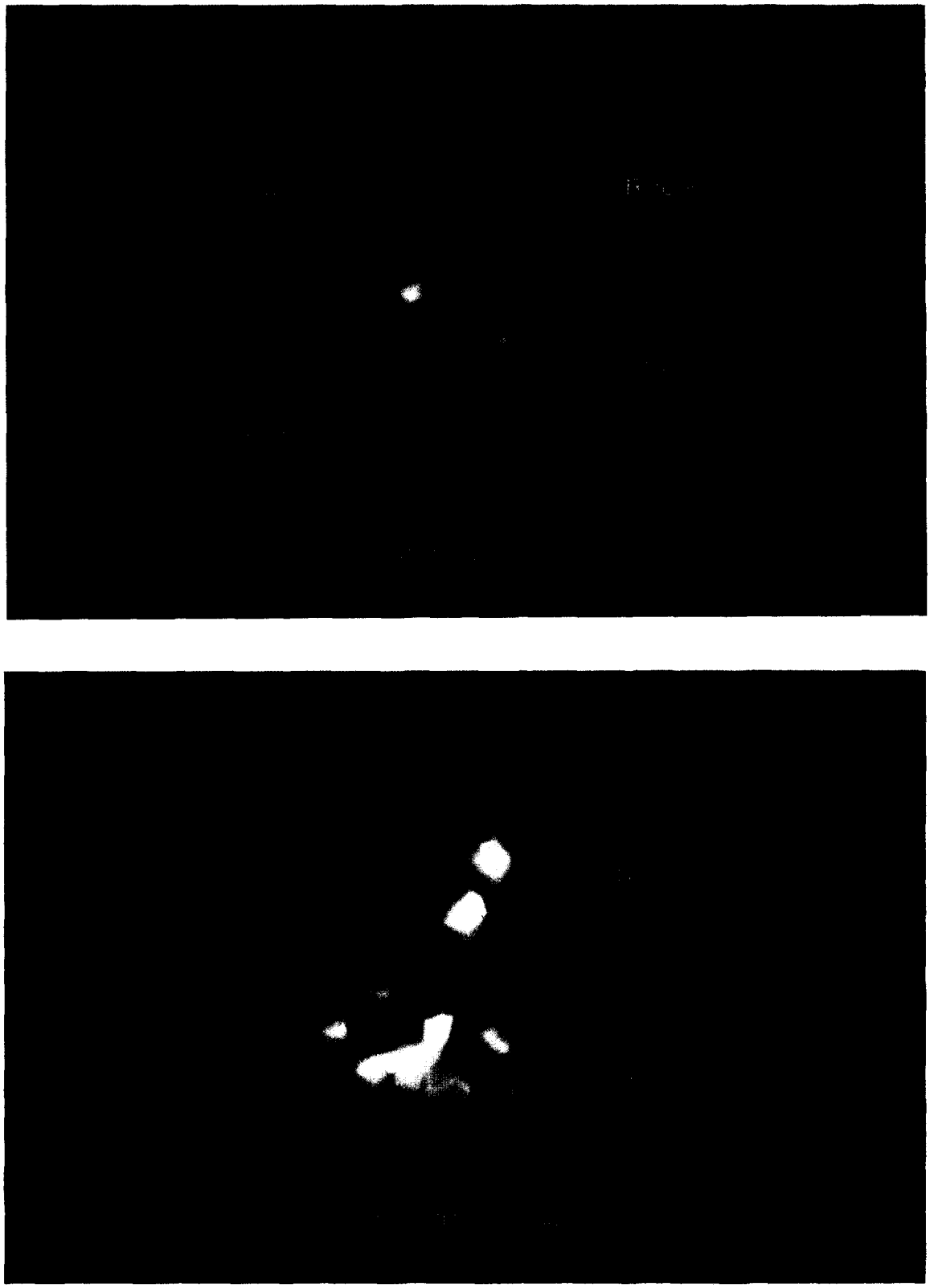


Figure 6. LUMO distribution on the Connolly surface for flunitrazepam and ligand **5a**. The scale is blue–red with red indicating the areas of significant LUMO distribution for the ligands. Values ranged from 0.0 (blue) to 0.15 (red).

three endpoints, with higher efficacy than Flu at the anxiolytic endpoint and equal to or lower efficacy than Flu at the other two. In that case, improvement in the affinity for ligand **5a** would make it an even more powerful anxiolytic agent. Second, ligand **5a** might not bind to all the BDZR receptors responsible for the

hypothermia and anticonvulsant effects, that flunitrazepam does. Finally, since the distribution in LUMO is more delocalized for ligand **5a**, it is possible that the different receptors responsible for these endpoints cannot be completely activated by ligand **5a**, resulting in a weak agonist effect.

Conclusions

In a continued effort to probe the role of the aromatic rings in the classical 1,4-benzodiazepine (BDZ) pharmacology, a series of new thiophene-containing benzodiazepine receptor (BDZR) ligands (**5a–d**) were synthesized. Since these ligands showed reasonable affinity for BDZR(s) in cerebellar and spinal cord CNS regions (60–200 nM), a complete pharmacological profile at five *in vivo* endpoints was determined for ligand **5a** and compared to the more classical BDZR ligand, flunitrazepam. The results were evaluated using the techniques of computational chemistry and a recently developed pharmacophore for recognition and activation of the 'Type I' BDZR. The computations showed all ligands synthesized fulfilled the minimum requirements for recognition, further validating the current pharmacophore. Comparisons of the LUMO made using the in-house program *Grapha*, to allow for display of the LUMO on the solvent accessible surface of the ligands showed the new ligands had significant differences in lowest occupied molecular orbital distribution when compared to the more classical BDZR agonist, flunitrazepam. Specifically, the thiophene ligands had a more delocalized electron distribution in LUMO, equally distributed over rings A and B, whereas flunitrazepam has LUMO distribution solely on ring B. These differences in LUMO could, in part, explain the different pharmacological profile found for this new class of thiophene BDZR ligands. Further work in which compounds are made that incorporate the nitro-group found in Flunitrazepam in the corresponding thiophene compounds will add additional information on this interesting series of compounds.

Experimental

Animals

Male hooded Long Evans rats (Charles River, Wilmington, MA, U.S.A.), weighing 300–450 g, housed in pairs and maintained on a reversed 12:12 h light/dark cycle were used in the behavioral studies. All the tests were conducted in the dark phase of the light cycle, and allow to habituate for at least 1 h to the dimly illuminated testing room prior to the administration of drug or vehicle and testing.

Drugs

Flunitrazepam were received as gift from Hoffman-LaRoche (Nutley, NJ, U.S.A.) and ligand **5a** were synthesized in our laboratory (see synthesis). Drugs were suspended in 40% Encapsin® (β-cyclodextrin) (American Maize, IN, U.S.A.), sonicated for 5 min before administration. Similar to Flu (15), ligand **5a** was also given 30 min before the assessment of behavioral activity.

Behavioral tests

Hypothermia. The body temperature was recorded using a rat rectal probe (Digital Thermometer, Fisher Scientific) prior to and 30 min after administration of the vehicle and test compounds. The first readings was taken to familiarize the animal with the experimental procedure and to ensure that drug responses are not masked by the small hyperthemic response caused by initial handling. For testing ligand **5a** as an antagonist, temperature readings were taken at time 0, 15, 30, 45 and 60 min.

Anxiolysis. A computer controlled elevated plus-maze test system was adapted to study the anxiolytic and anxiogenic properties of Flu and ligand **5a**. The apparatus consisted of two open arms (50 × 10 cm) and two enclosed arms (50 × 40 × 10 cm) made from dark Plexiglas and connected by a central platform (10 × 10 cm). The maze was mounted on a 50 cm high plastic base, equipped with 12 pairs of infrared photocell units that were attached to an IBM computer. Thirty minutes following drug or vehicle administration, the animal was placed in the center of the plus-maze, facing a closed arm. The number of entries and the time spent in the open, closed and center of the arms were recorded over a 5 min period.

Sedation. The testing was done immediately after the anxiety test for a duration of 10 min. The locomotor activity monitor, enclosed in a sound proof cubicle in a dimly illuminated room, consists of test cages 23 cm in diameter and 34 cm high each equipped with six pairs of photocell detectors. Interruptions of the photocell beams were recorded automatically by digital counter.

Hyperphagic test. The day before the testing day, rats were subjected to a sham experiment to allow habituation to the testing diet and handling, in which the procedures were exactly as the real test except no drug was administered. After 16 h of food deprivation, rats were individually caged and given palatable modified rat chow pellets (Purina Mill, diet no. 5729C-D) in preweighed cups. The day after the sham feeding test, animals injected with vehicle or drug at 30 min before the introduction of the food cups. The rats were allowed to eat for 1 h, after which time the remaining food in the cup was weighed and the amount consumed recorded.

Anticonvulsant test. The ability of Flu and ligand **5a** to prevent clonic convulsions was tested by i.p. injection of the test compound at 30 min before the injection of pentylenetetrazol (PTZ) (60 mg kg^{-1}). Rats were observed for 30 min and the occurrence of clonic seizures, and their duration were recorded. Statistical

analysis was not attempted with this test, and data is presented as the percentage of animals convulsing after PTZ injection.

Synthesis

Melting points were determined in open capillary tubes with a Gallenkamp melting point apparatus and are corrected. ^{1}H NMR spectra were recorded for $CDCl_3$ solutions (unless otherwise specified) on a Jeol GX 270 spectrometer, using Me_4Si as internal standard. Elemental analysis were performed by the Chemistry Department, University of Bath, U.K. Column chromatography was performed on silica gel 130–270 mesh (60 Å) with a medium pressure apparatus. Mass spectra were obtained on a VG 7070 EHF spectrometer. Reaction progress and purity of products were checked by analytical TLC using Merck silica gel F254 coated aluminum plates. Spots were visualized with UV254 light or iodine.

1,3-Dihydro-7-methyl-5-(2-thienyl)-2H-1,4-benzodiazepin-2-one (5b). The 5-methyl-2-nitrobenzoic acid (50.0 g, 0.276 M) was refluxed in thionyl chloride (65 mL, 0.574 M) for 1 h. The excess thionyl chloride was evaporated off under reduced pressure to give 5-methyl-2-nitrobenzoyl chloride (**2b**). A solution of **2b** (11.0 g, 55.1 mM) and thiophene (16.0 mL, 0.181 M) in CS_2 (80 mL) was treated with $AlCl_3$ (7.37 g, 55.3 mM) in small portions, over a period of 1 h. The reaction mixture was then stirred at 20 °C for 2.5 h. A further 3.70 g (27.8 mM) of $AlCl_3$ was added in small portions over 2 h. The reaction mixture was then stirred at 20 °C for 16 h. The polymeric byproduct was filtered off and the filtrate partitioned between CH_2Cl_2 and 2 M NaOH solution. The product was extracted with CH_2Cl_2 (thrice) and the combined extracts washed with H_2O (twice), dried ($MgSO_4$), filtered and evaporated leaving a green crystalline solid. Recrystallization from a mixture of light petroleum (bp 40–60°):Et_2O (1:2) gave 2-(5-methyl-2-nitrobenzoyl)thiophene (**3b**, 4.0 g, 30%) as a crystalline solid mp 121–124 °C. (Owing to unavoidable polymerization of the thiophene during the Friedel–Crafts reaction, yields can vary from less than 10 to over 45%. Typical yields were around 30–35%.) A mixture of **3b** (3.33 g, 13.5 mM), 15 wt% aqueous titanium(III)chloride solution containing 15 wt% hydrogen chloride (80 mL) and toluene (15 mL) was stirred at 20 °C under a nitrogen atmosphere for 17 h. The reaction mixture was made alkaline with Na_2CO_3, filtered and the solid byproduct washed with CH_2Cl_2. The filtrate was transferred to a separating funnel and the layers separated. The organic layer was dried (K_2CO_3), filtered, and evaporated under reduced pressure to give 2-(2-amino-5-methylbenzoyl)thiophene (**4b**) (2.66 g, 91%) as an orange oil. A mixture of **4b** (2.0 g, 9.22 mM) and glycine ethyl ester hydrochloride (2.17 g, 15.6 mM) in pyridine (20 mL) was refluxed under a nitrogen atmosphere for 16 h. The solvent was evaporated under reduced pressure and the residue partitioned between CH_2Cl_2 and 1% NaOH solution. The product was extracted with CH_2Cl_2 and the combined organic extracts washed with water, dried ($MgSO_4$), filtered, and evaporated to give a crude product that was purified on a silica gel column (CH_2Cl_2:MeOH, 19:1) to afford pure product (**5b**, 1.24 g, 52%).

1,3-Dihydro-1,7-dimethyl-5-(2-thienyl)-2H-1,4-benzodiazepine-2-one (5d). A solution of **5b** (0.48 g, 1.88 mM) in MeOH (11 mL) was treated with sodium methoxide (0.15 g, 2.78 mM) and the resulting mixture stirred at 20 °C for 3 h. The solvent was evaporated off under reduced pressure and the residue redissolved in dimethyl formamide (4 mL). The resulting solution was cooled to 5 °C and treated dropwise with MeI (0.80 g, 5.64 mM), then stirred at 20 °C for 1 h. The solvent was evaporated under reduced pressure and the residue partitioned between Et_2O and H_2O. The product was extracted with Et_2O (thrice), and the combined organic extracts washed with H_2O (five times), dried ($MgSO_4$), filtered and evaporated under reduced pressure to give an orange oil that was purified on a silica gel column (light petroleum bp 40–60 °C:EtOAc, 6:4) to afford pure product (**5d**, 0.338 g, 67%).

Acknowledgments

This work was supported by the National Institute on Drug Abuse (NIDA) through Grant DZ 06304-05. We would like to thank Dr Juan J. Perez, Dr Mervyn Maze, and Dr Hugo O. Villar for their helpful discussions, and Dr Richard Bone for his work on the *Grapha* toolkit.

References

1. (a) Luddens, H.; Wisden, W. *Trends Pharmacol. Sci.* **1991**, *12*, 49; (b) Klepner, C. A.; Lippa, A. S.; Benson, D. I.; Sano, M. C.; Beer, B. *Pharmacol. Biochem. Behav.* **1979**, *11*, 457; (c) Unnerstall, J. R.; Kuhlar, M. J.; Niehoff, D. L.; Palacios, J. M. *J. Pharmacol. Exp. Ther.* **1981**, *218*, 797.

2. (a) Wisden, W; Seeburg, P. H. *Curr. Opin. Neurobiol.* **1992**, *2*, 263; (b) Corda, M. G.; Giorgi, O.; Longoni, B.; Ongini, E.; Pesce, G.; Cruciani, R.; Biggio, G. *Eur. J. Pharmacol.* **1989**, *169*, 205; (c) Niehoff, D. L.; Marshal, R. D.; Horst, W. D.; O'Brien, R. A.; Palacios, J. M.; Kuhar, M. J. *J. Pharmacol. Exp. Ther.* **1982**, *221*, 670; (d) Perrault, G.; Morel, E.; Stanger, D. J.; Zivkovic, B. *Eur. J. Pharmacol.* **1990**, *187*, 487; (e) Pritchett, D. B.; Seeburg, P. H. *J. Neurochem.* **1990**, *54*, 1802; (f) Kleingoor, C.; Wieland, H. A.; Korpi, E. R.; Seeburg, P. H.; Kettenmann, H. *Neuroreport* **1993**, *4*, 187; (g) Wafford, K. A.; Whiting, P. J.; Kemp, J. A. *Mol. Pharmacol.* **1993**, *43*, 240.

3. (a) Sternbach, L. H. In *The Benzodiazepines;* Raven: New York, 1973, pp. 1–26; (b) Sternabach, L. H. *Prog. Drug Des.* **1978**, *22*, 229; (c) Sternbach, L. H.; Tandall, L. O.; Gustavson, S. R. *J. Med. Chem.* **1974**, *17*, 374.

4. (a) Blair, T.; Webb, G. A. *J. Med. Chem.* **1977**, *20*, 1206; (b) Lucek, R. W.; Garland, W. A.; Dairman, W. *Fed. Proc.* **1979**, *38*, 541; (c) Kaufman, J. J.; Kerman, E. *Int. J. Quantum Chem. Quantum Biol. Symp.*, **1974**, *1*, 259; (d) Borea, P. A. *Arzneim. Forsch.* **1983**, *33*, 1086.

5. Haefely, W.; Kyburz, E.; Gerecke, M.; Mohler, H. *Adv. Drug. Res.* **1985**, *14*, 165.

6. Zhang, Z.; Skolnick, P.; Cook, J. M. Poster Presentation, 207th American Chemical Society National Meeting, San Diego, 1994.

7. Codding, P. W.; Muir. A. K. S. *Mol. Pharmacol.* **1984**, *28*, 178.

8. Fryer, R. I.; Cool, C.; Gilman, N. W.; Walser, III A. *Life Sci.* **1986**, *39*, 1947.

9. Tebib, S.; Bourguignon, J. J.; Wermuth, C. G. *J. Comput.-Aided Drug Design* **1987**, *8*, 153.

10. Villar, H. O.; Uyeno, E. T.; Toll, L.; Polgar, W.; Davies, M. F.; Loew, G. H. *Mol. Pharmacol.* **1989**, *36*, 589.

11. (a) Gee, K.; Yamamura, H. *Life Sci.* **1982**, *31*, 1939; (b) Gee, K.; Yamamura, H.; Roeske, W.; Yamamura, S. *Fed. Proc.* **1984**, *43*, 2767; (c) Sieghart, W.; M. Karobath *Nature (Lond.)* **1980**, *286*, 285.

12. Maguire, P. A.; Davies, M. F.; Villar, H. O.; Loew, G. H. *Eur. J. Pharmacol.* **1992**, *214*, 85.

13. Maguire, P. A.; Villar, H. O.; Davies, M. F.; Loew, G. H. *Eur. J. Pharmacol. Mol. Pharmacol. Sec.* **1992**, *226*, 233.

14. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.

15. Davies, M. F.; Onaivi, E. S.; Chen, S.-W.; Maguire, P. A.; Tsai, N. F.; Loew, G. H. *Pharmacol. Biochem. Behav.* **1994**, *49*, 47.

16. Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187.

17. Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.

18. Fraga, S. *J. Comp. Chem.* **1982**, *3*, 329.

19. Villar, H. O.; Bone, R. G. A. *Grapha*, Molecular Research Institute, Palo Alto, CA, U.S.A.

20. Kantola, A.; Villar, H. O.; Loew, G. H. *J. Comp. Chem.* **1991**, *12*, 681.

21. Croizet, F.; Langlois, M. H.; Dubost, J. P.; Braquet, P.; Audry, E.; Dallet, P.; Colleter, J. C. *J. Mol. Graphics* **1990**, *8*, 153.

22. Heiden, W.; Moeckel, G.; Brickmann, J. *J. Comp-Aided Mol. Design* **1993**, *7*, 503.

23. (a) Cramer, C. J.; Truhlar, D. G.; *J. Am. Chem Soc.* **1991**, *113*, 8305; (b) Cramer, C. J.; Truhlar, D. G. *J. Comp. Aided Drug Des.* **1992**, *6*, 629; (c) Liotard, D. A.; Healy, E. F.; Ruiz, J. M.; Dewar, M. J. S. AMPAC v. 2.1, QCPE Program 506, Bloomington, IN, U.S.A., 1989.

24. Schove, L. T.; Perez, J. J.; Loew, G. H. *Bioorg. Med. Chem.*, **1994**, *2*, 1029.

25. Schove, L. T.; Perez, J. J.; Maguire, P. A.; Loew, G. H. *Med. Chem. Res.* **1994**, *4*, 307.

26. Georges, G. J.; Evrard, G. H.; Durant, F. V.; George, P. G.; Wick, A. E. *Eur. J. Med. Chem.* **1993**, *28*, 323.

(*Received in U.S.A.* 20 *March* 1995; *accepted* 18 *July* 1995)