A Concerted Study Using Binding Measurements, X-ray Structural Data, and Molecular Modeling on the Stereochemical Features Responsible for the Affinity of 6-Arylpyrrolo[2,1-*d*][1,5]benzothiazepines toward Mitochondrial Benzodiazepine Receptors

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The 7-(acyloxy)-6-arylpyrrolo[2,1-*d*][1,5]benzothiazepine derivatives have been recently proposed as a new class of ligands specific for the mitochondrial benzodiazepine receptor (Fiorini et al. *J. Med. Chem.* **1994**, *37*, 1427–1438) (Greco et al. *J. Med. Chem.* **1994**, *37*, 4100–4108). In this paper we report the X-ray crystallographic structures of three potent (**1**–**3**) and two inactive (**4** and **5**) previously described benzothiazepines, as well as binding affinity constants for two newly assayed analogs in which the acyloxy side chain was replaced by a methoxy group (**6**) or removed (**7**). Structure–affinity relationships and molecular mechanics calculations performed using crystal structures as references have led to a revised 3D pharmacophore model accounting for all the data available up until now. Interestingly, the hypothetical receptor-bound conformations of **1**–**3** display a considerable degree of similarity with their crystal geometries. Additional calculations have confirmed that the poor affinities of benzothiazepines bearing an aroyloxy group (**4** and **5**) should be ascribed to the steric and/or electronic features of the side chain aryl moieties rather than to unfavorable conformational properties.

Introduction

The principal site of action of benzodiazepines in the central nervous system (CNS) is believed to be a domain that allosterically regulates chloride channel gating activated by γ-aminobutyric acid (GABA) on $GABA_A$ receptors.[1-3] Initial studies to identify specific benzodiazepine receptors in the CNS unexpectedly also revealed additional binding sites in peripheral tissues having recognition properties slightly different from those associated with $GABA_A$ receptors in the brain.[4] Because of its initial identification outside the CNS, this class of recognition sites became known as peripheral sites. The CNS and peripheral benzodiazepine recognitions sites were later found to display distinct structural specificities.[5-8]

In rodents, diazepam is rather nonselective, while its 4′-chloro derivative, designated as Ro 5-4864, shows high affinity for peripheral and very low affinity for $GABA_A$ receptors.[9,10] Subsequently, other classes of organic compounds were found to have high affinity and specificity for peripheral benzodiazepine receptors. Some isoquinolinecarboxamides have shown great selectivity for peripheral receptors.[11-13] Among these derivatives, PK 11195 is currently the most widely used specific probe for peripheral benzodiazepine receptors.

Benzodiazepine peripheral-type receptors have been identified in nearly all the mammalian tissues, including heart,[11,12,14,15] several endocrine glands,[11,12,16] kidney,[7,15] and erythrocytes.[17,18] In the CNS, where their density is either comparable to or greater than that of the benzodiazepine recognition sites on $GABA_A$ receptors,[5,19] they are essentially located on glial cells, mainly astrocytes.[20,21] Subcellular fractionation studies have demonstrated that they are principally associated with the outer mitochondrial membrane[22-24] (hence the term mitochondrial benzodiazepine receptors or MBR).

The effects of benzodiazepines on MBR include modulation of the release of hormones, especially steroid hormones,[25] which seems to account for the high density of MBR in adrenal glands. Diazepam inhibits potassium-stimulated aldosterone secretion,[26] and a stimulatory effect of Ro 5-4864 on testosterone secretion has been described.[27] Several benzodiazepines, including Ro 5-4864, cause dose-dependent stimulation of the conversion of cholesterol to pregnenolone in bovine adrenal mitochondria.[28] It has been recently proposed that glial cells may be steroidogenic[29,30] and that this effect is mediated by MBR.[25] An enhanced production of neurosteroids, consequent to MBR activation, could explain the "antineophobic" properties of some indole derivatives in rodents.[31] An interaction between the MBR and calcium channels has also repeatedly been suggested.[32-34] Finally, stimulatory as well inhibitory effects of MBR ligands on the proliferative index of immune cells have been reported.[35] In general, immunostimulating effects were observed with nanomolar concentrations of Ro 5-4864, whereas immunodepressive effects were measured at micromolar concentration of diazepam or Ro 5-4864.

Recently, derivatives of the pyrrolobenzothiazepine system were proposed as a new class of ligands specific for MBR[36,37] (Table 1 lists the structures of some of these

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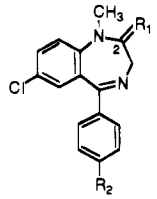
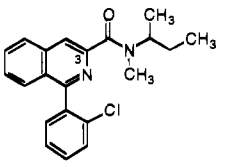
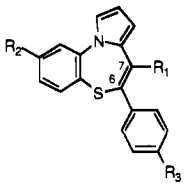
Table 1. Structures and Binding Affinity Constants (K_D or K_i) of the Investigated Ligands





	R_1	R_2	K_D (nM) ± SE		K_D (nM) ± SE
Ro 5-4864[a]	O	Cl	58 ± 30	PK 11195[a]	2 ± 0
diazepam	O	H	213 ± 70		
medazepam	H_2	H	> 10^4		



	R_1	R_2	R_3	K_i (nM) ± SE
1[a]	$OCON(CH_3)_2$	H	H	9 ± 1
2[b]	$OCOCH_3$	H	Cl	30 ± 3
3[a]	OSO_2CH_3	H	OCH_3	95 ± 10
4[a]	OCO-3-pyridyl	H	OCH_3	> 10^4
5[a]	$OCOC_6H_2$-3,4,5-$(OMe)_3$	Cl	OCH_3	> 10^4
6	OCH_3	H	H	7000 ± 600
7	H	H	H	3150 ± 530

[a] Reference 36. [b] Reference 37.

compounds). Radioligand-binding data have shown that acylation at the 7-position of the tricycle leads to binding constants in the micro- or nanomolar range, except for the 7-aroyloxy derivatives which lack affinity. Moreover, 7-(dimethylcarbamoyl)oxy (R_1 = $OCON(CH_3)_2$) derivatives have the highest inhibition potencies, compared to the acetoxy (R_1 = $OCOCH_3$) and mesyloxy (R_1 = OSO_2CH_3) analogs. Molecular modeling studies[36] on these benzothiazepines have highlighted structural requirements for a tight binding to MBR.

The present paper reports the MBR-binding affinities of four newly assayed ligands and the molecular structure determination by X-ray diffraction of five already described benzothiazepines.[36,37] These data have been useful for identifying, through a partial revision of our earlier pharmacophore hypothesis,[36] the stereochemical features responsible for the binding of Ro 5-4864, PK 11195, and benzothiazepine derivatives to MBR.

Results and Discussion

Structure–Affinity Relationships. Table 1 lists the MBR affinity constants of previously[36,37] tested ligands (**1**–**5**, Ro 5-4864, and PK 11195) and of newly assayed benzothiazepines (**6** and **7**) and Ro 5-4864 analogs (diazepam and medazepam). The synthesis of **6** is detailed in the Experimental Section. Compound **7** had already been prepared in our laboratories.[39]

The binding data suggest that the potency is connected with the presence of a C=O or S=O group capable of accepting a hydrogen bond from a specific receptor site. This is supported by the observation that the 7-methoxy (**6**) and 7-hydrogen (**7**) benzothiazepine derivatives as well as medazepam (the only benzodiazepine lacking a carbonyl group at the 2-position) have no appreciable binding abilities. Additionally, structure–affinity relationships on isoquinoline analogs of PK 11195 showed that the carboxamide function at the 3-position is needed for nanomolar affinity.[40]

Crystallographic Studies. Crystal data, data collection, and refinement details are given in Table 2. Table 3 reports selected bond distances, bond angles, torsional angles, and interatomic contacts for compounds **1**–**5**. The molecular conformations for the five compounds are illustrated in the ORTEP[41] views of Figures 1–5. Interplanar angles are listed in Table 4. In all compounds the thiazepine ring assumes a boat conformation with stern angles of 49.5–55.1° and bow angles of 35.3–37.2° (stern and bow angles are defined

Table 2. Crystal Data and Summary of Experimental Details for Compounds **1**–**5**

	1	**2**	**3**	**4**	**5**
formula	$C_{21}H_{18}N_2O_2S$	$C_{20}H_{14}ClNO_2S$	$C_{20}H_{17}NO_4S_2$	$C_{25}H_{18}N_2O_3S$	$C_{29}H_{24}ClNO_6S$
M_r	362.45	367.9	399.49	416.50	550.03
space group	*C*2/c	$P2_1/n$	*P*bca	$P\bar{1}$	$P\bar{1}$
crystal system	monoclinic	monoclinic	orthrombic	triclinic	triclinic
a (Å)	22.203(5)	10.950(3)	15.247(2)	9.488(1)	7.649(1)
b (Å)	11.852(1)	10.834(2)	7.949(3)	11.025(2)	9.418(3)
c (Å)	16.418(2)	14.866(4)	31.232(3)	11.274(2)	19.291
α (deg)	90.0	90.0	90	92.83(1)	97.02(2)
β (deg)	121.96(1)	100.95	90	113.24(1)	95.38(1)
γ (deg)	90.0	90.0	90	107.31(1)	106.54(2)
V (Å^3)	3666(1)	1732.9(8)	3785(2)	1015.7(3)	1301.1(5)
Z	8	4	8	2	2
D_{calcd} (g cm^{-3})	1.31	1.41	1.40	1.39	1.39
F(000)	1520	760	1664	444	572
μ (Mo Kα) (cm^{-1})	1.84	3.46	2.94	1.81	2.63
crystal size (mm^3)	0.14 × 0.19 × 0.36	0.17 × 0.24 × 0.33	0.14 × 0.21 × 0.48	0.17 × 0.26 × 0.52	0.14 × 0.24 × 0.43
independent refltns	3985	4182	4111	4419	5699
obsd refltns (N_o)	2255 [$I > 3\sigma(I)$]	2350 [$I > 3\sigma(I)$]	2223 [$I > 3\sigma(I)$]	2775 [$I > 3\sigma(I)$]	3409 [$I > 3\sigma(I)$]
$\theta_{min} - \theta_{max}$ (deg)	2 – 28	2 – 28	2 – 28	2 – 27	2 – 27
hkl range	−13,13; 0,14; 0,20	0,14; 0,14; −19,19	0,19; 0,10; 0,39	−9,9; −12,12; 0,24	−9,9; −12,12; 0,24
R[a]	0.040	0.042	0.044	0.037	0.050
R_w[b]	0.043	0.047	0.049	0.041	0.068
p[c]	0.020	0.030	0.03	0.02	0.05
N_o/N_v	7.3	8.3	7.1	7.9	7.2
max shift/error	0.03	0.03	0.01	0.01	0.02
no. of variables (N_v) (last cycle)	307	282	312	352	476
GOF[d]	1.73	1.62	1.63	1.71	1.87
largest ΔF peak (eÅ^{-3})	0.10	0.32	0.27	0.21	0.80

[a] $R = \Sigma|\Delta F|/\Sigma|F_o|$. [b] $R_w = (\Sigma_w|\Delta F|^2/\Sigma_w|F_o|^2)^{1/2}$. [c] $w = 4F_o^2/[\sigma^2(F_o^2) + (pF_o^2)^2]$. [d] GOF = $[\Sigma|\Delta F|^2/(N_o - N_v)]^{1/2}$.

Table 3. Selected Bond Distances (Å), Bond Angles (Deg), Torsional Angles (Deg), and Interatomic Contacts (Å) for Compounds 1–5

	1	2	3	4	5
N11–C14	1.388(2)	1.388(3)	1.389(4)	1.395(4)	1.399(3)
C14–C7	1.448(3)	1.455(4)	1.450(4)	1.448(3)	1.434(5)
C7–C6	1.338(4)	1.333(3)	1.343(4)	1.337(3)	1.335(4)
S5–C6	1.789(2)	1.778(3)	1.783(3)	1.792(2)	1.791(2)
S5–C13	1.766(3)	1.771(2)	1.766(3)	1.767(3)	1.761(3)
C12–C13	1.389(3)	1.396(3)	1.390(4)	1.394(3)	1.395(5)
N11–C12	1.415(4)	1.419(3)	1.424(4)	1.409(3)	1.427(3)
O1–C7	1.403(2)	1.406(3)	1.419(4)	1.417(3)	1.414(3)
O1–C15	1.380(3)	1.363(4)		1.363(2)	1.355(3)
O1–S15			1.616(2)		
O2–C15	1.204(3)	1.191(4)		1.194(3)	1.173(4)
S15–O2			1.411(3)		
C12–N11–C14	128.1(2)	128.3(2)	127.5(3)	127.3(2)	127.2(4)
N11–C14–C7	124.5(1)	125.4(2)	124.3(3)	124.5(2)	123.6(2)
C14–C7–C6	127.8(2)	128.5(2)	128.6(3)	128.4(2)	128.7(2)
S5–C6–C7	116.1(2)	118.2(2)	115.4(2)	114.7(2)	117.2(2)
C6–S5–C13	100.0(1)	102.2(1)	100.4(2)	99.7(1)	98.1(2)
S5–C13–C12	121.3(2)	122.2(2)	121.2(2)	120.5(2)	120.3(2)
N11–C12–C13	121.7(2)	121.5(2)	120.6(3)	120.6(2)	120.6(2)
C7–O1–C15	114.6(2)	116.7(2)		116.8(2)	118.1(2)
C3–O1–S15			118.7(2)		
O1–C15–O2	122.0(2)	122.9(3)		123.5(2)	122.6(2)
O1–S15–O3			109.6(1)		
C14–C7–O1–C15	−67.3(2)	−77.6(3)		−64.0(2)	106.6(3)
C14–C7–O1–S15			−75.1(3)		
C6–C7–O1–C15	119.5(2)	108.6(3)		120.9(2)	−74.9(3)
C6–C7–O1–S15			109.1(3)		
C7–O1–C15–O2	−19.8(3)	−2.6(4)		−11.9(3)	1.2(4)
C7–O1–S15–O2			−15.3(3)		
C14···O2	3.024(3)	3.098(4)	2.962(4)	2.957(2)	3.532(4)

Table 4. Dihedral Angles (Deg) Formed by Weighted Least-Squares Planes and Asymmetry Parameters (ΔC_s) of the Thiazepine Ring[a]

	1	2	3	4	5
1–2[b]	53.4(1)	49.5(1)	53.5(1)	55.1(1)	54.7(1)
1–3[c]	35.9(1)	35.3(1)	37.2(2)	37.1(1)	36.2(2)
1–4	32.4(1)	29.4(1)	33.7(1)	34.5(1)	36.6(1)
1–6	62.6(1)	29.8(1)	30.2(1)	25.1(1)	98.8(1)
3–5	4.2(1)	4.7(1)	6.4(2)	5.5(1)	6.7(1)
ΔC_s(S5)	0.0259(9)	0.017(1)	0.009(1)	0.0059(8)	0.027(1)

[a] Plane 1 = C7–C6–C13–C12; plane 2 = C13–S5–C6; plane 3 = C12–N11–C14–C7; plane 4 = C4–C3–C2–C1–C12–C13; plane 5 = N11–C14–C8–C9–C10; and plane 6 = 6-Phenyl Ring. [b] Stern angle. [c] Bow angle. [d] Asymmetry parameter according to ref 54.

in Table 4). The ring displays an almost perfect C_s symmetry with respect to a perpendicular plane crossing the S5 atom and the midpoint between the N11 and C14 atoms, as shown by the small value of the asymmetry parameter ΔC_s(S5).[42] The pyrrole ring is essentially coplanar with the bow plane, while the phenyl ring can assume different conformations in view of the very limited energy needed for its rotation around the C6–phenyl bond (in compound **1**, the energy difference calculated with the Tripos force field[43] does not exceed 1.5 kcal/mol for values of the torsional angle C7–C6–C14–C6a comprised between 30–60° and 120–150°).

The structures of the benzothiazepines **1**–**4** (Figures 1–4) show similar conformations for the O-C(=O) or O-S(=O) fragments. The observed torsional angles C14–C7–O1–C15(S15) and C7–O1–C15(S15)–O2 are in the ranges −64° to −78° and −3° to −20°, respectively; they thus produce a folding of the side chain so that the distances between the carbonyl/sulfonyl O2 and the endocyclic C14 atoms are essentially constant in the four compounds, i.e., 3.024(3) Å in **1** (Figure 1), 3.098 (4) Å in **2** (Figure 2), 2.962(4) Å in **3** (Figure 3), and 2.957(2) Å in **4** (Figure 4). Compound **5** (Figure 5) is the only one, among those examined, whose torsional angle C14–C7–O1–C15 has a positive sign (107°). This particular arrangement of the side chain projects the O2 atom further (3.532(4) Å) from the pyrrole C14 atom.

Hypothesis for a 3D Pharmacophore. The lack of affinity observed for compounds **6** and **7** required a partial revision of our earlier reported pharmacophore model[36] (Chart 1) which was made up by the following features: two aromatic rings (L1 and L3), an electron-rich moiety (π1), and a receptor hydrogen bond donor site (H2).

Compared with the old one, in the "updated" model (Chart 2) the H2 and π1 locations are no longer present and a pseudoatom (H1) has been positioned 2.0 Å away from the carbonyl oxygen in the plane of the >C=O system to simulate a hydrogen of a hypothetical receptor protic function. The centroids of the fused benzene ring (L1) and the pendant phenyl ring (L3) are retained.

In compound **3**, the only one featuring a sulfonyl in place of a carbonyl group, the H1 pseudoatom was not constrained to lie in any particular plane. In fact, while >C=O groups show a clear tendency to accept hydrogen bonds within the above-specified plane,[44–46] the same does not hold for >S(=O)$_2$ fragments.[45] Due to its different hydrogen-bonding behavior, **3** was included in the last step of the modeling.

As already done in our previous work,[36] the labels of the pharmacophoric elements coincide with those originally proposed by Cook et al.[47] in modeling "central" benzodiazepine receptor ligands (these authors defined an additional site (L2) for rationalizing more subtle differences in affinity among a large number of compounds).

Identification of the Receptor-Bound Conformations of the High-Affinity Ligands. The active analog approach[48] was employed to identify a common pharmacophoric 3D alignment for Ro 5-4864, PK 11195,

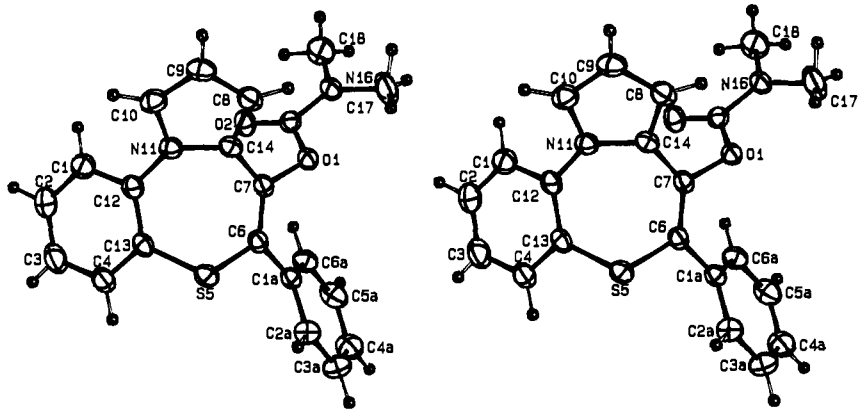


Figure 1. Stereoscopic view[41] of compound 1 showing the thermal ellipsoids at 30% probability.

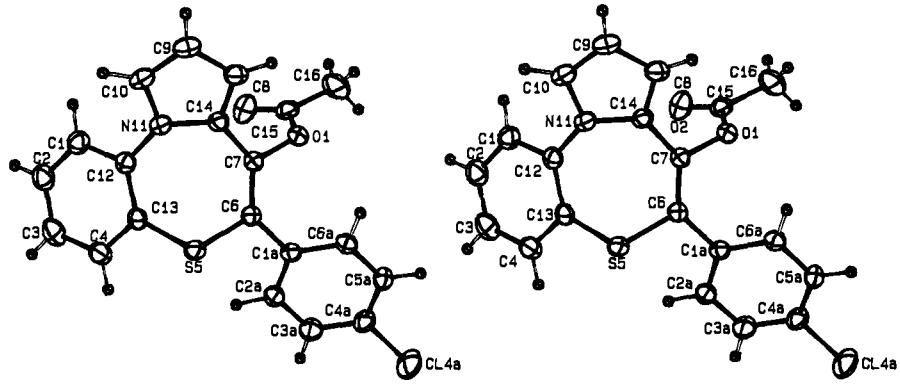


Figure 2. Stereoscopic view[41] of compound **2** showing the thermal ellipsoids at 30% probability.

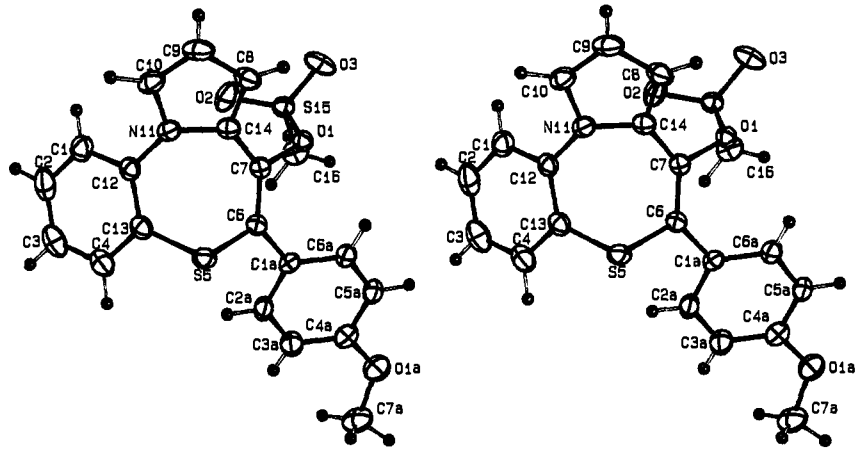


Figure 3. Stereoscopic view[41] of compound **3** showing the thermal ellipsoids at 30% probability.

and the potent benzothiazepine derivatives **1** and **2** (computational details are given in the Experimental Section). Ro 5-4864 was modeled in its global minimum conformation as in this ligand the H1−L1 and H1−L3 distances depend uniquely on variation of the C=O···H1 angle. For PK 11195 we used an input geometry characterized by the minimum steric hindrance around the carbonyl oxygen. In such a conformation (see Table 5), the CH_3 and C_2H_5 groups of the side chain are oriented away from the carbonyl so as to not interfere with the hydrogen bond engaged with the H1 receptor site.

The crystal structures of **1** and **2** were preliminarly submitted to optimization of the hydrogens' coordinates using the Tripos force field.[43] The energies computed on these partially relaxed geometries were later compared with those corresponding to hypothetical receptor-bound conformations.

The direction of the O···H1 vector was treated as an optimizable variable for all the investigated molecules. Specifically, the angle C=O···H1 (defined in Chart 2 and termed θ) was varied with 10° increments within a range of 90−270°. This angular interval was chosen according to experimental findings[44] and molecular mechanics force fields[45,46] handling hydrogen bond directionality. Since the current implementation of the SYBYL/SEARCH module[49] scans only torsional angles, we overcame this limitation by interposing two dummy atoms between the carbonyl carbon atom and the H1 site as illustrated in Figure 6. The dummy atom Du1 is located 0.5 Å away from the oxygen, along the normal to the plane of the >C=O system passing through the

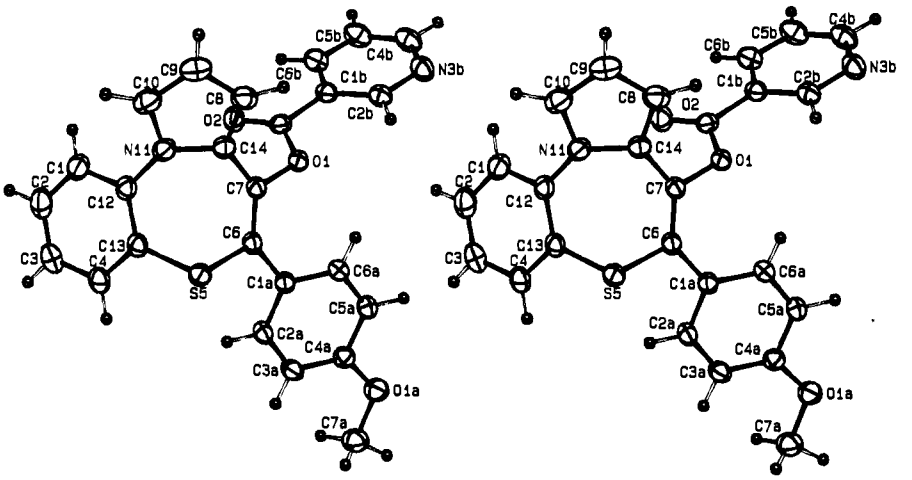


Figure 4. Stereoscopic view[41] of compound **4** showing the thermal ellipsoids at 30% probability.

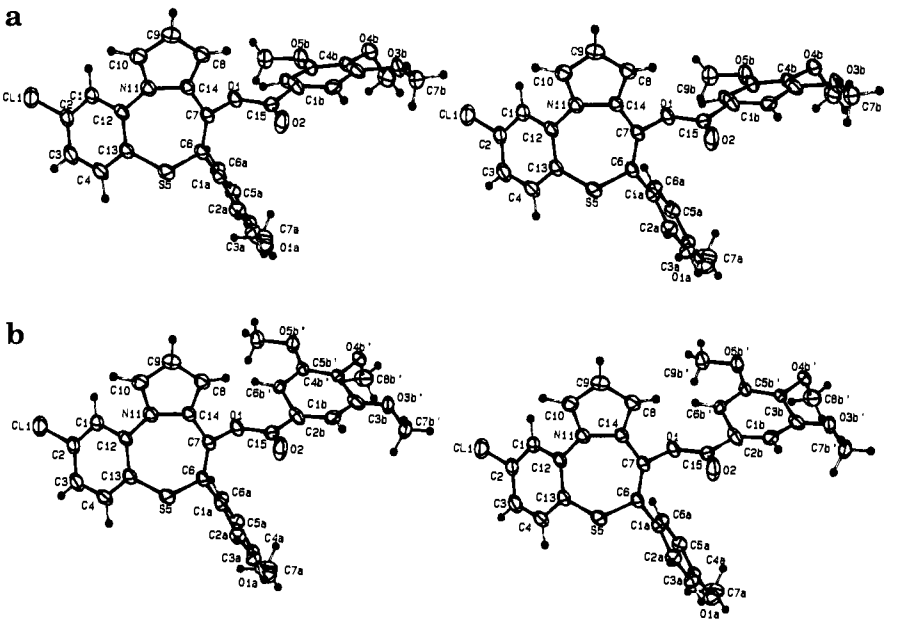
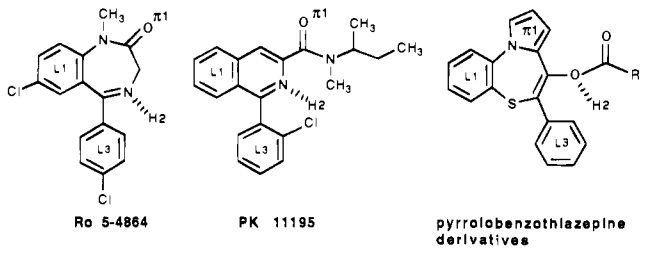


Figure 5. Stereoscopic views[41] of compound **5** showing the thermal ellipsoids at 30% probability and the two orientations of the trimethoxyphenyl group.

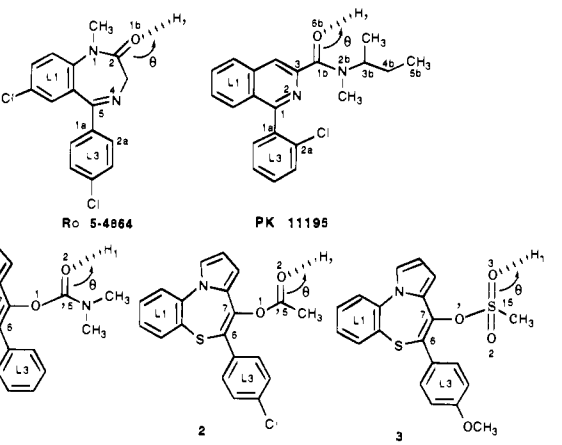
Chart 1



oxygen. The dummy atom Du2 and the oxygen are perfectly overlapping. Rotation of the torsional angle C−Du1−Du2−H1 results in a concomitant variation of the angle C=O···H1.

The orientation maps produced by systematic searches on Ro 5-4864, PK 11195, **1**, and **2** were compared one another to select the rotamers, one for each molecule, as the closest in the plane of the H1−L1 and H1−L3 distances (the distance between L1 and L3 is invariant in all the considered compounds). The geometries and relative energies of these rotamers, which can be viewed as hypothetical receptor-bound conformation models, are listed in Table 5. The calculated energy of the

Chart 2



bioactive conformation of PK 11195 was found nearly coincident with that of the corresponding global minimum (this latter was available from our previous work[36]).

Small differences in conformational energy between the crystal structure and the receptor-bound geometries

Table 5. Geometries[a] and Relative Energies of the Hypothesized Receptor-Bound Conformations

	Ro 5-4864[b]	PK 11195[c]
ΔE_{conf}[d] (kcal/mol)	0.0	0.1
rms dist[e] (Å)	0.0	0.2
L1−L3 dist (Å)	4.9	5.0
H1−L1 dist (Å)	6.5	6.2
H1−L3 dist (Å)	8.0	8.2
θ angle (deg)	210	200
N2−C3−C1b−O6b (deg)		135
C3−C1b−N2b−C3b (deg)		175

	1	**2**	**3**
ΔE_{conf}[f] (kcal/mol)	0.2	−0.7	−0.5
rms dist[e] (Å)	0.5	0.6	0.4
L1−L3 dist (Å)	6.1	6.3	6.1
H1−L1 dist (Å)	6.0	6.1	6.4
H1−L3 dist (Å)	7.8	7.9	8.2
θ angle (deg)	140	140	129
C7−C14−O1−C15	160.0	155.0	
C14−O1−C15−O2 (deg)	−19.8[g]	−2.6[g]	
C7−C14−O1−S15 (deg)			125.0
C14−O1−S15−O3 (deg)			55.0
O1−S15−O3−H1 (deg)			172.5

[a] See Chart 2 for atom labeling and definition of the angle θ. [b] C2a−C1a−C5−N4 = −152°. [c] Additional torsional angle values: C2a−C1a−C1−N2 = −118°, C1b−N2b−C3b−C4b = −125°, and N2b−C3b−C4b−C5b = 172°. [d] ΔE_{conf} for Ro 5-4864 and PK 11195 is the energy difference between the bioactive conformation and the calculated global minimum. [e] rms dist is the root mean square distance resulting from superimposition on Ro 5-4864 about L1, L3, and H1. [f] ΔE_{conf} for **1**−**3** is the energy difference between the bioactive conformation and the partially optimized crystal structure. [g] The same value is observed in the crystal structure.

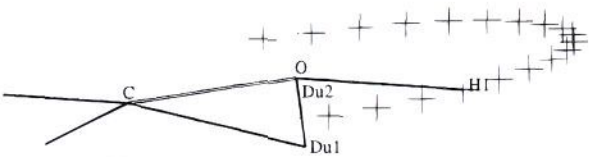


Figure 6. Torsional angle C−Du1−Du2−H1 built by connecting the carbon atom and the site H1 through the dummy atoms Du1 and Du2. Rotation about the Du1−Du2 bond allows variation of the angle C=O···H1.

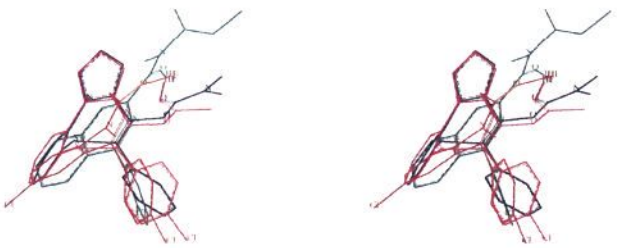


Figure 7. Stereopair picture showing the superposition of the hypothetical receptor-bound conformations of Ro 5-4864 (red), PK 11195 (green), **1** (black), and **2** (magenta).

of **1** and **2** were analogously detected. Interestingly, the bioactive conformations of the two high-affinity benzothiazepines differ only slightly from those determined by X-ray experiments (the values of the C6−C7−O1−C15 torsional angle in the crystal structures are respectively 40° and 46° lower).

Figure 7 shows the proposed bioactive conformations of PK 11195, **1**, and **2** least-squares fitted on Ro 5-4864 about the H1, L1, and L3 points. It can be seen that all the side chains occupy a unique region of space, probably corresponding to a cleft within the MBR binding site characterized by a certain steric tolerance.

Figure 8. Stereopair picture showing the biactive conformation of **3** (black) superimposed on those of **2** (magenta) and Ro 5-4864 (red).

Moreover, all the atoms of the aligned ligands, except the carbonyl oxygens, lie sufficiently apart from the H1 points so as to not interfere sterically with the postulated hydrogen bond. This condition was checked by replacing the H1 pseudoatoms with hydrogens and calculating intra- and intermolecular Lennard−Jones energies.

As already mentioned, **3** was modeled only after aligning its carbonyl analogs. A conformation for **3** was examined which gave the best overlap with **2** at the pyrrolobenzothiazepine skeleton and the sulfonyl/carbonyl oxygen (see Figure 8). In such a geometry (Table 5) the distance between one of the sulfonyl oxygens and the H1 point of Ro 5-4864 turned out to be 1.9 Å; the angle θ formed between S=O and the Ro 5-4864 H1 pseudoatom was 129°; the conformational energy was 0.5 kcal/mol lower than that calculated on the crystal structure; and no steric hindrance was produced on the H1 site. By looking at Figure 8 it can also be appreciated that the methyl groups of **2** and **3** share a common position in space. Taken together, these data convinced us that the considered conformation of **3** could be the bioactive one.

It is worth noting that all the θ values listed in Table 5 deviate by less than 25° from 135° or 225° (values reported by Vedani and Dunitz to be optimal for hydrogen bonds involving either carbonyl and sulfonamide oxygens[45]). The pharmacophore geometry of the five investigated molecules is simply given by the following distance ranges: L1−L3 = 4.9−6.3 Å, H1−L1 = 6.0−6.5 Å and H1−L3 = 7.8−8.2 Å.

Comparison of the Structures of the Active and Inactive Benzothiazepines. As last step in our analysis, we verified whether the two inactive 7-(aroyloxy)benzothiazepine derivatives **4** and **5** could attain low-energy conformations matching the pharmacophore features common to the highly potent congeners **1**−**3**. The crystal structures of **4** and **5** were thus modified by assigning to the C6−C7−O1−C15 torsional angle the same value (160°) characterizing the bioactive conformation of **1**. Such rotation did not significantly increase the internal energies of the compounds: compared to their solid state geometries, the modified conformations of **4** and **5** were calculated to have an extra energy of only 0.1 and 0.9 kcal/mol, respectively. Hence, the fact that compounds **4** and **5** are practically devoided of affinity cannot be accounted for in terms of unfavorable conformational behaviors.

Applying the comparative molecular field analysis (CoMFA) method,[38] we have recently attempted to explain why **4**, **5** and other aroyloxy analogs bind so weakly to MBR.[37] The obtained 3D quantitative structure−activity relationship (QSAR) model mapped, in the

3D space, possible unfavorable steric and electrostatic features of the side chain aromatic groups. Such aryl groups might be too large to easily fit into the receptor cavity. Alternatively (or additionally) they might give rise to a repulsive electrostatic interaction between their electron-rich aromatic moieties and a negatively charged receptor site. New benzothiazepine analogs, characterized by encumbering nonaromatic side chains, are currently being synthesized in order to unambiguously address this issue.

Conclusions

Using X-ray structural data and molecular modeling methods, we have identified the receptor-bound conformations and the mutual alignment of Ro 5-4864, PK 11195, and three potent benzothiazepines (**1**–**3**) binding selectively to the MBR. The developed pharmacophore model, which is a revised version of our earlier developed scheme of ligand–MBR interactions,[36] accounted for the very poor affinity of two newly assayed benzothiazepines, **6** and **7**, lacking a vital carbonyl/sulfonyl oxygen. In absence of experimental structures of ligand–MBR complexes, a putative pharmacophore pattern could guide the design of new diverse MBR ligands by molecular graphics comparisons as well as 3D database searching.[50]

It is widespreadly accepted that the bioactive conformation of a small ligand does not necessarily correspond to the calculated global minimum conformer nor to the geometry experimentally detected in the solid state or solution. On the other hand, accurate estimates of conformational energy are rarely achievable through commonly used computational techniques. In the light of the above considerations, approaches to conformational problems which combine experimental and theoretical tools should be particularly effective. Particularly, one can reasonably assume that solid state conformation of a ligand has an internal energy near that of the gas phase global minimum conformer. On the basis of this approximation, the former energy can be in turn used as a valuable reference to assess whether a given pharmacophore-derived conformation is energetically plausible. In this case, lengthy and often computer intensive conformational searches to identify the global minimum in vacuum are not strictly necessary.

The similarity found between putatively bioactive and crystal structures of the high-affinity benzothiazepines 1 and **2** supports the assumption, relying on simple molecular mechanics calculations, that their proposed receptor-bound conformations are energetically feasible. The hypothetical bioactive conformation of **3**, the only benzothiazepine derivative provided with a sulfonyl function, was selected on the basis of the pharmacophore model derived from the carbonyl-bearing compounds. For compound **3**, theoretical and crystal geometries were found slightly more dissimilar compared to the carbonyl analogs.

In agreement with our recently reported CoMFA model,[37] molecular mechanics calculations on two (aroyloxy)benzothiazepines (**4** and **5**) confirmed that their inability to bind to the receptor does not likely depend on difficulties to assume a proper conformation at the binding site but rather on repulsive specific interactions involving their side chains.

Experimental Section

Chemistry. Melting points (uncorrected) were determined using an Electrothermal 8103 apparatus. IR spectra were taken as Nujol mulls with a Perkin-Elmer 398 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 200 MHz spectrometer; chemical shifts (δ) are in ppm relative to TMS. Elemental analysis was performed with a Perkin-Elmer 240C elemental analyzer, and the results are within 0.4% of the theoretical values.

Preparation of 7-Methoxy-6-phenyl[2,1-*d*][1,5]benzothiazepine (6). The reaction was carried out in an argon atmosphere. A dispersion of potassium hydride (0.044 g, 1.11 mmol) in mineral oil was washed twice with anhydrous hexane, and the oil was removed. To the KH powder suspended in anhydrous tetrahydrofuran (2 mL) was slowly added a solution of 6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepin-7(6*H*)-one[36] (0.116 g, 0.398 mmol) in 5 mL of the same solvent, and the reaction mixture was stirred for 3 h at room temperature. A 0.037 mL (0.398 mmol) portion of dimethyl sulfate was then added, and the mixture was stirred for 1 h. The reaction suspension was carefully poured into crushed ice and extracted with ethyl acetate. The combined organic layers were washed with water and dried (Na_2SO_4), and the solvent was removed under reduced pressure; the residue was purified by flash chromatography (Merk silica gel 230–400 mesh) eluting with 50% chloroform–petroleum ether (bp 60–80 °C) (28% not optimized yield) and recrystallized from ethanol as white needles (mp 82–83 °C). IR: 1612, 1270, 998, 760 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 3.93 (s, 3H), 6.49 (m, 1H), 6.72 (m, 1H), 7.12 (m, 1H), 7.23–7.46 (m, 6H), 7.62-7.76 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 109.7, 112.9, 118.6, 124.2, 124.9, 126.9, 127.1, 127.7, 129.3, 129.5, 133.2, 134.1, 138.3, 142.2, 151.1. Anal. ($C_{19}H_{15}NOS$) C, H, N.

Binding Assays. Male CRL:CD(SD)BR (Charles River Italia, Calco, CO, Italy), weighing about 150 g, were used in these experiments. The rats were housed in groups of five in plastic cages, kept under standard conditions (room temperature 21 ± 1 °C, relative humidity 55 ± 10%, 12–12 h light–dark cycle), and given tap water and food pellets ad libitum. They were decapitated unanesthetized, and the brains were rapidly removed and dissected into anatomically recognizable areas.

Cortices were homogenized in about 50 vol of ice cold phosphate-buffered saline, 50 mM, pH 7.4, using an Ultra Turrax TP 1810 (2 × 20 s) instrument and centrifuged at 50000*g* for 10 min. The pellet was washed three more times by resuspension in fresh buffer and centrifuged as before. The last pellet was resuspended just before the binding assay.

For mitochondrial benzodiazepine binding,[51] 10 mg of original wet tissue weight was incubated with 1 nM [^{3}H]PK 11195 (specific activity 85.8 Ci/mmol; NEN) in 1 mL final volume for 120 min at 4 °C in the presence of 8–12 increasing concentrations of drugs. Nonspecific binding was determined using 1 μM PK 11195.

Incubation was stopped by rapid filtration under vacuum through glass fiber filters (Printed Filtermat B, Wallec) which were then washed with 12 mL of ice-cold buffer, using a Brandel M48 RP harvester. Filters were put into sample bags with 25 mL of Betaplate Scint (LKB) and counted in a 1204 BS Betaplate liquid scintillation counter, with a counting efficiency of about 45%. IC_{50}s were determined by nonlinear[52] fitting of binding inhibition curves, using the Allfit program running on an IBM AT personal computer. Each point was the mean of triplicate samples.

X-ray Crystallography. Suitable crystals of the studied compounds were obtained from ethanol solution. Crystal data, data collection, and refinement details are given in Table 2. Data for all the crystal structure determinations were collected at room temperature on an Enraf-Nonius CAD4 diffractometer using graphite monochromated Mo Kα radiation (λ = 0.71069 Å) with $\omega/2\theta$ scan technique. Lattice constants were determined by least-squares fitting of the setting angles of 25 reflections in the range $10° < \theta < 14°$. Intensities of three standard reflections were measured every 2 h and did not vary significantly for any of the five compounds investigated. All

intensities were corrected for Lorentz and polarization effects. The structures were solved by direct methods with the SIR88[53] system of programs. All other calculations were done by the MolEN[54] system of programs and PARST.[55]

All structures were refined by full matrix least-squares. For structures of **1**−**4**, the refinement was anisotropic for all the non-H atoms and isotropic for hydrogen atoms. All hydrogen positions were found from the ΔF synthesis carried out after the first cycles of isotropic refinement. The structure of **5** presents a partial disorder on the trimethoxyphenyl group. As a consequence, the O3b, O4b, O5b, C4b, C5b, C6b, C7b, C8b, and C9b atoms were refined anisotropically in two positions with occupancy of 0.5, and the corresponding hydrogen atoms were taken in calculated fixed positions. For the other three atoms, C1b, C2b, and C3b, it was impossible to determine the two positions; therefore, they were refined in the unusual way with occupancy 1. The two orientations of the trimethoxyphenyl group are shown in Figure 5. All other non-hydrogen atoms were refined anisotropically and hydrogens isotropically.

Molecular Modeling. All molecular modeling was performed with use of the software package SYBYL[49] running on a Silicon Graphics Iris Indigo XS24 workstation. Conformational energies were calculated employing the molecular mechanics TRIPOS force field[43] with neglect of electrostatics.[56] Energy minimizations were realized with the SYBYL/MAXIMIN2 option by applying the BFGS (Broyden, Fletcher, Goldfarb, and Shannon) algorithm and setting a δ energy value of 0.002 kcal/mol as convergence criterion. Molecular superpositions were carried out using the SYBYL/FIT command.

The modeling on the five benzothiazepines was conducted on their crystal structures after a preliminary partial geometry optimization of the hydrogens' coordinates.

Pharmacophore-consistent conformations for the structures reported in Chart 2 were searched using the Marshall's active analog approach.[48] We looked for geometries sharing similar distances among the points H1, L1, and L3 using the SYBYL/SEARCH routine.

The only chiral molecule in Chart 2, PK 11195, was modeled in the *S* configuration, as already done in our previous work,[36] even if its binding affinity was measured as a racemic mixture. However, chirality in this molecule does not involve any pharmacophoric element.

With reference to Chart 2, the torsional angles about C3−C1b (PK 11195), C7−O1 (**1**−**3**), and O1−S15 (**3**) were scanned with 5° increments within a 0−359° interval; the torsional angles about O1−C15 (**1** and **2**), in consideration of the resonance effects, were instead varied in the same interval with 180° increments. As illustrated in Figure 6, rotation of the C−Du1−Du2−H1 torsional angle in Ro 5-4864, PK 11195, **1**, and **2** allowed variation of the bond angle C=O···H1 (defined in Chart 2 as θ) with 10° steps within a 90−270° range.

A 5 kcal/mol energy window was applied to reduce the number of output conformations. In a systematic conformational search, energies are computed without allowing each geometry to relax to the nearest minimum conformer. To minimize the risk of missing conformations whose energies could be actually over estimated, a 0.75 van der Waals scaling factor was used to "soften" steric contacts in the rigid rotamers. Distances H1−L1 and H1−L3 were recorded in each search run at 0.2 Å resolution (the L1−L3 distance being invariant in all the molecules).

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Supporting Information Available: Final atomic positional parameters, atomic thermal parameters, and bond distances and angles for structures 1−**5** (32 pages). Ordering information is given on any current masthead page.

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(56) The investigated molecules cannot form internal hydrogen bonds or ionic interactions. As expected, inclusion of a Coulomb function in the force field did not alter significantly the conformational energies reported in Table 5. Specifically, using Gasteiger–Marsili charges (Gasteiger; Marsili. *Tetrahedron* **1980**, *36*, 3219–3228), the ΔE_{conf} values of compounds 1 and **2** increased both by only 0.2 kcal/mol. Analogous calculations for compound **3** were hampered by lack of parameters for the SO_2 sulfur. In the SYBYL/SEARCH routine, energetically disallowed geometries are filtered off mainly because of van der Waals repulsions. One limitation of molecular mechanics-based conformational analyses is that partial atomic charges are assumed to be invariant upon scanning of the torsional angles. Moreover, setting an appropriate value for the dielectric constant and taking into account intermolecular polarization effects remain serious problems. For the above reasons, we preferred to neglect the electrostatic term in the force field calculations. Notice that the question is completely different in heuristic methods such as CoMFA. Here, the electrostatic fields of the aligned ligands supply information regarding relative rather than absolute abilities to interact electrostatically with the biological target.

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