Novel Ligands Specific for Mitochondrial Benzodiazepine Receptors: 6-Arylpyrrolo[2,1-*d*][1,5]benzothiazepine Derivatives. Synthesis, Structure-Activity Relationships, and Molecular Modeling Studies†

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A novel class of ligands specific for MBR receptors has been identified: 6-arylpyrrolo[2,1-*d*]-[1,5]benzothiazepine derivatives. The majority of newly synthesized esters **37-64** as well as some intermediate ketones showed micro- or nanomolar affinity for [^{3}H]PK 11195 binding inhibition. A SAR study on 42 compounds and a molecular modeling approach led to a preliminary structural selectivity profile: the 6,7-double bond, the carbamoyloxy, alcanoyloxy, and mesyloxy side chains at the 7-position, and the prospective chloro substitution at the 4-position seemed to be the most important structural features improving affinity. Therefore, 7-[(dimethylcarbamoyl)oxy]- and 7-acetoxy-4-chloro-6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepine (**43** and **57**) were synthesized. With 7-[(dimethylcarbamoyl)oxy]-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (**65**), these were the most promising compounds with IC_{50}s of respectively 9, 8, and 9 nM, under conditions where PK 11195 had an IC_{50} of 2 nM.

Introduction

Benzodiazepines interact with two major classes of recognition sites, the "central" and "peripheral" types. The central benzodiazepine receptors (CBR), located in the neuronal tissues,[1,2] are functionally linked to a γ-aminobutyric acid (GABA) receptor-chloride ionophore complex[3] and are apparently located on synaptic membranes;[4] CBR mediate classical pharmacological properties (anxiolytic, anticonvulsant, sedative, and muscle relaxant) of the clinically widely used benzodiazepine.[2]

By contrast, the GABA-independent[5,6] peripheral or "mitochondrial" benzodiazepine receptors (MBR) have been identified in a wide range of peripheral tissues as well as in the central nervous system,[7,8] and their subcellular location has been reported to be mainly mitochondrial,[9-12] nuclear,[5,6] and in the plasma membrane.[13] The physiological role of MBR is still not clear; they are involved in various cellular functions[14-19] including inhibition of oxidative phosphorylation,[20,21] inhibition of cell proliferation,[22] and steroidogenesis.[23,24] They might be useful in the diagnosis of central nervous system tumors, and in fact, glial tumors selectively concentrate MBR.[25]

Specific drugs for MBR might serve as powerful probes to elucidate their role, but only a few compounds are available as yet: Ro 5-4864[5,26] and PK 11195[27] as prototype ligands and their 2-isothiocyanatoethyl derivatives[28,29] as irreversible ligands; a series of quinoline and naphthyridine derivatives is also known.[30]

Our previous report[31] described the synthesis of 6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine derivatives **24** and **65-72** (Table 2) and the preliminary assessment of their affinities for benzodiazepine and GABA receptor subtypes. Compounds **24**, **66**, and **67** showed selectivity for MBR binding, and **66** and **67** were excellent ligands, with nanomolar affinity (IC_{50}s respectively 34 ± 6 and 95 ± 10 nM). On the other hand, the 6,7-dihydro analog of **66**, *cis*-7-acetoxy-6,7-dihydro-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (**72**), showed no MBR affinity (IC_{50} >10^{-5} M). So, we investigated the relationships between the pyrrolo[2,1-*d*][1,5]benzothiazepine moiety and MBR, setting our first approach to check whether the phenyl group at the 6-position was important for affinity. Therefore, we tested the pyrrolo-[2,1-*d*][1,5]benzothiazepin-7(6*H*)-one[32] and its 6-phenyl derivative **23**[32] (Table 2), already synthesized in our laboratory, for MBR binding. The former (IC_{50} >14 000 nM) showed a 11-fold lower affinity than **23** (IC_{50} 1220 ± 110 nM). Encouraged by these preliminary results, which identified the 6,7-double bond and the 6-aryl substitution moieties as the key structural features for MBR affinity and specificity, we turned our attention to the 6-arylpyrrolo[2,1-*d*][1,5]benzothiazepine system.

We now propose the derivatives of this system as a new class of ligands specific for MBR and report their synthesis, radiobinding data, structure-activity relationships, and molecular modeling studies.

Chemistry

The new target compounds **25** and **27-64** (Table 2) together with **23**, **24**, and **26**[33b] were synthesized as shown in Scheme 1. Esters **7-12** (Table 1) were obtained in good yields starting from bis(2-*N*-pyrrolylphenyl)disulfides **1-6** (**4** and **5** being unknown) by reduction with sodium borohydride[34] followed by treatment with ethyl α-bromo-*p*-methoxyphenylacetate.[31] Compounds **4** and **5** were prepared by reaction of dimethoxytetrahydrofuran[33] with the appropriate bis(2-aminophenyl)disulfides.[35] Hydrolysis of esters **7-12** with sodium hydroxide afforded acids **14**,[31] **15**, **17**, **19**, **21**, and **22**. Acids **13**,[32] **16**,[33b] **18**, and **20** were one-step prepared starting from the appropriate

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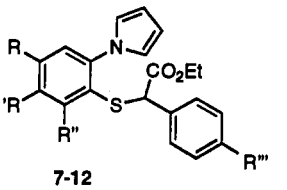
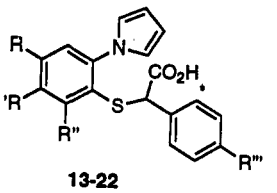
Table 1. Physicochemical Data of 7–22 Intermediates



7-12

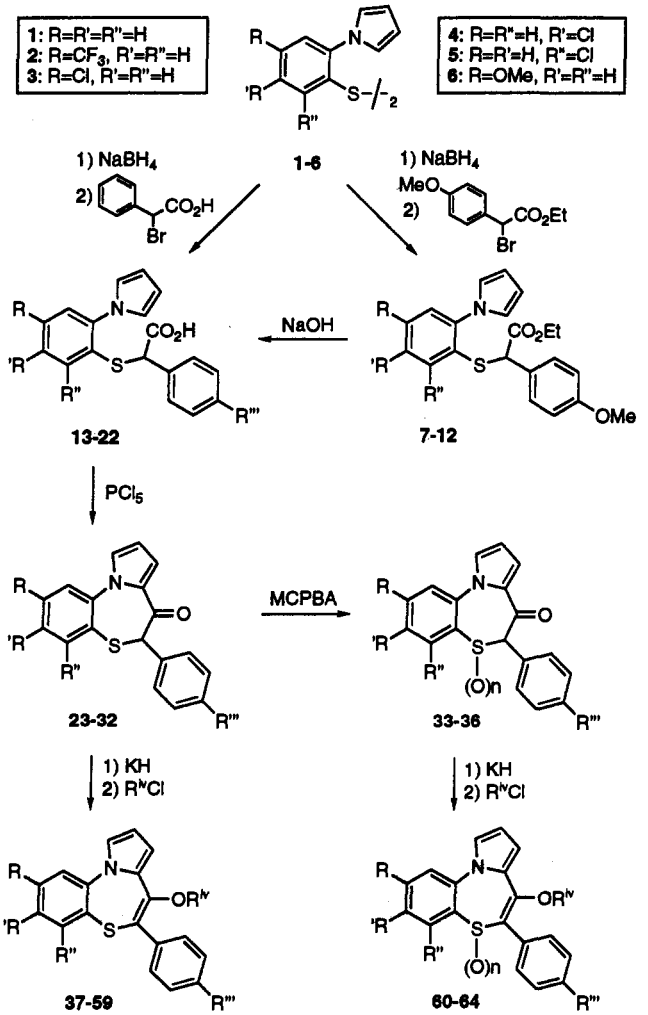


13-22

compd	R	R′	R″	R‴	% yield	recryst solv[a]	mp (°C)	formula
7	H	H	H	OCH_3	75.2	A	60–63[b]	$C_{21}H_{21}NO_3S$
8	CF_3	H	H	OCH_3	86.3	A	70–73	$C_{22}H_{20}F_3NO_3S$
9	Cl	H	H	OCH_3	80.5	A	74–75.5	$C_{21}H_{20}ClNO_3S$
10	H	Cl	H	OCH_3	85.9	B	58–61	$C_{21}H_{20}ClNO_3S$
11	H	H	Cl	OCH_3	80.1	A	89–91	$C_{21}H_{20}ClNO_3S$
12	OCH_3	H	H	OCH_3	45.5	A	93–95	$C_{22}H_{23}NO_4S$
13	H	H	H	H	69.1	C	124–126[c]	$C_{18}H_{15}NO_2S$
14	H	H	H	OCH_3	92.4	D	109–111[d]	$C_{19}H_{17}NO_3S$
15	CF_3	H	H	OCH_3	72.8	A	142–144	$C_{20}H_{16}F_3NO_3S$
16	Cl	H	H	H	70.5	C	138–140[e]	$C_{18}H_{14}ClNO_2S$
17	Cl	H	H	OCH_3	74.3	A	135–137	$C_{19}H_{16}ClNO_3S$
18	H	Cl	H	H	85.9	E	128–130	$C_{18}H_{14}ClNO_2S$
19	H	Cl	H	OCH_3	87.8	F	130–131	$C_{19}H_{16}ClNO_3S$
20	H	H	Cl	H	88.8	F	123–124	$C_{18}H_{14}ClNO_2S$
21	H	H	Cl	OCH_3	90.0	D	127–129	$C_{19}H_{16}ClNO_3S$
22	OCH_3	H	H	OCH_3	40.6		*f*	$C_{20}H_{19}NO_4S$

[a] Recrystallization solvent: A = ethanol; B = petroleum ether (40–60 °C); C = ligroin (75–120 °C); D = petroleum ether (60–80 °C); E = *n*-hexane; F = cyclohexane. [b] Lit.[31] mp 60–63 °C. [c] Lit.[32] mp 124–126 °C. [d] Lit.[31] mp 109–111 °C. [e] Lit.[33b] mp 138–140 °C. [f] Not crystallized and used crude.

Scheme 1



disulfides, sodium borohydride, and commercially available α-bromophenylacetic acid (Table 1). Intramolecular cyclization of acids **13–22** using phosphorus pentachloride gave ketones **23–32**. Oxidation of **23** and **24** with *m*-chloroperbenzoic acid (MCPBA) afforded sulfoxides **33** and **35** and sulfones **34** and **36** (see the Experimental Section for reaction conditions). Finally, **23–36** were converted to the desired esters **37–64**, using potassium hydride and the selected acid chlorides.

Results and Discussion

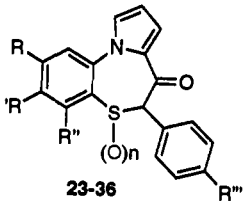
In Vitro SAR Study. Most of the new cyclic compounds were tested in radioreceptor assays and evaluated for their ability to displace [^{3}H]PK 11195 and [^{3}H]-flunitrazepam or [^{3}H]Ro 15-1788 from MBR and CBR, respectively. In Table 2, only the affinities for [^{3}H]PK 11195 binding expressed as IC_{50} are reported; in fact, none of the compounds inhibited [^{3}H]flunitrazepam or [^{3}H]-Ro 15-1788 binding, even at the highest concentration tested (10^{-5} M). Table 2 also reports the data of compounds **23**, **24**, and **65–71**, for comparison.

To single out further structural requirements capable of improving the MBR affinity of 6-arylpyrrolo[2,1-*d*]-[1,5]benzothiazepine derivatives, the SAR study was carried out as a function of: (a) the oxidation of the sulfur atom, (b) the nature and length of the ester side chain introduced at the 7-position, (c) the nature and position of one substituent on the ortho-condensed phenyl ring, and (d) the change at the 6-position from phenyl to *p*-methoxyphenyl moieties.

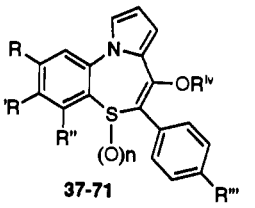
(a) Oxidation of the sulfur atom to sulfoxide or sulfone strongly lowered affinity (**23** vs **33** and **34**, **24** vs **35**, **38** vs **60** and **61**, **44** vs **64**, and **66** vs **62** and **63**).

(b) All aroyloxy derivatives (**48**, **54**, **68**, **69**, and **71**) lacked affinity, whereas all other esters showed micromolar (**45**, **46**, and **59**) or nanomolar values (**37–44**, **47**, **49–53**, **55–58**, **65–67**, and **70**). (Dimethylcarbamoyl)oxy derivatives had higher inhibition potencies, compared to the acetoxy and mesyloxy derivatives (**43** > **38** > **37** and **65** > **66** > **67**), suggesting that the nature of the ester side chain is an important structural feature for MBR binding. But, among the more active aliphatic acid esters, no substantial

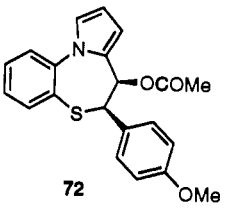
Table 2. Affinities for [^{3}H]PK 11195 Binding Inhibition



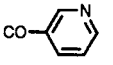
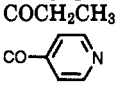
23-36



37-71



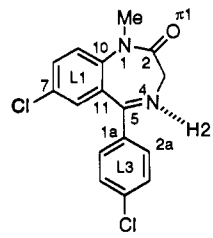
72

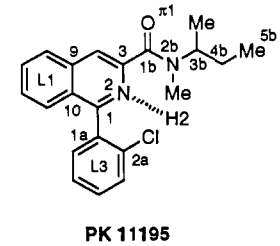
compd	R	R′	R″	R‴	n	R^{iv}	IC$_{50}$ (nM) ± ES
23[a]	H	H	H	H	0		1220 ± 110
24[b]	H	H	H	OCH_3	0		2090 ± 1610
25	CF_3	H	H	OCH_3	0		6210 ± 780
26[c]	Cl	H	H	H	0		NT[d]
27	Cl	H	H	OCH_3	0		1410 ± 360
28	H	Cl	H	H	0		NT[d]
29	H	Cl	H	OCH_3	0		NT[d]
30	H	H	Cl	H	0		NT[d]
31	H	H	Cl	OCH_3	0		NT[d]
32	OCH_3	H	H	OCH_3	0		NT[d]
33	H	H	H	H	1		14700 ± 1660
34	H	H	H	H	2		NA[e]
35	H	H	H	OCH_3	1		23000 ± 15700
36	H	H	H	OCH_3	2		NT[d]
37	H	H	H	H	0	SO_2CH_3	60 ± 10
38	H	H	H	H	0	$COCH_3$	20 ± 2
39	H	H	H	H	0	$COCH_2CH_3$	67 ± 10
40	H	H	H	H	0	$COCH_2CH_2CH_3$	22 ± 5
41	H	H	H	H	0	$COCH_2CH_2CH_2CH_3$	48 ± 5
42	H	H	H	H	0	$COCH_2CH_2CH_2CH_2CH_3$	23 ± 5
43	H	H	H	H	0	$CON(CH_3)_2$	9 ± 1
44	H	H	H	OCH_3	0	$COCH_2CH_2CH_3$	28 ± 3
45	CF_3	H	H	OCH_3	0	$COCH_3$	4530 ± 1060
46	CF_3	H	H	OCH_3	0	$COCH_2CH_3$	2950 ± 470
47	CF_3	H	H	OCH_3	0	$CON(CH_3)_2$	290 ± 40
48	CF_3	H	H	OCH_3	0	$COC_6H_2(OCH_3)_3$	NA[e]
49	Cl	H	H	H	0	$COCH_3$	560 ± 80
50	Cl	H	H	H	0	$COCH_2CH_3$	820 ± 200
51	Cl	H	H	OCH_3	0	$COCH_3$	650 ± 80
52	Cl	H	H	OCH_3	0	$COCH_2CH_3$	490 ± 90
53	Cl	H	H	OCH_3	0	$CON(CH_3)_2$	170 ± 20
54	Cl	H	H	OCH_3	0	$COC_6H_2(OCH_3)_3$	NA[e]
55	H	Cl	H	H	0	$COCH_3$	230 ± 30
56	H	Cl	H	OCH_3	0	$COCH_3$	240 ± 55
57	H	H	Cl	H	0	$COCH_3$	8 ± 1
58	H	H	Cl	OCH_3	0	$COCH_3$	20 ± 6
59	OCH_3	H	H	OCH_3	0	$COCH_3$	1810 ± 640
60	H	H	H	H	1	$COCH_3$	690 ± 110
61	H	H	H	H	2	$COCH_3$	1140 ± 330
62	H	H	H	OCH_3	1	$COCH_3$	2090 ± 140
63	H	H	H	OCH_3	2	$COCH_3$	640 ± 90
64	H	H	H	OCH_3	1	$COCH_2CH_2CH_3$	270 ± 27
65[b]	H	H	H	OCH_3	0	$CON(CH_3)_2$	9[f] ± 1
66[b]	H	H	H	OCH_3	0	$COCH_3$	34 ± 6
67[b]	H	H	H	OCH_3	0	SO_2CH_3	95 ± 10
68[b]	H	H	H	OCH_3	0		NA[e]
69[b]	H	H	H	OCH_3	0	$COC_6H_2(OCH_3)_3$	NA[e]
70[b]	H	H	H	OCH_3	0	$COCH_2CH_3$	30[f] ± 5
71[b]	H	H	H	OCH_3	0		NA[e]
72[b]							NA[e]
PK 11195							2 ± 0
RO 5-4864							58 ± 30

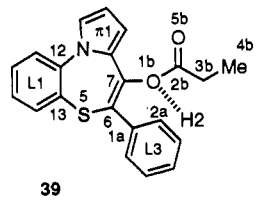
[a] Reference 32. [b] Reference 31. [c] Reference 33b. [d] Not tested. [e] Not active at the highest concentration tested (10^{-5} M). [f] Different stock of compounds 65 and 70 was previously reported as not active in inhibiting [^{3}H]PK 11195 binding. Although we have no clear explanation for such discrepancy, the present data, obtained in homogeneous experimental sessions, substitute previous results.

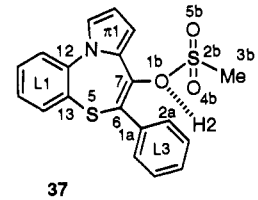
differences in the affinities were observed in the homologous derivatives (see **38–42, 44, 66,** and **70**). In summary, compounds **43, 57,** and **65** were the most active with binding values (IC_{50}s 9, 8, and 9 nM, respectively) only about 4 times smaller than that of reference PK 11195 (IC_{50} 2 nM).

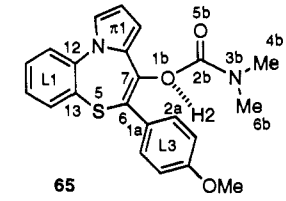
(c) In the active ester series, the introduction of one substituent (Cl, CF_3, OCH_3) at the 2-position, as well as of a chlorine at the 3-position, produced a significant decrease in the affinity, with a trend of $CF_3 > OCH_3 > Cl$ (**65** vs **47** and **53**, **66** vs **45**, **51**, and **59**, **70** vs **46** and **52**, and **66** vs **56**). On the other hand, the 4-chloro substitution

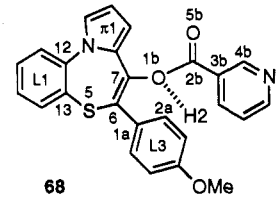


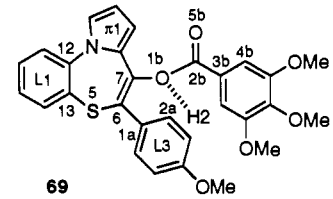


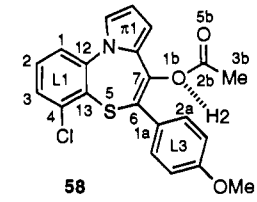


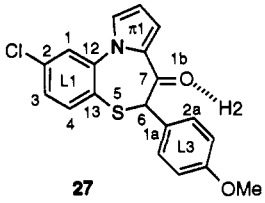


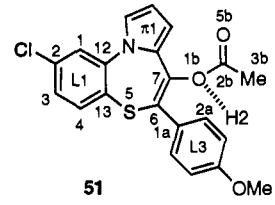












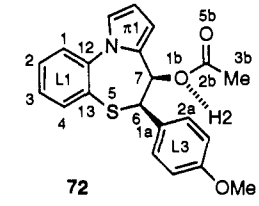


Figure 1. Structures of typical peripheral-mitochondrial-type BDZ receptor ligands (Ro 5-4864 and PK 11195) and of a structurally representative subset of benzothiazepine derivatives. L1, L3, H2, and $\pi 1$ are pharmacophoric points employed in molecular superimpositions. The scheme of atom labeling adopted to define torsional angles is conventional.

caused a moderate increase in affinity (**57** vs **38** and **58** vs **66**), which might indicate that this position is sensitive to steric and/or electronic effects.

(d) Variation at the 6-position between phenyl and *p*-methoxyphenyl moieties had relatively little effect on the affinity (**38** vs **66**, **40** vs **44**, **49** vs **51**, **50** vs **52**, and **55** vs **56**).

Compounds **38** and **44** were used in additional binding studies;[36] the two derivatives had no affinity for $\alpha 1$, $\alpha 2$, and β noradrenoceptors, 5-HT_{1A}, 5-HT_{1B}, 5-HT_2 and 5-HT_3 serotonin receptors, DA1 and DA2 dopaminergic receptors, GABA B, muscarinic, and opiate receptors, A1 and A2 adenosine receptors, cholecystokinin A and B receptors, NMDA, quisqualate and kainate receptors, σ receptors, and 5HT uptake sites.

Biology of 38 and 44. Preliminary pharmacological tests with compounds **38** and **44** indicated possible analgesic activity (phenylquinone writhing[37] and formalin algesia[38]) with no other marked behavioral effects in mice. The two compounds also inhibited 15-lipoxygenase activity[39] (indicative IC_{50}s were 2.3 and 6.2 μM for **44** and **38**, respectively) and platelet aggregation induced by arachidonic acid in vitro[40] (MICs were 1 μM for **44** and 0.1 μM for **38**). Whether these effects are mediated by an activation of MBR needs further investigation.

Molecular Modeling. The geometric and conformational properties of the tested compounds were investigated for their possible relevance in structure–affinity relationships. Nine pyrrolobenzothiazepines listed in Figure 1 (namely **39**, **37**, **65**, **68**, **69**, **58**, **27**, **51**, and **72**) were selected from the whole data set with the assumption that the sampling of their conformational space could provide information valid for other closely related analogs. As an example, most of the conformational features of compound **39** were expected to be in common with compounds **38**, **40–42**, **44–46**, **49–52**, **55–59**, **66**, and **70** which differ only in the length of the ester side chain and/or the presence of small substituents on benzene rings. Two typical ligands of the MBR, Ro 5-4864 and PK 11195, were also investigated and employed as templates for molecular superimpositions.

Systematic conformational searches were carried out on the structures shown in Figure 1 to locate steric energy minima (see the methods section for operational details). We were particularly interested in exploring the energetically feasible arrangements of the side chains at the 7-position of the thiazepine ring and the 3-position of the isoquinoline ring of PK 11195.

Conformational analysis on **39** indicated that two possible orientations of the ester side chain are energetically accessible for this compound: one with the carbon atom C3b (in α to the carbonyl) pointing away from the thiazepine nucleus ("extended", with τ(C7,O1b,C2b,C3b) = 173.2°) and one with the same carbon atom pointing back to this ring ("folded", with τ(C7,O1b,C2b,C3b) = 22.0°). The steric energy of the folded conformer was

estimated to be 3.8 kcal/mol greater than that of the extended conformer. Similar results were found for the mesyl ester derivative **37**, which is another potent ligand in our data set. This compound can acquire a folded conformation with steric energy not much higher than that of its global minimum conformer in the extended steric arrangement (the difference being 2.1 kcal/mol). The extended conformation of the potent carbamyl ester **65** was significantly more stable than the folded one (according to our calculation, the difference being 5.6 kcal/mol). The results of the conformational analysis led us to believe that the extended arrangement of the ester functions might characterize the receptor-bound conformation of the three potent pyrrolobenzothiazepines **39**, **37**, and **65**.

The molecular model of PK 11195 was constructed arbitrarily in the *S* configuration, although the compound was actually tested in vitro as a racemic mixture. The global minimum conformer of PK 11195, according to the molecular mechanics calculations, was found to have the nitrogen of the isoquinoline ring and the carbonyl oxygen of the amide moiety in a trans-type conformation (with reference to the atom-labeling scheme in Figure 1, τ(N2,C3,C1b,N2b) = −25.8°). However, conformers were also detected with these N and O atoms in a cis-type orientation and with energies not far from that of the global minimum (the lowest steric energy value among these "cis conformers" being only 1.6 kcal/mol over the global minimum). It is very common in drug design to examine how structurally diverse ligands might be mutually oriented at the receptor site.[46a,b]

In order to superimpose the ligands shown in Figure 1, we first had to define a set of pharmacophoric elements. These "key" substructures were identified on the basis of the following considerations. Many benzodiazepine derivatives, such as diazepam (which is the 4′-deschloro analog of Ro 5-4864), bind to CBR and MBR with comparable potency.[6,22,41–43] Structure–affinity relationship data[6,22,41–43] have shown that the CBR/MBR selectivity profiles of benzodiazepines often result from subtle structural modifications not altering the pharmacophoric functions considered critical for CBR binding.[44,45] Therefore, it seemed reasonable superimposing the MBR ligands investigated by following a pharmacophoric scheme proposed earlier for benzodiazepines binding to CBR.[44,45] The following are the pharmacophoric substructures (see labels in Figure 1) employed to align the ligands: (1) the centroid L1 of the ortho-condensed benzene ring, (2) the centroid L3 of the pendant phenyl ring, (3) the lone-pair H2, which extends from an iminic nitrogen in Ro 5-4864 and PK 11195 and, in the pyrrolobenzothiazepine derivatives, from a sp^2 (ketones) or sp^3 (esters) hybridized oxygen; this lone pair was elongated up to 2.0 Å in the direction of a hypothetical hydrogen-bond-donor function, and (4) the electronegative moiety π1, constituted by a carbonyl oxygen in Ro 5-4864 and PK 11195 and by the centroid of the ortho-condensed pyrrole ring. More specifically, L1 and L3 describe the positions of benzene rings interacting with lipophilic receptor areas; H2 indicates the position of a receptor complementary site donating a hydrogen bond; π1 represents an electron-rich moiety of the ligand capable of giving rise to an ionic dipole interaction with a positively charged receptor site. The conventional notations of the pharmacophoric points adopted in the present study are the same as those proposed originally by Cook et al.[44] (L1, L3, and H2 points) and Fryer[45] (π1 point) in their models of "central-type" BDZ receptors.

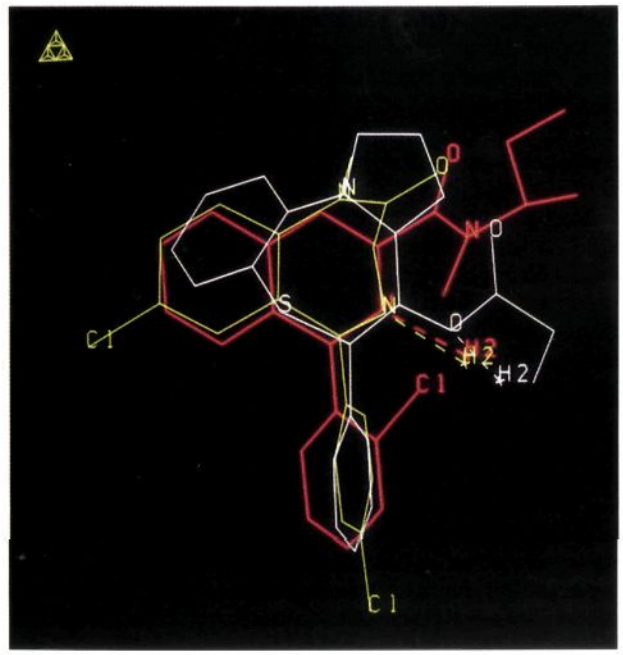


Figure 2. Structures of Ro 5-4864 (green), PK 11195 (red), and **39** (white) superimposed in their supposed receptor-bound conformations. The dashed lines indicate hydrogen bonds formed between each ligand and a receptor-donor site termed H2.

As already mentioned, the structure of Ro 5-4864, in which the distances between the four pharmacophoric points L1, L3, H2, and π1 are not significantly influenced by conformational flexibility, was used as template for molecular superimpositions. Among the various conformers of PK 11195, we selected the one which overlapped Ro 5-4864 with the lowest root-mean-square (rms) distance between these pharmacophoric points. In this geometry, which is the global minimum, the isoquinoline ring nitrogen and the amidic carbonyl oxygen of PK 11195 are in a trans-type arrangement.

The global minimum conformations of **39**, **37**, and **65** were found to be superimposable on the structures of both Ro 5-4864 and PK 11195. Figure 2 shows the superimposed conformations of Ro 5-4864, PK 11195, and **39**. Compounds **37** and **65**, like all the ester derivatives listed in Figure 1, fitted the template with almost identical rms distances (for the sake of clarity, only compound **39** is shown in Figure 2). To maximize the complanarity of the L3 ring planes, the pendant phenyl rings of Ro 5-4864 and PK 11195 were rotated a small amount to obtain geometries with steric energies still close to the global minimum (see Table 3). To further refine our model, the torsion angle τ(N2,C3,C1b,N2b) of PK 11195 was rotated about −20° from its energy minimum. This rotation made the carbon atom of the *N*-methyl group move up to 2.7 Å away from the H2 lone pair so as not to interfere with the postulated hydrogen-bond interaction at the H2 site. Table 3 summarizes the geometric and conformational parameters associated with the proposed bioactive conformations of Ro 5-4864, PK 11195, **39**, **37**, and **65**.

Figure 2 suggests the Ro 5-4864, PK 11195, and our pyrrolobenzothiazepine derivatives bind to the MBR by sharing the same type of fundamental interactions. Although the conformational search on PK 11195 also led to low-energy conformers characterized by a cis-type arrangement between the ring nitrogen and the carbonyl

Table 3. Geometric and Conformational Parameters of Receptor-Bound Conformations[a]

ligand	Econf	Emin	$\tau 1$	$\tau 2$	$\tau 3$	$\tau 4$	$\tau 5$	L1–L3	L1–H2	L1–π1	L3–H2	L3–π1	H2–π1	rms
Ro 5-4864	16.46	12.83	−104.9				42.2	4.94	5.75	4.91	3.84	6.40	4.19	0.00
PK 11195	3.55	9.07	−74.6	−44.7	173.4	72.4	173.4	4.91	5.58	5.57	3.71	7.01	4.08	0.64
39	27.51	27.51	−45.9	167.2	173.2	60.9	−71.2	5.88	6.62	3.90	3.77	6.39	4.75	0.95
37	25.10	25.10	−42.2	167.6	−157.0		−69.6	5.88	6.64	3.89	3.81	6.41	4.78	0.96
65	31.53	31.53	−45.9	167.1	174.2	179.8	−72.2	5.86	6.59	3.90	3.75	6.38	4.75	0.95

[a] Econf is the steric energy of the proposed receptor-bound conformation. Emin is the steric energy of the global minimum conformer. In the structure of Ro 5-4864, $\tau 1$ and $\tau 5$ (in degrees) correspond to the following torsional angles: $\tau 1 = \tau$(C2a,C1a,C5,C4) and $\tau 5 = \tau$(C10,C11,C5,N4). In the structure of PK 11195, $\tau 1$–$\tau 5$ (in degrees) correspond to the following torsional angles: $\tau 1 = \tau$(C2a,C1a,C1,N2), $\tau 2 = \tau$(N2,C3,C1b,N2b), $\tau 3 = \tau$(C3,C1b,N2b,C3b), $\tau 4 = \tau$(C1b,N2b,C3b,C4b), and $\tau 5 = \tau$(N2b,C3b,C4b,C5b). In the benzothiazepine set, $\tau 1$–$\tau 5$ (in degrees) correspond to the following torsional angles: $\tau 1 = \tau$(C2a,C1a,C6,C7), $\tau 2 = \tau$(C6,C7,O1b,C2b), $\tau 3 = \tau$(C7,O1b,X2b,X3b), $\tau 4 = \tau$(O1b,X2b,X3b,C4b), and $\tau 5 = \tau$(C12,C13,S5,C6). L1–L3, L1–H2, L1–π1, L3–H2, L3–π1, and H2–π1 are the distances (Å) between the pharmacophore points. rms is the root-mean-square distance (Å) resulting from the fit of the molecules on Ro 5-4864 at the pharmacophore points. See Figure 1 for atom labeling.

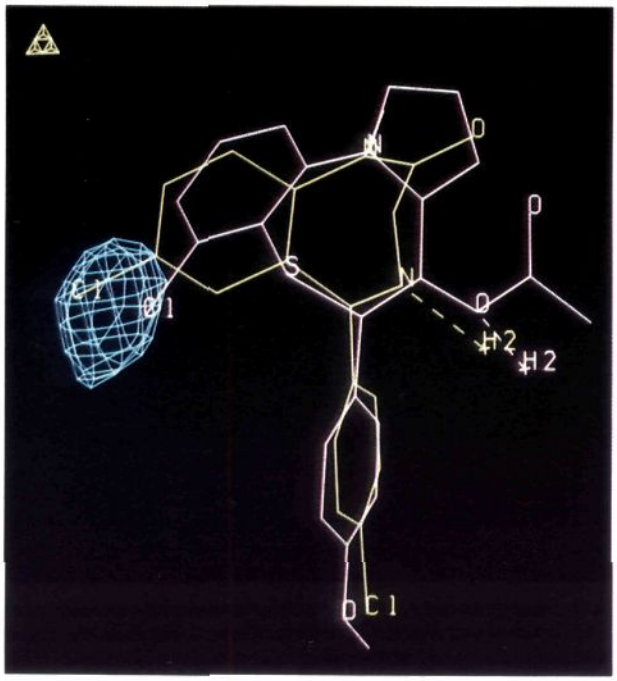


Figure 3. Structures of Ro 5-4864 (green) and **58** (white) superimposed in their bioactive conformations. The blue contour displays the volume in common to the chlorine atoms attached to the ortho-condensed benzene rings.

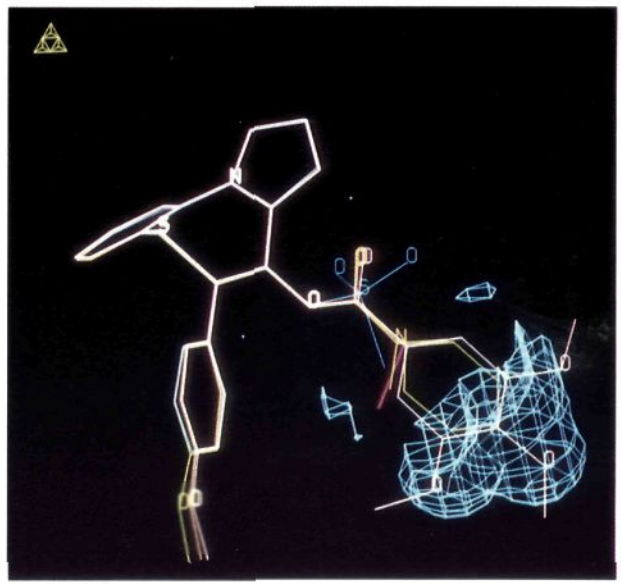


Figure 4. Superimposed structures of active ligands **39** (red), **37** (blue), and **65** (yellow) and inactive ligands **68** (green) and **69** (white). The extra volume of **68** and **69** responsible for their lack of affinity is depicted in blue.

oxygen, on the basis of the proposed molecular fit, these conformations were assumed to be biologically irrelevant. The amide and ester side chains of PK 11195 and **39**, respectively, occupy distinct receptor domains. This suggests there is a region of relative steric tolerance, somewhere "halfway" between the sites complementary to π1 and H2, capable of accommodating apolar functions. Finally, it becomes possible to define which of the two lone pairs of the ester oxygen (in Figure 1 labeled O1b) is involved in a hydrogen bond with the receptor. The direction of this lone pair is described by the torsional angle τ(C6,C7,O1b,H2) which takes the value of −71.0° in structures **39**, **37**, and **65**.

As previously reported, within the series of 7-alkanoyloxy derivatives, the introduction of small groups (Cl, CF_3, and OCH_3) at the 2- and 3-positions invariably led to loss of affinity, while chloro substitution at the 4-position caused a small increase in affinity. Superimposition of the 4-chloro derivative **58** and Ro 5-4864 indicates that the chlorine atoms attached to the ortho-condensed rings share a significant amount of volume (see Figure 3). This supports the validity of molecular alignment proposed since the 4-chloro substitution in the set of pyrrolobenzothiazepines improves the binding affinity similarly to the 7-chloro substitution in the benzodiazepine analogs of Ro 5-4864.[6]

We also tried to highlight the factors responsible for the partial or total lack of affinity in a number of pyrrolobenzothiazepines with functions at the 7-position different from alkanoyloxy groups. One way to explain the lack of affinity of the aroyloxy derivatives **68** and **69** is that their side chains, although capable of assuming conformations similar to those of active analogs such as **39**, violate steric complementarity requirements in the receptor cavity. In other words, one reason for the inactivity could be the competition between the receptor and the ligand for common space when the ligand assumes the appropriate conformation and orientation to present the pharmacophore.

To test this hypotesis, molecular volume manipulations were done on the structures of both active and inactive ligands aligned in their supposed receptor-bound conformations. The extra volume of the inactive compounds was generated according to the procedure proposed by Sufrin et al.[46a] Thus, the union volume of the active ligands (**39**, **37**, and **65**) was subtracted from the volume in common to the inactive ones (**68** and **69**). The resulting volume is shown in Figure 4 and can be viewed as a sterically hindered receptor region that prevents the binding of ligands with bulky side chains.

Compounds with 7-alkanoyloxy side chains longer than

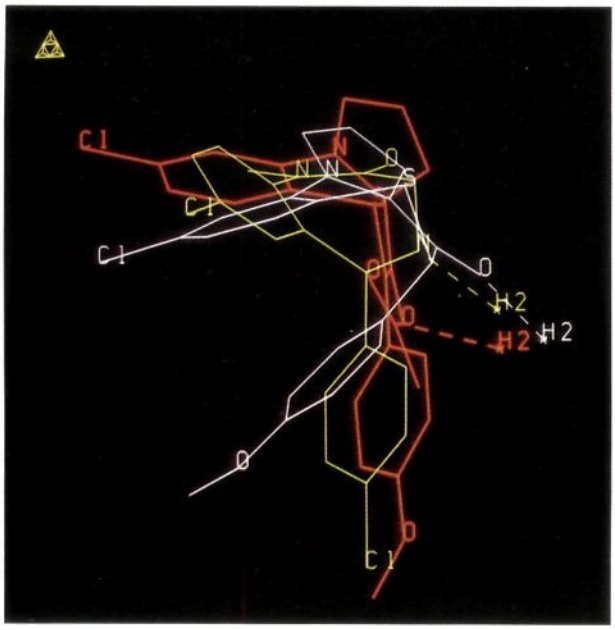


Figure 5. Structures of Ro 5-4864 (green), the *S* enantiomer of **27** in the phenyl-axial conformation (white), and **51** (red). Note that the L1 and L3 ring planes of **27** do not overlap those of **51**. Moreover, the chlorine atoms attached to the L1 rings point, in the three molecules, toward different directions.

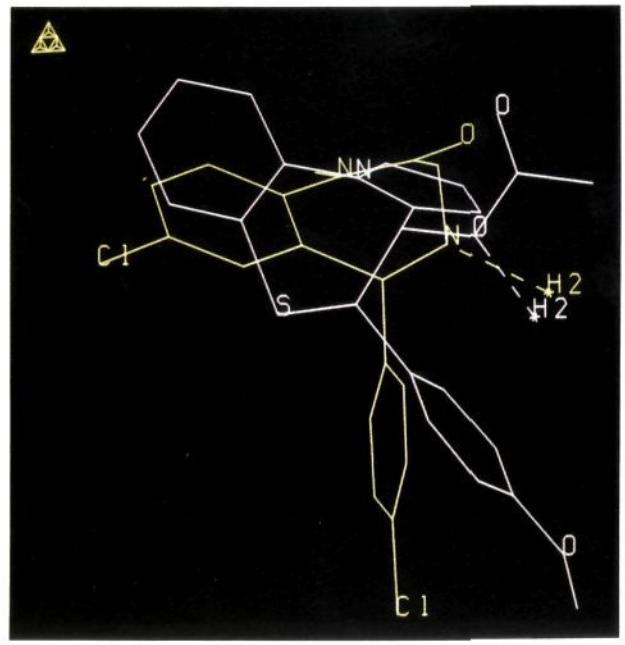


Figure 6. The *S,S* optical isomer of **72** in the phenyl-equatorial conformations (white) superimposed to Ro 5-4864 (green). Provided that **72** forms a hydrogen bond with the H2 receptor subsite, its benzene rings do not overlap those of Ro 5-4864.

that of **39** had comparable affinities (see biological data in Table 2 relative to **39** and their homologs **40**, **41**, and **42**). The high affinity of the latter compounds, in relation to the inactive 7-aroyloxy derivatives **68** and **69**, probably resides in their higher degree of torsional freedom. The flexible alkanoyloxy substituents can probably easily adopt energetically feasible conformations so as to interact within the boundaries of the receptor region surrounding the 7-position of the ligands.

The 7-ketone analogs **23**–**25**, **27**, and **33**–**35** were poorly active or not active at all; although they were all tested in vitro as racemic mixtures, it is reasonable to assume that both enantiomers have binding affinity values not far from those listed in Table 2. Since the compounds considered feature the basic pharmacophoric elements that we postulated as necessary for MBR binding, the reason for their low potency merited further investigation.

First, in the 7-ketone derivatives, contrary to the 7-alkanoyloxy series, the influence of the 2-substitution does not seem related to a decrease of affinity (compare the binding data of **24**, **25**, and **27** in Table 2, which are practically equipotent). This suggests that the 2-substituents of the 7-ketone derivatives are probably not oriented the same way at the receptor site as the 2-substituent of the 7-alkanoyloxy derivatives. To check this, we superimposed the molecular models of the 7-ketone derivative **27** and the 7-ester derivative **51**, both bearing a chlorine atom at the 2-position, on the template Ro 5-4864. For compound **27**, we considered both phenyl-equatorial and phenyl-axial conformers for each enantiomer, the latter being 4.3 kcal/mol lower in steric energy. Among the four possible geometries associated with **27**, the *S* enantiomer in the phenyl-axial conformation was selected for molecular comparison as it had the lowest fitting rms distance (0.85 Å). On examining the superimposed structures (see Figure 5), we see that the 2-chlorine atom of **27** points away from the 2-chlorine atom of **51**. This suggests that the potency of the 7-ketone and 7-alkanoyloxy analogs is not affected by the 2-substitution to the same extent because the small groups at the 2-positions of **27** and **51** fit into different receptor domains.

Further inspection of the molecular fit reveals that the planes of the benzene rings of **27** do not overlap well the corresponding ones of Ro 5-4864. The bad superimposition of the L1 and L3 pharmacophoric moieties of compounds **27** and Ro 5-4864 might partly account for the low potency of the 7-ketone derivatives.

To understand the lack of activity of the 6,6-dihydro ester derivative **72** (tested as a racemic mixture of the cis form), molecular superimpositions were performed on the template Ro 5-4864. For each enantiomer of **72**, there are two main conformers with close steric energy values: the phenyl-equatorial type and the phenyl-axial type (the former is 1.4 kcal/mol more stable than the latter). The molecular model of **72**, whose fitting on Ro 5-4864 yielded by far the lowest rms value (1.17 Å), was the phenyl-equatorial *S,S* enantiomer (see Figure 6). The spatial arrangements of the benzene rings of **72** differ dramatically from those of the template.

Conclusion

Novel effective ligands have been synthesized specific for mitochondrial benzodiazepine receptors: 6-arylpyrrolo-[2,1-*d*][1,5]benzothiazepine derivatives. A SAR study on 42 compounds and a molecular modeling study have been developed. Structural requirements for the binding affinity are proposed. The results allow for the design of novel derivatives with greater MBR affinity. The 6-arylpyrrolo[2,1-*d*][1,5]benzothiazepine system can thus be considered a highly promising basis for determination of the functional role of MBR.

Experimental Section

Melting points were determined using an Electrothermal 8103 apparatus and are uncorrected. IR spectra were taken as Nujol mulls with Perkin-Elmer 398 and 1600 spectrophotometers. ^{1}H-NMR spectra were recorded on a Varian XL 200 spectrometer with TMS as internal standard; the values of chemical shifts (δ)

are given in ppm and coupling costants (J) in Hz. All reactions were carried out in an atmosphere of dry nitrogen. Progress of the reaction was monitored by TLC on silica gel plates (Riedel-de-Haen, Art. 37341). Merck silica gel (Kieselgel 60) was used for chromatography (70–230 mesh) and flash chromatography (230–400 mesh) columns. Extracts were dried over Na_2SO_4, and solvents were removed under reduced pressure. Elemental analyses were performed on a Perkin-Elmer 240C elemental analyzer, and the results are within ±0.4% of the theoretical values, unless otherwise noted. Yields refer to the purified products and are not optimized.

Bis(5-chloro-2-*N*-pyrrolylphenyl)disulfide (4). To a suspension of bis(2-amino-5-chlorophenyl)disulfide (1.2 g, 37.8 mmol) in glacial acetic acid (40 mL) was slowly added a solution of 2,5-dimethoxytetrahydrofuran (9.8 g, 74 mmol) in the same solvent (15 mL), and the resulting mixture was heated at 100 °C for 30 min. After cooling, the reaction mixture was concentrated in vacuo and the residue was taken up in Et_2O, washed with saturated $NaHCO_3$ solution and H_2O, and dried. The solvent was removed, and the pasty residue was purified by flash column chromatography (CH_2Cl_2). Recrystallization from *n*-hexane gave the title compound 4 (85.3%) as yellow needles (mp 98–101 °C). ^1H NMR ($CDCl_3$): δ 6.36 (t, 4H, J = 2.3 Hz, β-pyrrole H), 6.78 (t, 4H, J = 1.9 Hz, α-pyrrole H), 7.10–7.55 (m, 6H, ArH). Anal. ($C_{20}H_{14}Cl_2N_2S_2$) C, H, N.

Bis(6-chloro-2-*N*-pyrrolylphenyl)disulfide (5). Compound 5 was prepared according to the procedure described for 4 starting from bis(2-amino-6-chlorophenyl)disulfide (5.4 g, 17.0 mmol). In this case, flash column chromatography (50% $CHCl_3$–petroleum ether (60–80 °C)) and the recrystallization from cyclohexane afforded **5** (40%) as yellow needles (mp 68–70 °C). ^1H NMR ($CDCl_3$): δ 6.16 (t, 4H, J = 2.1 Hz, β-pyrrole H), 6.46 (t, 4H, J = 2.1 Hz, α-pyrrole H), 7.10–7.50 (m, 6H, ArH). Anal. ($C_{20}H_{14}Cl_2N_2S_2$) C, H, N.

General Procedure for the Preparation of Esters 7–12. This procedure is illustrated for the preparation of *p*-methoxy-α-[[4-chloro-2-(1*H*-pyrrol-1-yl)phenyl]thio]phenylacetic acid ethyl ester (**9**). A suspension of bis(4-chloro-2-*N*-pyrrolylphenyl)-disulfide (**3**) (3.04 g, 7.28 mmol) in 50 mL of anhydrous EtOH was heated to reflux. Sodium borohydride (0.55 g, 14.5 mmol) was then added carefully, in portions, over a 30-min period. When adding stopped, the mixture was allowed to cool to room temperature, and ethyl α-bromo-*p*-methoxyphenylacetate (3.97 g, 14.5 mmol) in 25 mL of anhydrous EtOH was slowly added. After stirring 8 h at room temperature, the reaction mixture was reduced to one-half of the original volume in vacuo and poured into an equal volume of ice–water. The collected precipitate, washed with H_2O and dried, was purified by column chromatography (50% chloroform–hexane); **9** was obtained (80.5%) as an amorphous white solid. Recrystallization from EtOH afforded an analytical sample (mp 74–75.5 °C). ^1H NMR ($CDCl_3$): δ 1.08 (t, 3H, J = 7.3 Hz, CH_3), 3.78 (s, 3H, OCH_3), 4.01 (m, 2H, CH_2), 4.25 (s, 1H, CH), 6.36 (t, 2H, J = 2.1 Hz, β-pyrrole H), 6.79 (d, 2H, J = 8.9 Hz, ArH), 6.94 (t, 2H, J = 2.2 Hz, α-pyrrole H), 7.18–7.50 (m, 5H, ArH). IR: 1728 cm^{-1}. Anal. ($C_{21}H_{20}ClNO_3S$) C, H, N.

This procedure was repeated with the appropriate bis(2-*N*-pyrrolylphenyl)disulfide **2**, **4**, **5**, and **6** to give the esters **8**, **10**, **11**, and **12**, respectively. Yields and analytical data are given in Table 1. The IR and the ^1H NMR spectra were consistent with the assigned structures.

General Procedure for the Preparation of Acids 13, 16, 18, and 20. This procedure is illustrated for the preparation of α-[[5-chloro-2-(1*H*-pyrrol-1-yl)phenyl]thio]phenylacetic acid (**18**). The compound was prepared starting from bis(5-chloro-2-*N*-pyrrolylphenyl)disulfide (**4**) (2 g, 4.79 mmol), sodium borohydride (0.36 g, 9.58 mmol), and α-bromophenylacetic acid (2.06 g, 9.58 mmol) in anhydrous EtOH and operating likewise obtaining ester **9**. After stirring for one night at room temperature, the reduced-volume reaction mixture was poured into an equal volume of ice–water and then extracted with CH_2Cl_2. The combined organic extracts were washed with water, dried, and concentrated. The pasty product obtained was treated with petroleum ether (bp 40–60 °C) to give the crude title acid **18** (85.1%). Recrystallization from *n*-hexane gave an analytical sample (mp 128–130 °C) as a colorless solid. ^1H NMR (DMSO-d_6): δ 5.24 (s, 1H, CH), 6.26 (m, 2H, β-pyrrole H), 6.90 (m, 2H, α-pyrrole H), 7.20–7.65 (m, 8H, ArH), 13.26 (b s, 1H, COOH, D_2O exchanged). IR; 3420, 1720 cm^{-1}. Anal. ($C_{18}H_{14}ClNO_2S$) C, H, N.

Similarly **13**, **16**, and **20** were prepared starting from related disulfides **1**, **3**, and **5**. Yields and analytical data are given in Table 1. The IR and ^1H NMR spectra were consistent with the assigned structures.

General Procedure for the Preparation of Acids 15, 17, 19, 21, and 22. Basic Hydrolysis of Ester Derivatives. This procedure is illustrated for the preparation of *p*-methoxy-α-[[4-chloro-2-(1*H*-pyrrol-1-yl)phenyl]thio]phenylacetic acid (**17**). The ester **9** (2.63 g, 6.54 mmol) was dissolved in 22 mL of EtOH/tetrahydrofuran mixture (1:1), and 5% aqueous NaOH (23 mL) was slowly added. The reaction mixture was stirred at room temperature for 1 h, concentrated, and acidified with dilute HCl until pH 3–4. The yellow oil obtained was extracted with CH_2Cl_2, and the organic phase was washed, dried, and concentrated. The residue was triturated with petroleum ether (bp 40–60 °C) to afford the crude title compound (74.3%); **17** was recrystallized from EtOH (mp 135–137 °C). ^1H NMR ($CDCl_3$): δ 3.76 (s, 3H, OCH_3), 4.22 (s, 1H, CH), 6.35 (t, 2H, J = 2.1 Hz, β-pyrrole H), 6.79 (d, 2H, J = 8.6 Hz, ArH), 6.98–7.60 (m, 7H, α-pyrrole H and ArH), 9.80 (b s, 1H, COOH, D_2O exchanged). IR: 1715 cm^{-1}. Anal. ($C_{19}H_{16}ClNO_3S$) C, H, N.

Similarly, compounds **15**, **19**, **21**, and **22** were prepared. Yields and analytical data are reported in Table 1. The IR and ^1H-NMR spectra were consistent with the assigned structures.

6-(*p*-Methoxyphenyl)-2-(trifluoromethyl)pyrrolo[2,1-*d*][1,5]benzothiazepin-7(6*H*)-one (25). To a well-stirred solution of **15** (2.25 g, 5.52 mmol) in anhydrous CH_2Cl_2 (30 mL) was slowly added a suspension of PCl_5 (1.14 g, 5.55 mmol) in the same solvent (20 mL). The resulting mixture was heated at 70 °C for 5 h and allowed to stir at room temperature for 12 h. The solvent was removed under reduced pressure, and the oily dark residue was treated with Et_2O (90 mL, 3×). The ethereal solution was washed consecutively with 5% aqueous NaOH and water. The organic phase was dried, filtered, and concentrated to yield a residue that was purified by column chromatography using $CHCl_3$ as the eluant. The title compound was obtained (50.2%) as a white solid. An analytical sample was recrystallized from EtOH, (mp 132–134 °C, colorless prisms). ^1H NMR ($CDCl_3$): δ 3.70 (s, 3H, OCH_3), 4.83 (s, 1H, H-6), 6.52 (m, 1H, H-9), 6.68 (d, 2H, J = 8.8 Hz, ArH), 6.95 (d, 2H, J = 8.6 Hz, ArH), 7.15–7.65 (m, 5H, ArH, H-8, and H-10). IR: 1645 cm^{-1}. Anal. ($C_{20}H_{14}F_3NO_2S$) C, H, N.

2-Chloro-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepin-7(6*H*)-one (27). Starting from **17** (4.1 g, 10.96 mmol), the title compound **27** was prepared according to the procedure described above for **25**. Purification by flash chromatography ($CHCl_3$) gave the desired product (43.5%) as a colorless amorphous solid. An analytical sample was recrystallized from benzene as white needles (mp 194–195 °C). ^1H NMR ($CDCl_3$): δ 3.72 (s, 1H, OCH_3), 4.70 (s, 1H, H-6), 6.48 (m, 1H, H-9), 6.69 (d, 2H, J = 8.8 Hz, ArH), 6.95 (d, 2H, J = 8.8 Hz, ArH), 7.04–7.50 (m, 5H, ArH, H-8, and H-10). IR: 1645 cm^{-1}. Anal. ($C_{19}H_{14}ClNOS$) C, H, N.

3-Chloro-6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepin-7(6*H*)-one (28). Starting from 1.9 g (5.52 mmol) of **18**, the title compound **28** was obtained according to the procedure described above for **25**, except that the reaction mixture was heated at 80 °C for 3 h. Column chromatography ($CHCl_3$) afforded **28** (78.9%) as a white amorphous solid. An analytical sample was obtained from cyclohexane (mp 118–120 °C). ^1H NMR ($CDCl_3$): δ 4.83 (s, 1H, H-6), 6.47 (t, 1H, J = 2.9 Hz, H-9), 6.95–7.50 (m, 10H, ArH, H-8, and H-10). IR: 1650 cm^{-1}. Anal. ($C_{18}H_{12}ClNOS$) C, H, N.

3-Chloro-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepin-7(6*H*)-one (29). Starting from 2.1 g (5.61 mmol) of **19**, the title compound **29** was prepared using an identical procedure as for **25**. After chromatography column purification ($CHCl_3$), **29** was obtained (70.3%) and recrystallized from EtOH (mp 116–119 °C). ^1H NMR ($CDCl_3$): δ 3.72 (s, 3H, OCH_3), 4.79 (s, 1H, H-6), 6.47 (t, 1H, J = 1.8 Hz, H-9), 6.70 (d, 2H, J = 8.4 Hz, ArH), 6.96 (d, 2H, J = 8.7 Hz, ArH), 7.10 (t, 1H, J = 2.4 Hz, H-8), 7.20–7.50 (m, 4H, ArH and H-10). IR: 1650 cm^{-1}. Anal. ($C_{19}H_{14}ClNO_2S$) C, H, N.

4-Chloro-6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepin-7(6*H*)-one (30). Starting from 3.5 g (10.17 mmol) of **20**, the title compound **30** was prepared according to the procedure described

for **25** (5 h, 70 °C). Column chromatography (50% $CHCl_3$–petroleum ether, bp 60–80 °C) afforded **30** (55.6%). An analytical sample was obtained from EtOH (mp 107–110 °C). ^1H NMR ($CDCl_3$): δ 4.92 (s, 1H, H-6), 6.44 (t, 1H, J = 3.3 Hz, H-9), 6.90–7.55 (m, 10H, ArH, H-8, and H-10). IR: 1650 cm^{-1}. Anal. ($C_{18}H_{12}ClNOS$) C, H, N.

4-Chloro-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepin-7(6*H*)-one (31). Starting from 3 g (8.02 mmol) of **21**, the title compound **31** was prepared with a procedure similar to the preparation of **25** (5 h, 70 °C). Column chromatography ($CHCl_3$) afforded **31** (49.5%) as a white amorphous solid. An analytical sample was obtained from EtOH (mp 122–124 °C). ^1H NMR ($CDCl_3$): δ 3.69 (s, 3H, OCH_3), 4.92 (s, 1H, H-6), 6.47 (t, 1H, J = 1.9 Hz, H-9), 6.65 (d, 2H, J = 8.4 Hz, ArH), 6.95 (d, 2H, J = 8.7 Hz, ArH), 7.10 (t, 1H, J = 2.4 Hz, H-8), 7.18–7.48 (m, 4H, ArH and H-10). IR: 1642 cm^{-1}. Anal. ($C_{19}H_{14}ClNO_2S$) C, H, N.

2-Methoxy-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepin-7(6*H*)-one (32). Starting from 1.38 g (3.73 mmol) of crude **22**, the title compound was obtained according to the procedure described above for **25**, except that the reaction mixture was allowed to stir overnight at room temperature. After column chromatography ($CHCl_3$), the pasty clear residue was used crude. IR: 1645 cm^{-1}.

6-Phenylpyrrolo[2,1-*d*][1,5]benzothiazepin-7(6*H*)-one 5-Oxide (33). To a well-stirred and cooled (−2 °C) solution of **23** (1 g, 3.43 mmol) in 10 mL of dry chloroform was added 3-chloroperbenzoic acid (0.59 g, 3.43 mmol) in the same solvent (20 mL) dropwise over 1 h. After an additional 12 h at 0 °C, the reaction mixture was filtered and the filter cake was rinsed with chloroform. The combined solutions were washed twice with 5% aqueous potassium carbonate, dried, and evaporated to give crude **33** (63.2%) which was recrystallized from ligroin (bp 75–120 °C) as a white solid (mp 117–118 °C). ^1H NMR ($CDCl_3$): δ 5.23 (s, 1H, H-6), 6.54 (m, 1H, H-9), 6.68–7.70 (m, 11H, ArH, H-8, and H-10). IR: 1643, 1053 cm^{-1}. Anal. ($C_{18}H_{13}NO_2S$) C, H, N.

6-Phenylpyrrolo[2,1-*d*][1,5]benzothiazepin-7(6*H*)-one 5,5-Dioxide (34). To a well-stirred and cooled (−2 °C) solution of **23** (1.35 g, 4.64 mmol) in 25 mL of anhydrous chloroform was slowly added 3-chloroperbenzoic acid (2.39 g, 13.89 mmol) in the same solvent (70 mL). After an additional 1 h at the same temperature, the reaction mixture was stirred for 12 h at room temperature and filtered. The precipitate was washed with chloroform and discarded, and the combined solutions were treated twice with 5% aqueous potassium carbonate, dried, and evaporated. Crude **34** was purified by a chromatography column (chloroform/ethyl acetate 9:1) (35.9%). An analytical sample was obtained by recrystallization from ligroin (bp 75–120 °C) as white microcrystals (mp 155–156 °C). ^1H NMR ($CDCl_3$): δ 5.37 (s, 1H, H-6), 6.57 (m, 1H, H-9), 7.00–7.80 (m, 11H, ArH, H-8, and H-10). IR: 1636, 1323, 1123 cm^{-1}. Anal. ($C_{18}H_{13}NO_3S$) C, H, N.

6-(*p*-Methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepin-7(6*H*)-one 5-Oxide (35). The title compound was prepared according to the procedure described for **33**, starting from **24** (0.85 g, 2.64 mmol). Crude **35** was purified by column chromatography eluting with $CHCl_3$ (78.8%) and recrystallized from ethyl acetate as colorless needles (mp 156–157 °C). ^1H NMR ($CDCl_3$): δ 3.71 (s, 3H, OCH_3), 4.78 (s, 1H, H-6), 6.47 (t, 1H, J = 2.0 Hz, H-9), 6.68 (d, 2H, J = 8.8 Hz, ArH), 6.96 (d, 2H, J = 8.6 Hz, ArH), 7.08–7.80 (m, 6H, ArH, H-8, and H-10). IR: 1640, 1040 cm^{-1}. Anal. ($C_{19}H_{15}NO_3S$) C, H, N.

6-(*p*-Methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepin-7(6*H*)-one 5,5-Dioxide (36). The title compound was prepared according to the procedure described for **34**, starting from **24** (0.9 g, 2.8 mmol). Crude **36** (85%) was recrystallized from ligroin (75–120 °C) as a white solid (mp 80–82 °C). ^1H NMR ($CDCl_3$): δ 3.71 (s, 3H, OCH_3), 5.30 (s, 1H, H-6), 6.58 (m, 1H, H-9), 6.67 (d, 2H, J = 8.8 Hz, ArH), 6.95 (d, 2H, J = 8.7 Hz, ArH), 7.12–7.80 (m, 6H, ArH, H-8, and H-10). IR: 1640, 1324, 1123 cm^{-1}. Anal. ($C_{19}H_{15}NO_4S$) C, H, N.

General Procedure for the Preparation of Compounds 37–64. This procedure is illustrated for the preparation of 7-(mesyloxy)-6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepine (**37**). To a stirred solution of compound **23** (2 g, 6.86 mmol) in anhydrous tetrahydrofuran (25 mL) was slowly added a suspension of potassium hydride (0.27 g, 6.86 mmol) in 10 mL of the same solvent. The reaction mixture was allowed to stir for 5 h at room temperature. A solution of mesyl chloride (0.78 g, 6.86 mmol) in anhydrous tetrahydrofuran (5 mL) was then added dropwise, and the mixture was stirred overnight. The reaction mixture was poured onto crushed ice, washed with petroleum ether (bp 40–60 °C), and extracted with Et_2O. The combined organic layers were washed with water and dried, and the solvent was removed. The obtained pasty green residue was purified by chromatography ($CHCl_3$) affording **37** (40.1%) which, after crystallization from EtOH, melted at 124.5–125 °C (green crystals). ^1H NMR ($CDCl_3$): δ 2.36 (s, 3H, CH_3), 6.48 (t, 1H, J = 3.4 Hz, H-9), 6.88 (m, 1H, H-8), 7.00–7.85 (m, 10H, ArH and H-10). IR: 1372, 1180 cm^{-1}. Anal. ($C_{19}H_{15}NO_3S_2$) C, H, N.

Similarly, **38–64** were obtained. The reaction times after the proper acid chloride adding are reported.

7-Acetoxy-6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepine (38). Starting from **23** (2.70 g, 9.26 mmol), the title compound was obtained (reaction time 12 h) (20.2%) and recrystallized from EtOH as white microcrystals (mp 91–93 °C). ^1H NMR ($CDCl_3$): δ 1.96 (s, 3H, CH_3), 6.42 (t, 1H, J = 3.3 Hz, H-9), 6.63 (m, 1H, H-8), 7.10–7.80 (m, 10H, ArH and H-10). IR: 1775 cm^{-1}. Anal. ($C_{20}H_{15}NO_2S$) C, H, N.

6-Phenyl-7-(propionyloxy)pyrrolo[2,1-*d*][1,5]benzothiazepine (39). Starting from **23** (1 g, 3.43 mmol), the title compound was obtained (reaction time 12 h) (46.2%) and recrystallized from EtOH as a white solid (mp 94–96 °C). ^1H NMR ($CDCl_3$): δ 0.97 (t, 3H, J = 7.5 Hz, CH_3), 2.25 (q, 2H, J = 7.6 Hz, CH_2), 6.42 (m, 1H, H-9), 6.59 (m, 1H, H-8), 7.10–7.80 (m, 10H, ArH and H-10). IR: 1760 cm^{-1}. Anal. ($C_{21}H_{17}NO_2S$) C, H, N.

7-(Butyryloxy)-6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepine (40). Starting from 1.89 g (6.48 mmol) of compound **23**, the title compound was obtained (reaction time 7 h) (74.3%) and recrystallized from EtOH as colorless needles (mp 90–91 °C). ^1H NMR ($CDCl_3$): δ 0.76 (t, 3H, J = 7.4 Hz, CH_3), 1.48 (m, 2H, CH_2CH_3), 2.21 (t, 2H, J = 7.3 Hz, $CH_2CH_2CH_3$), 6.42 (m, 1H, H-9), 6.61 (m, 1H, H-8), 7.10–7.80 (m, 10H, ArH and H-10). IR: 1752 cm^{-1}. Anal. ($C_{22}H_{19}NO_2S$) C, H, N.

6-Phenyl-7-(valeryloxy)pyrrolo[2,1-*d*][1,5]benzothiazepine (41). Via **23** (1.16 g, 3.98 mmol) as starting material, **41** was obtained (reaction time 2 h) (66.2%) and recrystallized from EtOH as colorless prisms (mp 103–104 °C). ^1H NMR ($CDCl_3$): δ 0.78 (t, 3H, J = 7.3 Hz, CH_3), 1.00–1.55 (m, 4H, $CH_2CH_2CH_3$), 2.21 (t, 2H, J = 7.2 Hz, $COCH_2$), 6.40 (t, 1H, J = 3.4 Hz, H-9), 6.58 (m, 1H, H-8), 7.12 (m, 1H, H-10), 7.20–7.78 (m, 9H, ArH). IR: 1750 cm^{-1}. Anal. ($C_{23}H_{21}NO_2S$) H, N; C: calcd, 73.57; found, 73.15.

7-(Hexanoyloxy)-6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepine (42). Starting from **23** (1.14 g, 3.91 mmol), **42** was obtained (reaction time 0.5 h) (74.7%) and recrystallized from EtOH as a colorless solid, melting at 74–75 °C. ^1H NMR ($CDCl_3$): δ 0.79 (t, 3H, J = 6.9 Hz, CH_3), 0.97–1.60 (m, 6H, $CH_2CH_2CH_2CH_3$), 2.20 (t, 2H, J = 7.4 Hz, $COCH_2$), 6.40 (t, 1H, J = 3.2 Hz, H-9), 6.59 (t, 1H, J = 2.0 Hz, H-8), 7.13 (t, 1H, J = 2.3 Hz, H-10), 7.22–7.78 (m, 9H, ArH). IR: 1768 cm^{-1}. Anal. ($C_{24}H_{23}NO_2S$) C, H, N.

7-[(Dimethylcarbamoyl)oxy]-6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepine (43). Starting from **23** (0.98 g, 3.36 mmol), the title compound was obtained (reaction time 1 h) and purified using 50% chloroform–petroleum ether (bp 60–80 °C), for column chromatography (61.4%). Compound **43** was recrystallized from EtOH as white needles (mp 161–163 °C). ^1H NMR ($CDCl_3$): δ 2.75 (s, 3H, CH_3), 2.90 (s, 3H, CH_3), 6.41 (t, 1H, J = 3.2 Hz, H-9), 6.59 (m, 1H, H-8), 7.13 (t, 1H, J = 2.3 Hz, H-10), 7.16–7.75 (m, 9H, ArH). IR: 1725 cm^{-1}. Anal. ($C_{21}H_{15}N_2O_2S$) C, H, N.

7-(Butyryloxy)-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (44). Starting from **24** (2 g, 6.22 mmol), **44** was obtained (reaction time 0.5 h) (41.8%) and recrystallized from EtOH as a white solid melting at 69–70 °C. ^1H NMR ($CDCl_3$): δ 0.77 (t, 3H, J = 7.4 Hz, CH_3), 1.50 (m, 2H, CH_2CH_3), 2.21 (t, 2H, J = 7.4 Hz, $CH_2CH_2CH_3$), 3.80 (s, 3H, OCH_3), 6.39 (m, 1H, H-9), 6.56 (m, 1H, H-8), 6.84 (d, 2H, J = 8.8 Hz, ArH), 7.11 (m, 1H, H-10), 7.23–7.78 (m, 6H, ArH). IR: 1745 cm^{-1}. Anal. ($C_{23}H_{21}NO_3S$) C, H, N.

7-Acetoxy-6-(*p*-methoxyphenyl)-2-(trifluoromethyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (45). A 1.23-g (3.15 mmol) portion of compound **25** gave the title compound (reaction time 12 h) (44.8%), which crystallized from EtOH as colorless prisms

(mp 136–137 °C). ^{1}H NMR ($CDCl_3$): δ 1.98 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 6.41 (t, 1H, J = 3.3 Hz, H-9), 6.60 (m, 1H, H-8), 6.86 (d, 2H, J = 8.9 Hz, ArH), 7.12 (m, 1H, H-10), 7.22–7.79 (m, 5H, ArH). IR: 1775 cm^{-1}. Anal. ($C_{22}H_{16}F_3NO_3S$) C, H, N.

6-(*p*-Methoxyphenyl)-7-(propionyloxy)-2-(trifluoromethyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (46). Via **25** (1 g, 2.56 mmol) as starting product, **46** was obtained (reaction time 12 h) (48.2%) and recrystallized from EtOH (mp 112–113 °C, green prisms). ^{1}H NMR ($CDCl_3$): δ 0.99 (t, 3H, J = 7.5 Hz, CH_3), 2.27 (q, 2H, J = 7.4 Hz, CH_2), 3.91 (s, 3H, OCH_3), 6.44 (t, 1H, J = 3.3 Hz, H-9), 6.59 (m, 1H, H-8), 6.85 (d, 2H, J = 8.9 Hz, ArH), 7.13 (t, 1H, J = 2.3 Hz, H-10), 7.46 (d, 2H, J = 8.8 Hz, ArH), 7.50–7.90 (m, 3H, ArH). IR: 1772 cm^{-1}. Anal. ($C_{23}H_{16}F_3NO_3S$) C, H, N.

7-[(Dimethylcarbamoyl)oxy]-6-(*p*-methoxyphenyl)-2-(trifluoromethyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (47). A 0.81-g (2.08 mmol) portion of compound **25** gave **47** according to the general procedure (reaction time 12 h), except that column chromatography was not necessary; after the reaction mixture was poured into crushed ice, the solid precipitate was collected (68.4%), washed with petroleum ether (bp 40–60 °C), dried, and crystallized from EtOH (yellow prisms, mp 113–115 °C). ^{1}H NMR ($CDCl_3$): δ 2.76 (s, 3H, CH_3), 2.95 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 6.44 (t, 1H, J = 3.3 Hz, H-9), 6.60 (m, 1H, H-8), 6.87 (d, 2H, J = 8.9 Hz, ArH), 7.13 (t, 1H, J = 2.2 Hz, H-10), 7.35–7.90 (m, 5H, ArH). IR: 1718 cm^{-1}. Anal. ($C_{23}H_{16}F_3N_2O_3S$) C, H, N.

6-(*p*-Methoxyphenyl)-2-(trifluoromethyl)-7-[(3,4,5-trimethoxybenzoyl)oxy]pyrrolo[2,1-*d*][1,5]benzothiazepine (48). Starting from **25** (1.26 g, 3.23 mmol), the title compound was obtained (reaction time 5 h at 70 °C) (55%) and recrystallized from EtOH as white crystals (mp 167–169 °C). ^{1}H NMR ($CDCl_3$): δ 3.76 (s, 3H, OCH_3), 3.85 (s, 6H, 2 OCH_3), 3.89 (s, 3H, OCH_3), 6.44 (t, 1H, J = 3.3 Hz, H-9), 6.64 (m, 1H, H-8), 6.81 (d, 2H, J = 8.9 Hz, ArH), 7.17 (t, 1H, J = 2.3 Hz, H-10), 7.20–7.93 (m, 7H, ArH). IR: 1735 cm^{-1}. Anal. ($C_{30}H_{24}F_3NO_6S$) C, H, N.

7-Acetoxy-2-chloro-6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepine (49). Starting from **26** (2 g, 6.13 mmol), the title compound was obtained (reaction time 3 h) (60.2%) and recrystallized from EtOH (mp 157–158 °C, white needles). ^{1}H NMR ($CDCl_3$): δ 1.97 (s, 3H, CH_3), 6.43 (m, 1H, H-9), 6.62 (m, 1H, H-8), 7.12 (m, 1H, H-10), 7.20–7.70 (m, 8H, ArH). IR: 1751 cm^{-1}. Anal. ($C_{20}H_{14}ClNO_2S$) C, H, N.

2-Chloro-6-phenyl-7-(propionyloxy)pyrrolo[2,1-*d*][1,5]benzothiazepine (50). Starting from 2 g (6.13 mmol) of **26**, the title compound was obtained (reaction time 12 h) (47.5%) and recrystallized from benzene as a white solid (mp 108–110 °C). ^{1}H NMR ($CDCl_3$): δ 0.97 (t, 3H, J = 7.4 Hz, CH_3), 2.26 (q, 2H, J = 7.6 Hz, CH_2), 6.42 (t, 1H, J = 3.3 Hz, H-9), 6.59 (m, 1H, H-8), 7.12 (m, 1H, H-10), 7.23–7.74 (m, 8H, ArH). IR: 1760 cm^{-1}. Anal. ($C_{21}H_{16}ClNO_2S$) C, H, N.

7-Acetoxy-2-chloro-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (51). Starting from 1.5 g (4.21 mmol) of **27**, the title compound was obtained (reaction time 5 h) (43.1%) and recrystallized from EtOH as white prisms (mp 110–112 °C). ^{1}H NMR ($CDCl_3$): δ 1.98 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 6.41 (t, 1H, J = 3.3 Hz, H-9), 6.59 (m, 1H, H-8), 6.86 (d, 2H, J = 8.8 Hz, ArH), 7.10 (t, 1H, J = 2.3 Hz, H-10), 7.23–7.70 (m, 5H, ArH). IR: 1770 cm^{-1}. Anal. ($C_{21}H_{16}ClNO_3S$) C, H, N.

2-Chloro-6-(*p*-methoxyphenyl)-7-(propionyloxy)pyrrolo[2,1-*d*][1,5]benzothiazepine (52). Starting from 2 g (5.62 mmol) of compound **27**, **52** was obtained (reaction time 1 h) (51.7%) and recrystallized from EtOH (mp 109–110 °C, white solid). ^{1}H NMR ($CDCl_3$): δ 1.00 (t, 3H, J = 7.5 Hz, CH_3), 2.29 (q, 2H, J = 7.5 Hz, CH_2), 3.82 (s, 3H, OCH_3), 6.42 (m, 1H, H-9), 6.58 (m, 1H, H-8), 6.86 (d, 2H, J = 9.0 Hz, ArH), 7.11 (m, 1H, H-10), 7.22–7.78 (m, 5H, ArH). IR: 1769 cm^{-1}. Anal. ($C_{22}H_{18}ClNO_3S$) C, H, N.

2-Chloro-7-[(dimethylcarbamoyl)oxy]-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (53). Starting from 3.14 g (8.82 mmol) of **27**, the title compound was obtained (reaction time 12 h) and purified by chromatography ($CHCl_3$–petroleum ether (bp 60–80 °C) 3:1) (42.7%). Compound **50** was recrystallized from EtOH as white crystals (mp 164–165 °C). ^{1}H NMR ($CDCl_3$): δ 2.77 (s, 3H, CH_3), 2.96 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 6.42 (m, 1H, H-9), 6.57 (m, 1H, H-8), 6.87 (d, 2H, J = 8.9 Hz, ArH), 7.10 (t, 1H, J = 2.4 Hz, H-10), 7.22–7.73 (m, 5H, ArH). IR: 1730 cm^{-1}. Anal. ($C_{22}H_{19}ClN_2O_3S$) C, H, N.

2-Chloro-6-(*p*-methoxyphenyl)-7-[(3,4,5-trimethoxybenzoyl)oxy]pyrrolo[2,1-*d*][1,5]benzothiazepine (54). A 1.3-g (3.65 mmol) portion of compound **27** gave **54** over a reaction time of 18 h (83.1%). The title compound was recrystallized from EtOH as white crystals (mp 174–175 °C). ^{1}H NMR ($CDCl_3$): δ 3.76 (s, 3H, OCH_3), 3.84 (s, 6H, 2 OCH_3), 3.88 (s, 3H, OCH_3), 6.40 (m, 1H, H-9), 6.61 (m, 1H, H-8), 6.80 (d, 2H, J = 8.8 Hz, ArH), 7.13 (m, 1H, H-10), 7.18–7.75 (m, 7H, ArH). IR: 1753 cm^{-1}. Anal. ($C_{29}H_{24}ClNO_6S$) C, H, N.

7-Acetoxy-3-chloro-6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepine (55). Starting from 0.5 g (1.53 mmol) of compound **28**, the desired **55** was obtained (reaction time 12 h) and purified by chromatography eluting with benzene (51.2%). The title compound was recrystallized from EtOH as a white solid (mp 118–120 °C). ^{1}H NMR ($CDCl_3$): δ 1.96 (s, 3H, CH_3), 6.42 (t, 1H, J = 3.4 Hz, H-9), 6.62 (m, 1H, H-8), 7.08 (t, 1H, J = 2.3 Hz, H-10), 7.20–7.75 (m, 8H, ArH). IR: 1780 cm^{-1}. Anal. ($C_{20}H_{14}ClNO_2S$) C, H, N.

7-Acetoxy-3-chloro-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (56). A 0.5-g (1.40 mmol) portion of **29** was used as starting product and gave, over the reaction time of 20 h, the title compound (65%). Compound **56** was recrystallized from EtOH as colorless plates (mp 126–127 °C). ^{1}H NMR ($CDCl_3$): δ 1.95 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 6.37 (t, 1H, J = 3.3 Hz, H-9), 6.59 (m, 1H, H-8), 6.85 (d, 2H, J = 8.8 Hz, ArH), 7.02 (t, 1H, J = 2.1 Hz, H-10), 7.15–7.75 (m, 5H, ArH). IR: 1780 cm^{-1}. Anal. ($C_{21}H_{16}ClNO_3S$) C, H, N.

7-Acetoxy-4-chloro-6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepine (57). Starting from **30** (0.5 g, 1.53 mmol), **57** was obtained (reaction time 12 h) (37.5%) and recrystallized from EtOH as colorless bright plates (mp 131–132 °C). ^{1}H NMR ($CDCl_3$): δ 1.97 (s, 3H, CH_3), 6.42 (t, 1H, J = 3.3 Hz, H-9), 6.63 (m, 1H, H-8), 7.10 (m, 1H, H-10), 7.20–7.78 (m, 8H, ArH). IR: 1775 cm^{-1}. Anal. ($C_{20}H_{14}ClNO_2S$) C, H, N.

7-Acetoxy-4-chloro-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (58). Starting from **31** (0.41 g, 1.15 mmol), the title compound was obtained (reaction time 12 h at 80 °C) (32.6%). Compound **58** was recrystallized from EtOH as colorless microcrystals (mp 152–153 °C). ^{1}H NMR ($CDCl_3$): δ 2.00 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 6.42 (m, 1H, H-9), 6.63 (m, 1H, H-8), 6.90 (d, 2H, J = 8.8 Hz, ArH), 7.09 (m, 1H, H-10), 7.20–7.80 (m, 5H, ArH). IR: 1780 cm^{-1}. Anal. ($C_{21}H_{16}ClNO_3S$) C, H, N.

7-Acetoxy-2-methoxy-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (59). Via compound **32** (0.64 g, 1.82 mmol) as crude starting material, the title compound was obtained (reaction time 12 h) (27.8%) and recrystallized from EtOH as a white solid (mp 120–121 °C). ^{1}H NMR ($CDCl_3$): δ 2.00 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.40 (m, 1H, H-9), 6.58 (m, 1H, H-8), 6.75–7.00 (m, 4H, ArH), 7.13 (m, 1H, H-10), 7.40–7.68 (m, 3H, ArH). IR: 1732 cm^{-1}. Anal. ($C_{22}H_{15}NO_4S$) C, H, N.

7-Acetoxy-6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepine 5-Oxide (60). A 1.21-g (3.93 mmol) portion of **33** was used as starting product and gave, over the reaction time of 18 h, the title compound. Compound **60** was purified by column chromatography eluting with chloroform–ethyl acetate 9:1 (46.5%) and recrystallized from EtOH as white crystals (mp 157–158 °C). ^{1}H NMR ($CDCl_3$): δ 1.91 (s, 3H, CH_3), 6.49 (m, 1H, H-9), 6.68 (m, 1H, H-8), 7.10–7.90 (m, 10H, ArH and H-10). IR: 1767, 1049 cm^{-1}. Anal. ($C_{20}H_{15}NO_3S$) C, H, N.

7-Acetoxy-6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepine 5,5-Dioxide (61). Starting from **34** (0.35 g, 1.08 mmol), the title compound was obtained according to the general procedure (reaction time 1.30 h), but column chromatography was not necessary. Compound **61** was collected and recrystallized from EtOH (white solid, mp 219–220 °C). ^{1}H NMR ($CDCl_3$): δ 1.96 (s, 3H, CH_3), 6.56 (m, 1H, H-8), 6.86 (m, 1H, H-9), 7.22–8.20 (m, 10H, ArH and H-10). IR: 1758, 1318, 1154 cm^{-1}. Anal. ($C_{20}H_{15}NO_4S$) C, H, N.

7-Acetoxy-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine 5-Oxide (62). Starting from 0.25 g (0.74 mmol) of compound **35**, the title compound was obtained over a 12-h reaction time and using chloroform/ethyl acetate 18:2 for the chromatography column (29%). **40** was recrystallized from EtOH as colorless needles (mp 157–158 °C). ^{1}H NMR ($CDCl_3$): δ 1.93 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 6.47 (t, 1H, J = 3.3 Hz, H-9), 6.65 (m, 1H, H-8), 6.89 (d, 2H, J = 8.9 Hz, ArH), 7.10 (d, 2H, J = 8.9 Hz, ArH), 7.24–7.92 (m, 5H, ArH and H-10). IR: 1753, 1069 cm^{-1}. Anal. ($C_{21}H_{17}NO_4S$) C, H, N.

7-Acetoxy-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]-benzothiazepine 5,5-Dioxide (63). Starting from 0.28 g (0.8 mmol) of **36**, the title compound was obtained over a 1-h reaction time. After the reaction mixture was poured into crushed ice, crude **63** was collected (73.2%) and crystallized from EtOH as white crystals (mp 209–210 °C). ^{1}H NMR ($CDCl_3$): δ 1.99 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 6.54 (m, 1H, H-9), 6.83 (m, 1H, H-8), 6.90 (d, 2H, J = 8.9 Hz, ArH), 7.21 (d, 2H, J = 8.9 Hz, ArH), 7.45–8.15 (m, 5H, ArH and H-10). IR: 1769, 1314, 1154 cm^{-1}. Anal. ($C_{21}H_{17}NO_5S$) C, H, N.

7-(Butyryloxy)-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]-benzothiazepine 5-Oxide (64). A 1.05-g (3.26 mmol) portion of **35** gave the title compound after a 0.5-h reaction time and column chromatography using chloroform–ethyl acetate 9:1 (43.7%). **64** was recrystallized from ethyl acetate–petroleum ether 1:2 (bp 40–60 °C) as bright microcrystals (mp 113–115 °C). ^{1}H NMR ($CDCl_3$): δ 0.75 (t, 3H, J = 7.4 Hz, CH_3), 1.47 (m, 2H, CH_2CH_3), 2.17 (t, 2H, J = 7.3 Hz, $CH_2CH_2CH_3$), 3.81 (s, 3H, OCH_3), 6.47 (m, 1H, H-9), 6.64 (m, 1H, H-8), 6.90 (d, 2H, J = 8.9 Hz, ArH), 7.11 (d, 2H, J = 8.9 Hz, ArH), 7.22–7.87 (m, 5H, ArH and H-10). IR: 1759 cm^{-1}. Anal. ($C_{23}H_{21}NO_4S$) C, H, N.

Molecular Modeling. Computational Procedures. All molecular modeling was performed with the use of an Evans & Sutherland PS390 graphics system tethered to a MicroVAX 3500 as host machine. Molecular models were constructed with standard bond distances and bond angles using the molecular modeling software package SYBIL 5.41.[47] These structures were used as original coordinates for further geometry optimization by means of the molecular mechanics TRIPOS force field[48] with neglect of electrostatics. Energy minimizations were realized with the SYBYL/MAXIMIN2 option by applying the BFGS (Broyden, Flecher, Goldfarb, and Shannon) algorithm and setting a δ energy value of 0.002 kcal/mol as convergence criterion. Molecular superimpositions and volume manipulations were performed respectively with the FIT and MVOLUME commands within SYBYL.

Systematic conformational searches were carried out on the structures of the ligands shown in Figure 1 with the aim to detect geometries close to energy minima. For this purpose, we used the SEARCH routine within SYBYL. Steric energies were measured, as previously done for energy minimizations, with the TRIPOS force field by neglecting the electrostatic contribution. The most relevant torsional angles were generally scanned with 20° increments in the absolute range of 360°. In case of torsional angles about amidic bounds, the increment was set to 180°. Whenever the torsional angle was associated to the rotation of a symmetrically substituted phenyl ring, the absolute interval of variation was restricted to 180°. A 0.75 van der Waals scaling factor was used to "soften" steric contacts in the rigid rotamers.

Finally, a 5 kcal/mol energy window was set to reject, on the basis of energetic criteria, many of the theoretically possible conformations. The resulting output conformations were clustered into a smaller number of "families" according to the values of their torsional angles (for this purpose, the FAMILY option of the SYBYL/TABLE routine was used). For each family we energy minimized, with the SYBYL/MAXIMIN2 command, the conformation characterized by the lowest value of steric energy.

Since the bioactive conformation of a ligand (Econf) is not necessarily the one of a global minimum or even a local minimum conformer, we took into consideration also partially optimized geometries. In any case, the conformations which we classified as energetically feasible had steric energies not higher than 4 kcal/mol above that of the corresponding global minimum conformer (Emin).

Binding Assays. Male CRL:CD(SD)BR rats (Charles River Italia, Calco, BG, Italy), weighing about 150 g, were used in these experiments. Before being killed by decapitation (unanesthetized), the rats had been 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. After decapitation, the brains were rapidly removed from the skulls and dissected out into various anatomically recognizable areas. Cortices were homogenized in about 50 volumes of ice cold phosphate-buffered saline, 50 mM, pH 7.4, using an Ultra Turrax TP-1810 (2 × 20 s), and centrifuged at 50000*g* for 10 min. The pellet was then washed three times more by resuspensions in fresh buffer and centrifugations as before. The last pellet was resuspended just before the binding assay.

For mitochondrial benzodiazepine binding,[49] 10 mg of original wet tissue weight was incubated with 1 nM [^{3}H]PK 11195 (s.a. 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 by using 1 μM PK 11195.

For central benzodiazepine binding,[41] 8.3 mg of original wet tissue weight was incubated with 1 nM [^{3}H]Ro 15-1788 (s.a. 87 Ci/mmol; NEN) or 1 nM [^{3}H]flunitrazepam (s.a. 81 Ci/mmol; NEN) in a final volume of 1 mL at 4 °C for 120 min. Nonspecific binding was determined in the presence of 1 μM clonazepam.

Incubations were stopped by rapid filtration under vacuum through GF/B fiber filters which were then washed with 12 mL of ice-cold buffer and counted in 8 mL of Filter Count (Packard) in a liquid scintillation spectrometer LKB, Model Rakbeta 1214, with a counting efficiency of about 56%. IC_{50}s were determined by nonlinear[50] fitting of binding inhibition curves, using the Allfit program running on an IBM AT personal computer. Each point was the mean of triplicate samples.

Biology. Analgesic Activity. The two compounds **38** and **44** administered orally at the dose of 100 mg/kg to mice 1 h before injection of phenylquinone[37] (PQ) (2 mg/kg ip) or formalin[38] (0.02 mL, 1% solution, subplanctal into the right hind paw) reduced by 60–70% the number of writhes per group of animals observed during 5–10 min after PQ or by 60% the paw-licking time recorded during 2 min following formalin injection relative to a vehicle-treated group.

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