Cardiovascular Characterization of Pyrrolo[2,1-*d*][1,5]benzothiazepine Derivatives Binding Selectively to the Peripheral-Type Benzodiazepine Receptor (PBR): From Dual PBR Affinity and Calcium Antagonist Activity to Novel and Selective Calcium Entry Blockers

Giuseppe Campiani,[‡] Isabella Fiorini,[‡] Maria P. De Filippis,[‡] Silvia M. Ciani,[‡] Antonio Garofalo,[‡] Vito Nacci,*[,‡] Gianluca Giorgi,[†] Alessandro Sega,[||] Maurizio Botta,[‡] Alberto Chiarini,[¶] Roberta Budriesi,[¶] Giancarlo Bruni,[§] Maria R. Romeo,[§] Cristina Manzoni,[▽] and Tiziana Mennini[▽]

Dipartimento Farmaco Chimico Tecnologico, Banchi di Sotto 55, Istituto di Farmacologia, Via delle Scotte 6, Istituto di Chimica Organica, Pian dei Mantellini, and Centro Interdipartimentale di Analisi e Determinazioni Strutturali, Via Mattioli 10, Universita' di Siena, 53100 Siena, Italy, Dipartimento di Scienze Farmaceutiche, Via Belmeloro 6, Universita' di Bologna, 40126 Bologna, Italy, and Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea 62, 20127 Milano, Italy

Received February 27, 1996[⊗]

The synthesis and cardiovascular characterization of a series of novel pyrrolo[2,1-*d*][1,5]-benzothiazepine derivatives (**54**−**68**) are described. Selective peripheral-type benzodiazepine receptor (PBR) ligands, such as PK 11195 and Ro 5-4864, have recently been found to possess low but significant inhibitory activity of L-type calcium channels, and this property is implicated in the cardiovascular effects observed with these compounds. In functional studies both PK 11195 (1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide) and Ro 5-4864 (4′-chlorodiazepam) did not display selectivity between cardiac and vascular tissue. Therefore, several 7-(acyloxy)-6-arylpyrrolo[2,1-*d*][1,5]benzothiazepines, potent and selective peripheral-type benzodiazepine receptor ligands recently developed by us (**3**, **7**−**20**), were subjected to calcium channel receptor binding assay. Some of these compounds showed an unexpected potency in displacing the binding of [^{3}H]nitrendipine from L-type calcium channels, much higher than that reported for PK 11195 and Ro 5-4864 and equal to or higher than that of reference calcium antagonists such as verapamil and (+)-*cis*-diltiazem. Specifically, in rat cortex homogenate, our prototypic PBR ligand 7-acetoxy-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*]-[1,5]benzothiazepine (**3**) showed an IC_{50} equal to 0.13 nM for inhibition of [^{3}H]nitrendipine binding. Furthermore, in functional studies this compound displayed a clear-cut selectivity for cardiac over vascular tissue. Comparison of calcium antagonist activity on guinea pig aorta strips with the negative inotropic activity, determined by using isolated guinea pig left atria, revealed that **3** displayed higher selectivity than the reference (+)-*cis*-diltiazem. Thus, the pyrrolobenzothiazepine **3** might represent a new tool for characterizing the relationship between the PBR and cardiac function. Furthermore, we have also investigated the structural dependence of binding to PBR and L-type calcium channels, and this study allowed us to identify a new class of potent calcium channel blockers selective for cardiac over vascular tissue, with no affinity for PBR. A number of structure−activity relationship trends have been identified, and a possible explanation is advanced in order to account for the observed differences in selectivity. Three structural features, namely, (i) the saturation of the C(6)−C(7) double bond, with a consequent higher molecular flexibility, (ii) the presence of a substituent in the benzo-fused ring, and (iii) a basic side chain at C-10 of the pyrrolobenzothiazepine ring system, were found to be responsible for potent L-type calcium channel antagonism and clear-cut selectivity for cardiac over vascular tissue. Among the synthesized compounds the pyrrolobenzothiazepine **62** was found to be the most promising selective calcium channel blocker. Additionally, the molecular structure determination of the key intermediate **48** by X-ray diffraction, molecular modeling, and NMR analysis is reported.

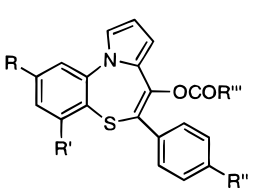
Introduction

During the last decade, the "peripheral-type" benzodiazepine receptor (PBR) has been the object of extensive studies aimed at defining its biochemical and pharmacological role.[1] Several PBR-mediated pharmacological effects have been identified[2] including inhibition of cell proliferation,[3] modification of mitochondrial respiration,[4] and stimulation of steroidogenesis.[5] The involvement of PBR in cardiac function has also been pointed out.[2b,6] The correlation between PBR ligands, namely, PK 11195 (**1**, a PBR antagonist) and Ro 5-4864 (**2**, a PBR agonist), and dihydropyridine (DHP) calcium entry blocker (CEB) binding site in brain and cardiac tissues[7a] has attracted considerable interest. In binding studies performed on cultured astroglia, Ro 5-4864 has been found to displace [^{3}H]nitrendipine from the calcium channel protein in a micromolar range.[7a] Furthermore, nitrendipine and nifedipine were able to displace [^{3}H]-Ro 5-4864 from PBR *in vitro*.[7b]

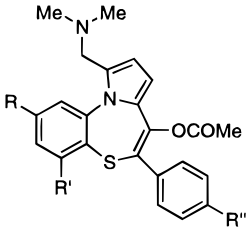
* To whom correspondence should be addressed.
[‡] Dipartimento Farmaco Chimico Tecnologico, Universita' di Siena.
[¶] Universita' di Bologna.
[§] Istituto di Farmacologia, Universita' di Siena.
[▽] Istituto di Ricerche Farmacologiche "Mario Negri".
[†] Centro Interdipartimentale di Analisi e Determinazioni Strutturali, Universita' di Siena.
[||] Istituto di Chimica Organica, Universita' di Siena.
[⊗] Abstract published in *Advance ACS Abstracts,* June 15, 1996.

S0022-2623(96)00162-8 CCC: $12.00

Table 1. Receptor Binding Affinity: Comparison of PBR and L-Type Calcium Channel Affinities for Benzothiazepines **3**, **7**−**20**, and **54**−**56**



3, 7-20



54-56

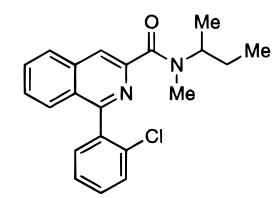
compd	R	R′	R″	R‴	IC_{50} (nM) (±SEM) CCR[a]	PBR[b]
3	H	H	OMe	Me	0.13 ± 0.03	34 ± 6
7	H	H	H	Me	6 ± 0.5	20 ± 2
8	H	H	OMe	$MeCH_2CH_2$	16 ± 1.5	28 ± 3
9	H	H	H	$MeCH_2CH_2$	4.88 ± 0.3	22 ± 5
10	H	H	H	$MeCH_2CH_2CH_2$	NA	48 ± 5
11	H	H	H	$MeCH_2CH_2CH_2CH_2$	NA	23 ± 5
12	H	H	OMe	$(Me)_2N$	420 ± 12.8	9 ± 1
13	H	H	OMe	$3,4,5\text{-}(OMe)_3C_6H_2$	NA	NA
14	H	H	OMe	$4\text{-}C_5H_4N$	NA	NA
15	Cl	H	H	Me	150 ± 61	560 ± 80
16	H	Cl	OMe	Me	NA	20 ± 5
17	CF_3	H	OMe	$3,4,5\text{-}(OMe)_3C_6H_2$	NA	NA
18	CF_3	H	OMe	Me	>2000	NA
19	Cl	H	OMe	Me	132 ± 53	651 ± 80
20	Cl	H	OMe	$3,4,5\text{-}(OMe)_3C_6H_2$	NA	NA
54	H	H	H		NA	>2000
55	Cl	H	H		960 ± 73	>2000
56	Cl	H	OMe		NA	>2000
6[c]					0.42 ± 0.15	>2000
PK 11195						2 ± 0.1
verapamil					3.7 ± 1.3	
diltiazem					46 ± 6.5	

[a] The concentration of the tested compounds that inhibited [^{3}H]nitrendipine binding to rat cortex homogenate by 50% (IC_{50}) was determined by log−probit analysis with six concentrations of the displacers, each performed in triplicate. [b] The concentration of the tested compounds that inhibited [^{3}H]PK 11195 binding to rat cortex homogenate by 50% (IC_{50}) was determined by log−probit analysis with six concentrations of the displacers, each performed in triplicate. Values are the mean ± SE of at least three separate experiments, performed in triplicate, refs 14d,e for compounds **3** and **7**−**20**. NA = not active. [c] Reference 15c.

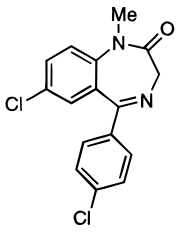
These initial results prompted a deeper investigation of the relationship between PBR and L-type calcium channels in the cardiovascular system.[8] Several data support the hypothesis that PBRs are functionally coupled to DHP-modulated voltage-operated calcium channels (VOCCs) (receptor-operated calcium channels (ROCCs) are not modulated by PK 11195[9a]) in the heart[9a−c] (although which subpopulation of PBR is coupled to VOCC is still controversial).[9d] On the other hand, the effects of PBR ligands in the cardiovascular system are not mediated through the DHP binding site, and since the activity of these PBR ligands in the cardiovascular system is a calcium-dependent process,[10] it is possible that, although these two receptors share a common effector mechanism, they are discrete entities, packaged into the same membrane compartment in the cardiac tissue.[8,11] Accordingly, at the electrophysiological level in the cardiac tissue, Ro 5-4864 is able to decrease, in a dose-dependent manner, the duration of intracellular action potentials, while clonazepam, a selective central benzodiazepine receptor ligand, is inactive.[2b,12] In guinea pig heart, Ro 5-4864 displayed a negative inotropic activity associated with a depressant effect in the contractile amplitude, while PK 11195, in guinea pig atrium, is capable of blocking the activation of cardiac L-type VOCC induced by BAY K 8644 (a DHP calcium activator).[9a,b] Positive chronotropy is also reduced by Ro 5-4864, with a mechanism that probably involves an interaction with the c-AMP-mediated events.[9c] A small but significant depressant effect on contractility of Ro 5-4864 was also confirmed in the human atrial muscle.[12] In the vascular system both compounds diminished the contractility of the vascular smooth muscle in large arteries and arterioles (especially in the brachial vascular bed) with a DHP-like action.[2b,13] Moreover, while PK 11195 was found to reduce early and delayed cardiac arrhythmias induced by myocardial ischemia and reperfusion in dog, nicardipine and PK 11195 were capable of reducing the vascular resistance of arteries in humans.[13]

These observations, coupled with strong evidence of an interaction between L-type VOCC and PBR ligands *in vitro*, suggest that these latter compounds might represent novel potential therapeutic tools for cardiovascular diseases.[2b,10] We have recently developed a novel class of specific PBR ligands based on a pyrrolobenzothiazepine skeleton.[14] In the light of the close relationship between PBR and the cardiovascular function and as part of our program aimed at developing novel cardioactive agents (**6**),[15] we decided to further investigate our PBR ligands in order to define their cardiovascular activity. Several pyrrolobenzothiazepine PBR ligands (**3**, **7**−**20**)[14d,e] have been screened *in vitro* for Ca^{2+} channel binding. Several of these compounds showed a surprising inhibitory activity of [^{3}H]nitrendipine binding higher than that reported for Ro 5-4864[7a]

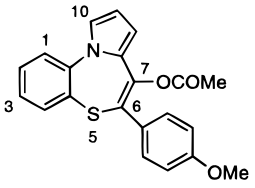
Chart 1



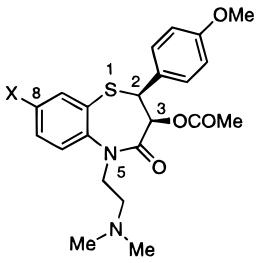
1 PK 11195



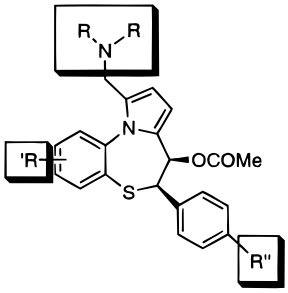
2 Ro 5-4864



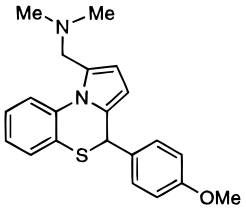
3



4a X = H, diltiazem
4b X = Cl



5 General Structure for Title Compounds

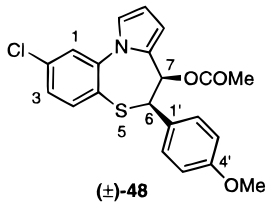


6

and equal to or higher than that of verapamil and (+)-*cis*-diltiazem, representative calcium channel antagonists (Table 1). In functional studies our prototypic PBR ligand **3** proved to be a potent negative inotropic agent with a clear-cut selectivity for cardiac over vascular tissue, while PK 11195 and Ro 5-4864 have been found to be nonselective. So, in this paper we describe the pharmacological profile of the representative pyrrolobenzothiazepine **3** as a new selective probe for studying the interaction between PBR and L-type calcium channels in cardiac tissue.

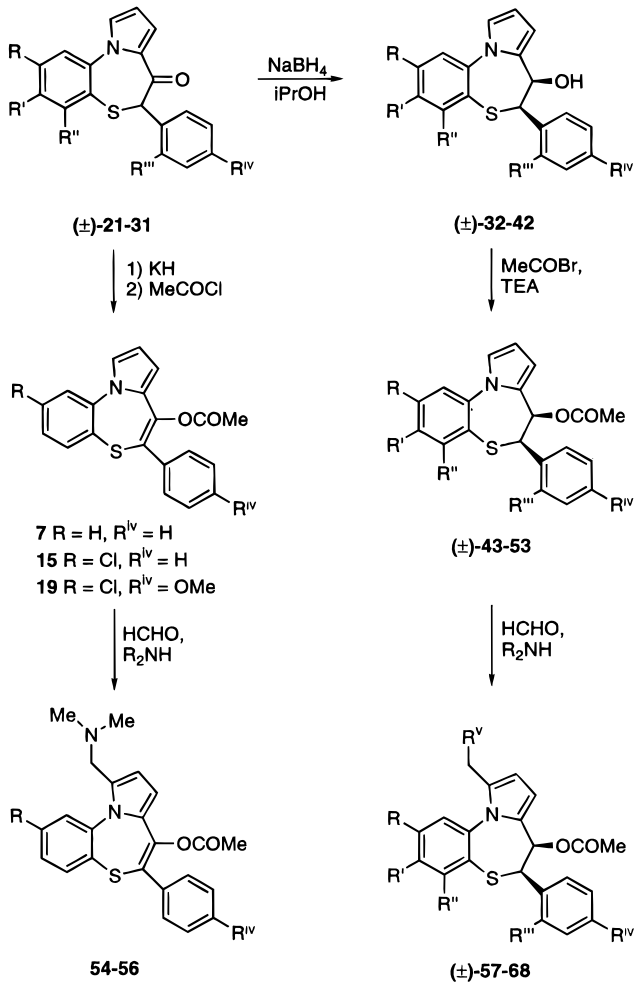
Furthermore, in the course of this work, we have also investigated the structural dependence of PBR affinity and calcium antagonist activity. The suitable alterations of the molecular structure of our PBR ligand **3** allowed us to identify a novel class of calcium channel antagonists (**5**), with no affinity for PBR. We detail herein the synthesis of pyrrolobenzothiazepine derivatives **5** (Chart 1) and their structure−activity relationships (SARs) for calcium antagonist activity associated with variation of the substituents in the heterocyclic system. This study led to the identification of the benzothiazepine derivative **62** as a potent prototypic calcium antagonist selective for cardiac over vascular tissue, with no affinity for PBR. Moreover, a comprehensive study using X-ray diffraction, molecular modeling, and NMR analysis has been performed in order to determine the conformational properties and the mutual orientation of the pharmacophoric groups at positions

Chart 2



(±)-**48**

Scheme 1



6 and 7 of 7-acetoxy-2-chloro-6,7-dihydro-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (**48**) (Chart 2), a precursor of the novel prototypic calcium channel blocker **62**.

Chemistry

The synthesis of pyrrolobenzothiazepinones **21**−**31**, which served as starting materials to obtain esters **54**−**68**, has been accomplished following an already published procedure (see ref 14a−e and references cited therein). As highlighted in Scheme 1, sodium borohydride reduction of the ketones **21**−**31** provided the alcohols **32**−**42** in 80−90% yield. By ^{1}H NMR analysis the alcohols consisted of a 85/15 mixture of diastereoisomers. Treatment of alcohols **32**−**42** with acetyl bromide in the presence of triethylamine at 80 °C[15c] afforded the acetyl derivatives **43**−**53** in 60−85% yield. At this stage the diastereoisomers were separated by fractional crystallization or flash chromatography. The major diastereoisomers showed a *cis* configuration for

Table 2. Physical Data for Compounds **21**−**53**

compd	R	R′	R″	R‴	R^{iv}	yield[a] (%)	mp (°C)	recryst solvent	formula	anal.[b]
21[c]	H	H	H	H	H					
22[d]	H	H	H	H	OMe					
23[e]	CF_3	H	H	H	H					
24[f]	CF_3	H	H	H	OMe					
25[e]	Cl	H	H	H	H					
26[f]	Cl	H	H	H	OMe					
27[f]	H	H	Cl	H	H					
28[f]	H	H	Cl	H	OMe					
29[f]	H	Cl	H	H	OMe					
30[g]	Me	H	H	H	H					
31[g]	H	H	H	F	H					
32[c]	H	H	H	H	H					
33[d]	H	H	H	H	OMe					
34[e]	CF_3	H	H	H	H					
35	CF_3	H	H	H	OMe	97	97−99	EtOH	$C_{20}H_{16}F_3NO_2S$	C,H,N
36[e]	Cl	H	H	H	H					
37	Cl	H	H	H	OMe	42	92−93	ligroine	$C_{19}H_{16}ClNO_2S$	C,H,N
38	H	H	Cl	H	H	61	95−96	ligroine	$C_{18}H_{14}ClNOS$	C,H,N
39	H	H	Cl	H	OMe	94	63−65	hexanes	$C_{19}H_{16}ClNO_2S$	C,H,N
40	H	Cl	H	H	OMe	83	65−67	hexanes	$C_{19}H_{16}ClNO_2S$	C,H,N
41	Me	H	H	H	H	85	64−65	ligroine	$C_{19}H_{17}NOS$	C,H,N
42	H	H	H	F	H	88	73−74	ligroine	$C_{18}H_{14}FNOS$	C,H,N
43[c]	H	H	H	H	H					
44[d]	H	H	H	H	OMe					
45[e]	CF_3	H	H	H	H					
46	CF_3	H	H	H	OMe	67	108−109	cyclohexane	$C_{22}H_{18}F_3NO_3S$	C,H,N
47[e]	Cl	H	H	H	H					
48	Cl	H	H	H	OMe	65	160−165	EtOAc/hexane	$C_{21}H_{18}ClNO_3S$	C,H,N
49	H	H	Cl	H	H	59	110−112	EtOH	$C_{20}H_{16}ClNO_3S$	C,H,N
50	H	H	Cl	H	OMe	40	132−133	EtOAc	$C_{21}H_{18}ClNO_3S$	C,H,N
51	H	Cl	H	H	OMe	77	127−128	EtOAc	$C_{21}H_{18}ClNO_3S$	C,H,N
52	Me	H	H	H	H	69	152−156	cyclohexane	$C_{21}H_{19}NO_2S$	C,H,N
53	H	H	H	F	H	33	136−138	*i*-PrOH	$C_{20}H_{16}FNO_2S$	C,H,N

[a] Yields refer to isolated and purified materials. [b] All the compounds were analyzed within ±0.4% of the theoretical values. [c] Reference 14a. [d] Reference 14b. [e] Reference 14c. [f] Reference 14d. [g] Reference 14e.

Table 3. Physical Data for Compounds **54**−**68**

compd	R	R′	R″	R‴	R^{iv}	R^{v}	yield[a] (%)	recryst solvent	mp (°C)	formula	anal.[b]
54	H	H	H	H	H		38	EtOAc	181−182	$C_{23}H_{22}N_2O_2S$	C,H,N
55	Cl	H	H	H	H		31	EtOAc	197−200	$C_{23}H_{21}ClN_2O_2S$	C,H,N
56	Cl	H	H	H	OMe		75	EtOAc	123−125	$C_{24}H_{23}ClN_2O_3S$	C,H,N
57	H	H	H	H	H	$N(Me)_2$	74	EtOAc	138−140	$C_{23}H_{24}N_2O_2S$	C,H,N
58	H	H	H	H	OMe	$N(Me)_2$	66	EtOAc/hexanes	147−148	$C_{24}H_{26}N_2O_3S$	C,H,N
59	CF_3	H	H	H	H	$N(Me)_2$	77	hexanes	111−113	$C_{24}H_{23}F_3N_2O_2S$	C,H,N
60	CF_3	H	H	H	OMe	$N(Me)_2$	82	EtOAc	130−131	$C_{25}H_{25}F_3N_2O_3S$	C,H,N
61	Cl	H	H	H	H	$N(Me)_2$	48	EtOAc	141−143	$C_{23}H_{23}ClN_2O_2S$	C,H,N
62	Cl	H	H	H	OMe	$N(Me)_2$	41	cyclohexane	163−165	$C_{24}H_{25}ClN_2O_3S$	C,H,N
63	H	H	Cl	H	H	$N(Me)_2$	43	EtOAc/hexanes	167−168	$C_{23}H_{23}ClN_2O_2S$	C,H,N
64	H	H	Cl	H	OMe	$N(Me)_2$	39	EtOAc/hexanes	169−172	$C_{24}H_{25}ClN_2O_3S$	C,H,N
65	H	Cl	H	H	OMe	$N(Me)_2$	58	EtOAc	151−152	$C_{24}H_{25}ClN_2O_3S$	C,H,N
66	Me	H	H	H	H	$N(Me)_2$	63	EtOAc/hexanes	138−139	$C_{24}H_{26}N_2O_2S$	C,H,N
67	H	H	H	F	H	$N(Me)_2$	62	EtOAc/hexanes	101−103	$C_{23}H_{23}FN_2O_2S$	C,H,N
68	Cl	H	H	H	OMe	$O(CH_2CH_2)_2N$	84	EtOH	136−137	$C_{26}H_{27}ClN_2O_4S$	C,H,N

[a] Yields refer to isolated and purified materials. [b] All the compounds were analyzed within ±0.4% of the theoretical values.

protons at C-6 and C-7 (see the Experimental Section) (Table 2). Finally, Mannich condensation with the pyrrole ring[15c] of the *cis*-esters **43**−**53** provided the desired *cis*-benzothiazepines **57**−**68** (Table 3). Furthermore, O-acylation[14d] of **21**, **25**, and **26** performed by exposure of the corresponding potassium enolate to acetyl chloride afforded the esters **7**, **15**, and **19**. Mannich condensation with the pyrrole ring of **7**, **15**, and **19** gave the (dimethylamino)methyl derivatives **54**−**56** (see Table 3).

Results and Discussion

Table 1 lists the PBR affinity (as IC_{50}) of previously tested ligands (**3**, **7**−**20**) and newly assayed pyrrolobenzothiazepines (**54**−**56**) and the affinity of these compounds for the displacement of [^{3}H]nitrendipine binding to the calcium channel receptor (CCR). The inhibition of [^{3}H]nitrendipine binding of compounds **3**, **7**−**20**, and **57**−**68** was measured in rat cortex and heart homogenates (Tables 4 and 5),[16,17] while the inotropic, chronotropic, and vasorelaxing effects of selected compounds were evaluated in functional studies (see the Experimental Section) (Table 6). Chiral compounds were tested in the racemic form. Data for compound **6**, PK 11195, diltiazem, and verapamil are included for comparison in Tables 1 and 4−6.

L-Type Calcium Channel Affinity of the PBR Ligands 3 and 7−20: *In Vitro* SAR Study. A series of pyrrolobenzothiazepines, recently described as potent and selective PBR ligands (**3**, **7**−**20**),[14d,e] were examined for their ability to displace [^{3}H]nitrendipine binding to

CCR in rat cortex and heart homogenates. Table 1 summarizes the IC_{50}s for the displacement of [^{3}H]PK 11195 and [^{3}H]nitrendipine binding in rat cortex, while Table 5 summarizes the IC_{50}s of a subset of compounds for the displacement of [^{3}H]nitrendipine in heart homogenate compared to rat brain. PBR affinity is usually associated to calcium antagonist activity,[9a−d] and a direct correlation between the inhibitory potencies of [^{3}H]PK 11195 and [^{3}H]nitrendipine binding (Table 1) is pointed out (**3, 7**−**9**, **17**). However, although the present data seem to confirm the hypothesis that peripheral benzodiazepine receptors are associated to VOCC and that this property is implicated as the possible cause of the cardiovascular effects observed with PK 11195, Ro 5-4864, and our prototypic benzothiazepine **3**, a discrepancy between PBR affinity and inhibition of [^{3}H]nitrendipine binding in rat brain for compounds **10**, **11**, and **16** was found. As reported in Table 1, the latter compounds do not displace [^{3}H]-nitrendipine binding in rat brain, although they interact with PBR in the nanomolar range. This fact might be the consequence of the interaction with a different binding site in the PBR protein associated to that of PK 11195 (or with another PBR subpopulation) but not to VOCC, probably located in another membrane compartment. Surprisingly, **10** appears to be selective for cardiac calcium channels, from which it displaces [^{3}H]-nitrendipine binding in the submicromolar range (see Table 5). In this series of PBR ligands, the calcium antagonist activity is essentially influenced by the same structural elements responsible for PBR affinity. While the structural features responsible for PBR affinity have been already discussed,[14e] we focus herein on the structure−activity relationships for calcium antagonist activity. In particular, three structural features were taken into account for SAR study: (i) the substitution on the phenyl ring at C-6, (ii) the nature and length of the acyl chain at C-7, and (iii) the nature and position of the substituent in the benzo-fused ring. The binding data are shown in Table 1.

(i) The ability to displace [^{3}H]nitrendipine binding to CCR is increased by the presence of a hydrogen bond acceptor (4′-OMe) on the phenyl ring at C-6 (**3** *vs* **7**), although the unsubstituted **9** is 3-fold more potent than the corresponding 4′-OMe derivatives **8** as calcium antagonist. Compound **9** shows an affinity for CCR comparable to **7**. It is possible that in compounds **8** and **9** the length of the chain at C-7 plays a more important role in the interaction with the calcium channel protein than any substituent in the C-6 phenyl ring.

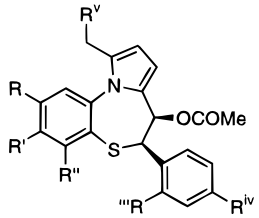
(ii) The L-type calcium channel affinity of these benzothiazepines is also dependent upon the nature and length of the acyl chain at C-7. Specifically, an amide moiety provided a compound (**12**) that showed lower affinity for CCR than the corresponding C-7 ester **3**, even if it was a more potent PBR ligand. In the ester series the affinity for CCR was dependent on the length of the alkyl chain. In fact, a small lipophilic group such as methyl was preferred (**3** *vs* **8**), though **9** showed an ability to displace [^{3}H]nitrendipine comparable to compound **7**. *n*-Butyl or *n*-pentyl chains led to compounds selective for PBR with no affinity on rat brain calcium channel receptors (**10** and **11** *vs* **7**). The introduction of an aroyl group at position 7 provided inactive compounds toward PBR and L-type calcium channels (**13**, **14**, and **20** *vs* **3** and **15**).

(iii) The electron-withdrawing trifluoromethyl group at C-2 led to a compound inactive toward both receptors (**18** *vs* **3**), while the compounds with a chloro substituent at position 2 maintained a weak calcium channel and PBR affinity (**15** and **19**). It was somewhat surprising to find out that the presence of a chlorine atom at position 4 (**16**) provided a PBR ligand unable to displace [^{3}H]nitrendipine binding to brain CCR. The pharmacological profile of these compounds might be a consequence of the binding to a different PBR subpopulation not associated to VOCC.[9d]

Pyrrolo[2,1-*d*][1,5]benzothiazepine Calcium Entry Blockers 54−**68: *In Vitro* SAR Study.** The object of this study was the identification of a novel class of CEBs based on a pyrrolobenzothiazepine skeleton (**57**−**68**) (Table 4). The previously described pyrrolobenzothiazepines (**3**, **7**−**20**)[14d,e] proved to be potent ligands of PBR with significant inhibitory activity of [^{3}H]-nitrendipine binding for L-type calcium channels as well (Table 1). Starting from these compounds we investigated the structural dependence of PBR affinity and L-type calcium antagonist activity with the aim to diminish, by suitable structural alterations, the PBR affinity while maintaining the calcium antagonist activity. Recently an approach to increase the calcium channel affinity of unfunctionalized compounds by appending a basic side chain has been described.[15a,c] This strategy showed that a (dialkylamino)methyl side chain increased the calcium channel affinity, leading to a series of CEBs (**6**) with no affinity for central benzodiazepine receptors and PBR.[15c] Therefore, a first approach to lessen the PBR affinity was the introduction of a basic side chain at C-10 of compounds **7**, **15**, and **19** (resembling the basic side chain of **6** and diltiazem). Unfortunately, this structural modification was detrimental to calcium antagonist activity as well, leading to weak (**55**) or inactive (**54** and **56**) compounds (Table 1). However, the basic (*N*,*N*-dimethylamino)methyl side chain drammatically reduced the PBR affinity (**54** *vs* **7**). Furthermore, the double bond at C(6)−C(7) of the thiazepine ring was successively saturated (**44** and **48**) but led to inactive compounds against both receptors (the potency for displacement of [^{3}H]PK 11195 and [^{3}H]-nitrendipine binding in rat brain homogenate was **44**, $IC_{50} > 50$ and $= 15\ \mu$M, respectively; **48**, $IC_{50} > 50$ and $= 3.2\ \mu$M, respectively).

On the contrary, the combination of the basic side chain at C-10 with suitable alteration of the geometric properties of the thiazepine ring of our original PBR ligands (**3** and analogous compounds) proved to be successful in diminishing the PBR affinity while maintaining high affinity for CCR. Geometrically, compound **3** presents a "folded" conformation[14e] with a reduced conformational flexibility of the thiazepine ring due to the C(6)−C(7) double bond. By altering the geometry at C(6)−C(7) from sp^2 to sp^3, we succeeded in reducing affinity for PBR, obtaining a series of compounds selective for calcium channels with the C-6 and C-7 substituents in a *cis* relationship (see molecular structure determination section). So, in order to identify the structural requirements capable of improving the calcium antagonist activity of our pyrrolobenzothiazepines, the SAR study (see Table 4) was carried out as a

Table 4. Receptor Binding Affinity to CCR in Rat Brain: Effect of Substitution at C-10, at C-6, and on the Benzo-Fused Ring



(±)-57-68

compd	R	R′	R″	R‴	R^{iv}	R^v	IC_{50} (nM)[a] (±SEM)	K_i (nM) (±SEM)
57	H	H	H	H	H	$N(Me)_2$	5200 ± 311	2407 ± 144
58	H	H	H	H	OMe	$N(Me)_2$	136 ± 68	63 ± 31
59	CF_3	H	H	H	H	$N(Me)_2$	300 ± 60	139 ± 28
60	CF_3	H	H	H	OMe	$N(Me)_2$	NA	
61	Cl	H	H	H	H	$N(Me)_2$	1230 ± 138	569 ± 64
62	Cl	H	H	H	OMe	$N(Me)_2$	3.3 ± 0.7	1.5 ± 0.213
63	H	H	Cl	H	H	$N(Me)_2$	29 ± 3.6	13 ± 1.7
64	H	H	Cl	H	OMe	$N(Me)_2$	22 ± 3.0	10 ± 1.4
65	H	Cl	H	H	OMe	$N(Me)_2$	NA	
66	Me	H	H	H	H	$N(Me)_2$	NA	
67	H	H	H	F	H	$N(Me)_2$	NA	
68	Cl	H	H	H	OMe	$N(CH_2CH_2)_2O$	NA	
6[b]							0.42 ± 0.15	0.19 ± 0.07
verapamil							3.7 ± 1.3	1.4 ± 0.5
diltiazem							46 ± 6.5	21 ± 2.9

[a] The concentration of the tested compounds that inhibited [^{3}H]nitrendipine binding to rat cortex homogenate by 50% (IC_{50}) was determined by log−probit analysis with six concentrations of the displacers, each performed in triplicate. The IC_{50} values obtained were used to calculate apparent inhibition constants (K_i) by Prusoff's method. Values are the mean ± SE of at least three separate experiments performed in triplicate. NA = not active. [b] Reference 15c.

function of (i) the nature of the substituent on the phenyl ring at C-6 and on the benzo-fused ring and (ii) the basic side chain at C-10.

(i) In general, the affinity for CCR is increased by the presence of a hydrogen bond acceptor (OMe) at position 4′ (**58**, **62**, and **64** *vs* **57**, **61**, and **63**, respectively). The introduction of a 2′-fluorine atom was detrimental to affinity (**67**). Concerning the substitution in the benzo-fused ring, the unsubstituted **57** and **58** show lower affinity for CCR (**57** and **58** *vs* **61** and **62**, respectively), while an electron-withdrawing trifluoromethyl group at C-2 (**59** and **60**) and the 3-chloro and 2-methyl substituents resulted in weak or inactive analogues (**65** and **66**). On the other hand, a chlorine atom substituent at C-2 and C-4 led to the most potent pyrrolobenzothiazepines of this series. In fact, the introduction in position 2 of a chlorine atom together with the presence of the (dimethylamino)methyl side chain at C-10 provided compound **62** which was as potent as the reference standards in radioligand binding studies. A chlorine atom at C-4 was well tolerated, providing analogues **63** and **64**, the affinity of which for CCR was remarkably high.

(ii) The further essential pharmacophore is the basic side chain at C-10 on the pyrrole ring.[18] The (dimethylamino)methyl side chain is required for optimum activity (**62**). A different amine substituent, morpholine, resulted in an inactive compound (**68**).

Compounds **60**, **61**, and **63** were also tested in a radioligand binding assay using rat heart homogenate, following Ehlert's procedure (Table 5).[17a] Compounds **60** and **61** confirmed their inactivity, while **63** showed an affinity comparable to that obtained in rat brain preparation (IC_{50} = 12 nM).

These data support the conclusion that a chlorine atom at position 2, the (dimethylamino)methyl side chain at C-10, the saturation of the C(6)−C(7) double bond, and the methoxy group at C-4′ are essential for the interaction with calcium channel receptors.

Table 5. Comparison of Inhibition of [^{3}H]Nitrendipine Binding in Rat Brain and Heart

	IC_{50} (nM)[a] (±SEM)	
	cerebral cortex	heart
3	0.13 ± 0.03	340 ± 88
7	6 ± 0.5	220 ± 17
8	16 ± 1.5	150 ± 12
10	NA	610 ± 13
12	420 ± 32	NA
60	NA	NA
61	1230 ± 131	NA
63	29 ± 4	12 ± 1.8
6[b]	0.42 ± 0.06	17 ± 4
diltiazem	46 ± 6.5	54 ± 2.9
verapamil	3.7 ± 1.3	39 ± 3.3

[a] IC_{50} values for displacement of [^{3}H]nitrendipine binding to rat brain and heart L-type calcium channels. The concentration of the tested compounds that inhibited [^{3}H]nitrendipine binding by 50% (IC_{50}) was determined by log−probit analysis with six concentrations of the displacers, each performed in triplicate. Values are the mean ± SE of at least three separate experiments performed in triplicate. NA = not active. [b] Reference 15c.

Pharmacological Studies. Vasorelaxing activity, which is a measure of calcium antagonism, was assessed on guinea pig aorta strips, while negative inotropy and chronotropy were determined on guinea pig atria. Essentially equivalent results were obtained for the tested compounds in radioligand binding assays and functional studies, although there is a discrepancy between the negative inotropic effect and binding affinity of **63**. In fact, its affinity for calcium channel receptor was not associated with high negative inotropic potency (Table 6). The reasons for such discrepancies between cardiovascular activity and binding affinity are not straightforward, but they may be related to tissue

Table 6. Cardiovascular Activity of Tested Compounds

	percent decrease (M ± SEM)		ED_{50} of inotropic negative potency on stimulated guinea pig left atrium		ED_{30} of chronotropic negative potency of tested compounds on guinea pig spontaneously beating right atrium		Ca^{2+} antagonist activity on K^+-depolarized guinea pig aortic strips of tested compounds at 5×10^{-5} M ($n = 4-5$),	IC_{50} of Ca^{2+} antagonist potency on K^+-depolarized guinea pig aortic strips	
compd	negative inotropic activity on isolated guinea pig left atrium[a] at 5×10^{-5} M ($n = 5-7$)	negative chronotropic activity on isolated guinea pig spontaneously beating right atrium[b] at 10^{-5} M ($n = 5-7$)	ED_{50}[c] (μM)	95% conf lim ($\times 10^{-6}$)	ED_{30}[c] (μM)	95% conf lim ($\times 10^{-6}$)	% inhib of Ca^{2+} contraction[d] (M ± SEM)	IC_{50}[c] (μM)	95% conf lim ($\times 10^{-6}$)
3	86 ± 3.4	26 ± 5.3	0.31	0.28−0.35			27 ± 1.6		
58	87 ± 1.5	90 ± 2.3	0.71	0.62−0.83	1.5	1.1−2.0	56 ± 2.6	4.45	3.9−5.2
62	83 ± 1.6	61 ± 1.7	0.66	0.61−0.72	0.88	0.82−0.95	40 ± 3.6		
63	41 ± 2.2	75 ± 0.1[f]			0.85	0.81−0.89	32 ± 2.5		
6[i]	75 ± 4.1[e]	30 ±1.7[h]	0.81	0.69−0.94			10 ± 0.7		
diltiazem	78 ± 3.4[e]	94 ± 5.6[g]	0.79	0.7−0.85	0.07	0.064−0.075	88 ± 2.3[h]	2.6	2.2−3.1

[a] The left atria were driven at 1 Hz. The 5×10^{-5} M gave the maximum effect for most compounds. [b] Pretreatment ranged from 170 to 200 beats/min. The 10^{-5} M gave the maximum effect for most compounds. [c] Calculated from log concentration−response curves (probit analysis by Litchfield and Wilcoxon with $n = 6-8$). When the maximum effect was <50%, the ED_{50}, ED_{30}, and IC_{50} were not calculated. [d] The 5×10^{-5} M gave the maximum effect for most compounds. [e] At 10^{-5} M. [f] At 5×10^{-6} M. [g] At 10^{-6} M. [h] At 10^{-4} M. [i] Reference 15c.

selectivity and the use of different tissues for the two assays. Compound **62** was confirmed as the most potent negative inotropic agent of this series in isolated left atria, while **58** was found to be as potent as the reference **6** and diltiazem.[15c] Even for the benzothiazepine **3**, the high PBR affinity (IC_{50} = 34 nM) was associated with potent negative inotropic activity, without affecting the chronotropy, with a potency 3-fold higher than that of diltiazem. These data clearly confirm the close relationships between PBR and cardiac function.

Furthermore, these studies revealed a different degree of selectivity of the tested compounds for cardiac over vascular tissue. Significant differences in the relative cardiac depression/smooth-muscle relaxant activities between representative PBR ligands, PK 11195 and Ro 5-4864, and CEB agents such as verapamil, diltiazem, compound **6**, and nifedipine have been recognized.[15c,19] While verapamil and diltiazem are approximately equiactive in cardiac and vascular tissue, benzothiazine **6** proved to be selective for cardiac over vascular tissue. Nifedipine is significantly more active in smooth muscle (vascular and nonvascular), and PK 11195 and Ro 5-4864 are equiactive in cardiac and vascular tissue. Accordingly, PK 11195 is a vasodilating agent with a nicardipine-like effect. In our case, the prototypic PBR ligand **3** showed a potent depressant activity on the myocardial inotropic parameter with selectivity for cardiac over vascular tissue.

In addition, even the novel CEBs described in this paper showed a different degree of selectivity, dependent upon the substituent in the benzo-fused ring. The unsubstituted benzothiazepine **58** resembled the pharmacological profile of diltiazem (**4a**) with negative inotropic activity associated with a vasorelaxing effect, although weaker than that of **4a**. The introduction of a chlorine at C-2 (**62**) led to a more potent negative inotropic agent with a clear-cut selectivity for cardiac over vascular tissue. The mechanism by which the presence of certain structural features alter the tissue selectivity is not fully understood among CEBs. The improvement in selectivity may be the consequence of increased potency or lipophilicity as well as differences in primary Ca^{2+} channel protein structure (isochannel concept)[20a,c] or the consequence of differences in the coupling between the receptor and the effector components. Anyway, further advances are required to fully understand the reason of variation of tissue selectivity among CEBs, since, even though the primary sequence of the channel subunits has already been established,[20b,c,21] the structure of the binding site of CEBs has not yet been elucidated.

Crystal and Molecular Structure of 48. The molecular structure of **48** is depicted in Figure 1. Selected bond lengths and angles are reported in Table 7. The data have been compared to those of diltiazem (**4a**)[22] and its 8-chloro analogue **4b**,[23] representative calcium entry blockers, with a *cis* relationship for protons at C-2 and C-3 (see Chart 1).

1. The Tricyclic System. In the benzo-fused ring, the C−C distances mean 1.388(3) Å, ranging from 1.374(3) to 1.402(3) Å, the latter representing the fusion with the thiazepine ring. The Cl−C(2) bond length (1.741(2) Å) is in agreement with the values found in analogous structures.[24] In the thiazepine ring, the two S−C

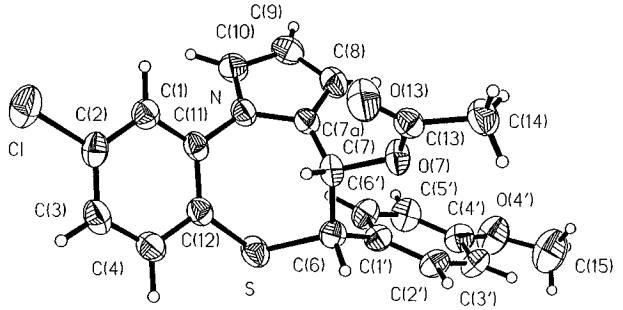


Figure 1. SHELXTL[39] drawing of compound **48** showing the thermal ellipsoids at 50% probability.

Table 7. Selected Bond Distances (Å), Bond Angles (Deg), and Torsion Angles (Deg) for Compound **48**

Bond Distances			
S−C(6)	1.842(3)	N−C(11)	1.425(3)
S−C(12)	1.772(2)	C(6)−C(7)	1.525(3)
O(7)−C(7)	1.444(3)	C(6)−C(1′)	1.518(3)
O(7)−C(13)	1.349(3)	C(7)−C(7a)	1.484(4)
O13−C(13)	1.196(3)	C(7a)−C(8)	1.371(4)
O(4′)−C(15)	1.414(4)	C(10)−C(9)	1.361(4)
O(4′)−C(4′)	1.370(3)	C(9)−C(8)	1.412(5)
N−C(7a)	1.379(3)	C(13)−C(14)	1.490(4)
N−C(10)	1.389(4)	[C(1′)−C(6′)]av	1.384(4)

Bond Angles			
C(6)−S−C(12)	105.1(2)	N−C(10)−C(9)	107.2(3)
S−C(6)−C(7)	109.3(2)	C(10)−C(9)−C(8)	108.2(3)
S−C(6)−C(1′)	109.7(2)	C(7A)−C(8)−C(9)	107.9(2)
C(7)−C(6)−C(1′)	113.9(2)	N−C(11)−C(12)	119.4(2)
O(7)−C(7)−C(6)	106.4(2)	S−C(12)−C(11)	122.9(2)
O(7)−C(7)−C(7a)	111.3(2)	O(7)−C(13)−C(14)	111.0(2)
C(7)−C(7a)−C(8)	133.7(2)	O(7)−C(13)−O13	122.8(2)
N−C(7a)−C(7)	119.0(2)	O(13)−C(13)−C(14)	126.2(3)
N−C(7a)−C(8)	133.7(2)	C(15)−O(4′)−C(4′)	118.1(3)
C(7a)−N−C(10)	109.4(2)	C(7)−O(7)−C(13)	117.2
C(9)−N−C(11)	124.5(2)		

Torsion Angles			
S−C(6)−C(7)−C(7a)	−54.7(2)	S−C(6)−C(7)−O(7)	−177.7(2)
C(6)−C(7)−C(7a)−N	84.8(2)	C(13)−O(7)−C(7)−C(6)	−143.5(2)
C(11)−N−C(7a)−C(7)	0.2(3)	C(7)−C(6)−C(1′)−C(2′)	97.0(3)
C(7a)−N−C(11)−C(12)	−48.2(4)	C(7)−O(7)−C(13)−O13	−4.6(4)
S−C(12)−C(11)−N	−8.9(4)	S−C(6)−C(1′)−C(6′)	44.4(3)
C(6)−S−C(12)−C(11)	67.4(3)	C(6)−C(7)−O(7)−C(13)	−143.5(2)
C(12)−S−C(6)−C(7)	−27.6(2)		

bond lengths are unequal: the S−C(6) and S−C(12) distances are 1.842(3) and 1.772(2) Å, respectively, the latter indicating a conjugation of the sulfur with the adjacent π-system. This difference is commonly encountered in other 1,5-benzothiazepine derivatives.[22−26] The C(7)−C(7a) bond length is 1.484(4) Å, shorter than those observed in diltiazem (1.539(7) Å)[22] and **4b** (1.527 Å).[23] This is due to the fact that in the latter two structures C-8 belongs to a carbonyl group, while in **48** it is involved in the fusion with the pyrrole ring. Other metrical parameters of the thiazepine moiety are similar to those found in other analogous structures.[22−26] The torsion angles (Table 7) are close to those observed in **4a**,[22]**b**.[23] A significant difference was found for C(12)−S−C(6)−C(7). In **48** the torsion angle is −27.6(2)°, while in **4a**,**b** the values are −42.0(4)° and −34.7°, respectively.[22,23] The thiazepine ring has a boat conformation: S, N, and C-8 are above its least-squares plane, while C-6, C-7, C-11, and C-12 are below it. The dihedral angle with the fused phenyl ring is 35.3°, close to the values found in **4a** (39.8°)[22] and **4b** (36.6°).[23] Concerning the pyrrole ring, the two C−N distances are equal to 1.379(3) and 1.389(4) Å, respectively, while C(10)−C(9) and C(7a)−C(8) bond lengths mean 1.366(4) Å. It is noteworthy that the unweighed mean over 58 independent observations of C−N and C−C bond lengths in substituted 1*H*-pyrroles are 1.372(16) and 1.375(18) Å, respectively, as measured by X-ray and neutron diffraction techniques.[27] The pyrrole ring forms dihedral angles with the fused phenyl and thiazepine rings equal to 48.4° and 44.2°, respectively. Two chiral centers, namely, C-6 and C-7, are present in the molecule, but owing to its racemic nature, both the enantiomers are present. This is in full agreement with the fact that **48** crystallizes in a centrosymmetric space group (*i.e.*, *P*1).

2. Substituents. The analyzed isomer of **48** (major) presents a *cis* orientation for substituents at C-6 and C-7. Their mutual orientation, as well as the conformation of the tricyclic system, is essential for the pharmacological activity of these compounds. In the aryl ring at C-6, the C−C bond length average is 1.384(4) Å, and it is tilted with respect to the thiazepine ring. The torsional angle C(7)−C(6)−C(1′)−C(2′) is equal to 97.0(3)°, and the dihedral angle with the thiazepine ring is 104.8°, with respect to 58.4° found in **4b**.[23] The C-7 acetoxy group presents a bond angle C(7)−O(7)−C(13) equal to 117.2(2)°, while O(7)−C(13)−C(14) is 111.0(2)°. The torsional angle C(6)−C(7)−O(7)−C(13) is equal to −143.5(2)°. The dihedral angle between the least-squares planes defined by this substituent and the thiazepine ring is 56.9°, wider than that observed in **4a** (22°)[22] and **4b** (32°).[23] The crystal packing is stabilized by intra- and intermolecular van der Waals interactions [C(2′)···O(7) = 3.164(4), C(7a)···O(13) = 3.303(4), C(1′)···O(7) = 2.846(3), C(14)···O(4′)(x, $-1 + y$, z) = 3.407(4) Å].

3. Molecular Conformation. To evaluate the energy barriers associated with the rotations of the C-6 aryl and C-7 acetoxy groups of **48**, van der Waals potential energy calculations have been carried out on **48** and **4a**,**b** (see Figure 2). The energy values refer to the conformation in the crystal, to which an energy value equal to zero is assigned. The three compounds show a quite similar behavior. Concerning the rotation of the *p*-methoxyphenyl group (Figure 2a), the orientation found in the crystal structure of **48** corresponds to that of the potential energy minimum. However values of C(7)−C(6)−C(1′)−C(2′) enclosed in the range 92° ÷ 107° produce energy variations lower than 1 kJ/mol.

The crystalline conformer of **4b** (C(7)−C(6)−C(1′)−C(2′) = −72°) is slightly shifted with respect to the calculated energy minimum (−62°, $\Delta E = -0.4$ kJ/mol). Freedom of rotation of the torsion angle C(7)−C(6)−C(1′)−C(2′) is found in the range −72° ÷ −62° ($\Delta E \leq$ 0.5 kJ/mol). **4b** shows two different regions (−64° ÷ −44° and 116° ÷ 156°) in which the potential energy is lower than that calculated for the crystal structure. The absolute minimum has a value of the torsional angle C(7)−C(6)−C(1′)−C(2′) equal to 131° ($\Delta E = -3.3$ kJ/mol). Owing to intramolecular steric hindrance, the rotation of the acetoxy moiety involves energy barriers higher than 100 times those relevant to the rotation of the *p*-methoxyphenyl group. Thus, the rotation is possible only in a limited region of space. The lowest energy region of the potential energy is reported in Figure 2b. For the three analyzed compounds, the crystal conformation has a potential energy close to that of the minimum obtained by the molecular modeling calculations. The data show that the three compounds investigated possess quite similar potential energy

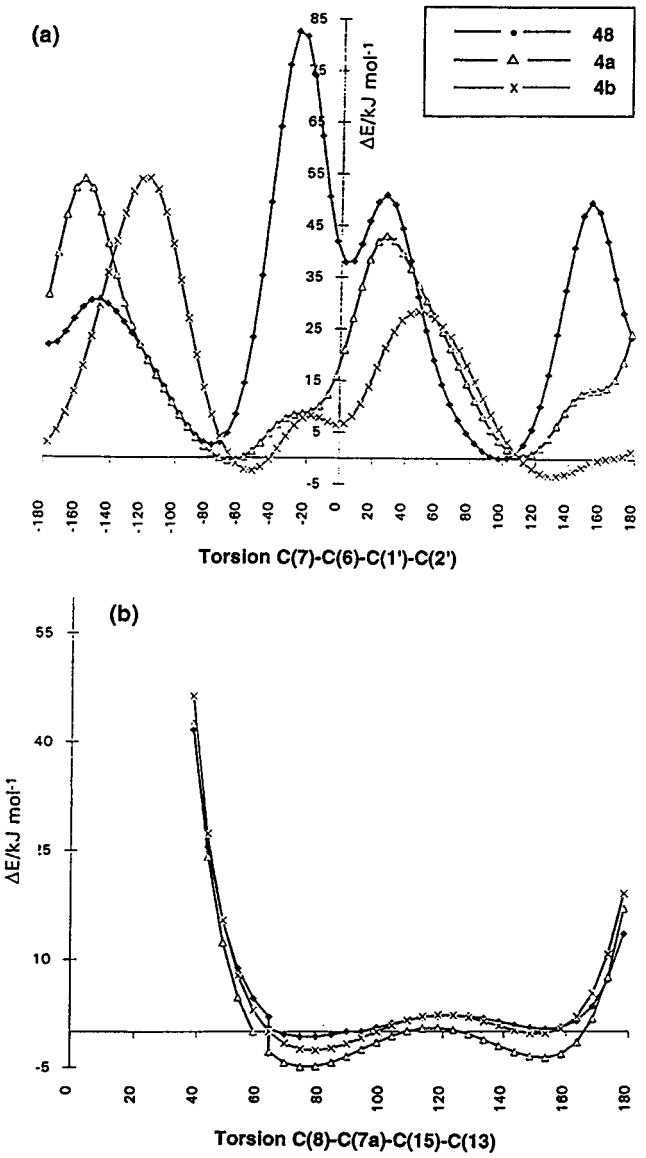


Figure 2. Calculated difference potential energy profiles for the rotation of the *p*-methoxyphenyl (a) and acetoxy (b) groups of **48** and the related structures **4a,b**.

profiles, relevant to the orientations of the substituents at positions 6 and 7, with common regions at low energy values. While the rotation of the *p*-methoxyphenyl group seems to be quite free for these three compounds, within wide ranges, the possible orientations of the acetoxy group are restricted to a narrow degree range owing to steric hindrance.

Nuclear Magnetic Resonance Analysis. The complete assignment of the spectrum of compound **48** has been made by using ^{1}H homonuclear (COSY) and ^{13}C–^{1}H heteronuclear (HETCOR) shift-correlated 2D-NMR experiments.[28,29] ^{1}H and ^{13}C NMR parameters are reported in Table 8. The chemical shifts of protons H-6 and H-7 are in agreement with those found for diltiazem,[22] although it is interesting to note that for a series of *cis*-2,3-dihydro-3-acetoxy-2-aryl-5-methyl-1,5-benzothiazepin-4(5*H*)-one[30] derivatives the proton corresponding to H-6 has been assigned downfield in respect to that corresponding to H-7.[30] The relative configuration at the C-6 and C-7 chiral centers has been determined by means of nuclear Overhauser effect

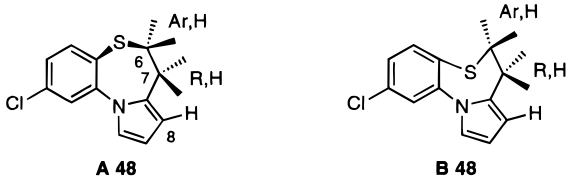
Table 8. ^{1}H and ^{13}C NMR Chemical Shifts (δ), H–H Vicinal Coupling Constants (Hz), and Relevant NOE Effects for *cis*-**48**

atom no.	δ ^{1}H	δ ^{13}C
1	7.42	124.01
2		135.96
3	7.28	127.07
4	7.70	136.19
6	5.13	57.16
7	5.92	70.47
8	6.18	129.09
9	6.35	
10	6.97	121.01
11		109.54
12		109.01
13		144.56
14		127.89
15		169.18
16	1.81	20.54
17	3.81	55.17
1′		125.77
2′	7.19	130.68
3′	6.84	113.41
4′		159.59

$J_{H,H}$
$^4J_{1-3} = 2.2$, $^3J_{3-4} = 8.1$, $^3J_{6,7} = 6.1$, $^3J_{8,9} = 3.2$, $^4J_{8,10} = 1.7$, $J_{9,10} = 3.0$, $^3J_{2'-3'} = 8.7$

	NOE Effects	
satd	obsd	NOE (%)
H(6)	H(2′)	7.9
	H(3′)	−0.8
	H(7)	24.3
H(7)	H(8)	2.5
	H(6)	18.9
H(8)	H(9)	5.2
	H(7)	1.0

Chart 3. Relevant Dreiding Distances for Conformations A and B of Compound (±)-**48**[a]



[a] A **48**: $r_{7,8}$ = 2.8 or 3.8 Å; $r_{6,7}$ = 2.2 Å (*cis*); $r_{6,7}$ = 2.7 or 2.9 Å (*trans*). B **48**: $r_{7,8}$ = 2.8 or 3.7 Å; $r_{6,7}$ = 2.2 Å (*cis*); $r_{6,7}$ = 2.6 or 3.0 Å (*trans*).

(NOE) analysis. NOEs involving protons H-6, H-7, and H-8 have been taken into account (Table 8).

In our case it is possible to exploit quantitative NOE analysis and eq 1 can be written, providing that the cross-saturation terms are negligible:[31]

$$\frac{f_7(6)}{f_7(8)} = \frac{r^6_{7,8}}{r^6_{6,7}} = 1.7 \qquad (1)$$

The distances between H-7 and H-8 ($r_{7,8}$) and H-6 and H-7 ($r_{6,7}$) protons depend on the conformation of the benzothiazepine ring (A or B, Chart 3) and the relative configuration at C-6 and C-7 chiral centers. From Dreiding's model of **48**, $r_{6,7}$ can be estimated to equal 2.2 for a *cis* configuration, while for a *trans* relationship this distance varies from 2.6 to 3.0 Å (Chart 3). From eq 1, using the distance between H-6 and H-7 of 2.2 Å (*cis*), we obtained a value for $r_{7,8}$ equal to 3.7 Å, while a *trans* relationship for H-6 and H-7 gave for $r_{7,8}$ a value ranging from 4.4 to 5.1 Å. Thus, only a *cis* relationship between H-6 and H-7 protons gave a value for $r_{7,8}$ (3.7

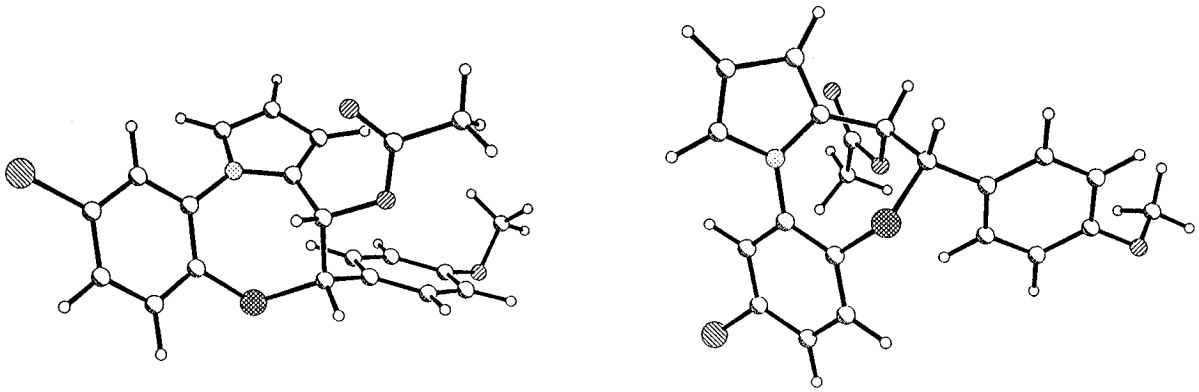


Figure 3. Diagrams of minimum energy conformers **48a**,**b** obtained by the conformational analysis.

Å) in agreement with one of the values estimated from Dreiding's model ($r_{7,8}$ = 2.8 or 3.7−3.8 Å; Chart 3). We have also been able to establish that H-6 and H-7 are on the opposite side of the thiazepine ring with respect to the pyrrole ring ($r_{7,8}$ = 3.7−3.8 Å and $r_{7,8exp}$ = 3.7 Å). On the other hand, on the basis of the NOE analysis, we could not establish the conformation (if A ($r_{7,8}$ = 3.8 Å) or B ($r_{7,8}$ = 3.7 Å)) of **48** (see Chart 3). This problem has been solved by X-ray analysis.

Molecular Modeling Studies. 1. Conformational Analysis of 48. The geometric and conformational properties of **48** were further investigated by a molecular modeling study. Diltiazem X-ray crystal structure (**4a**), as a typical CEB, was employed as a template for molecular superimposition. The X-ray crystal coordinates of **48** were used as reference geometry for computer-assisted molecular modeling (CAMM) studies. The first step of this study was to verify the accuracy of the force field implemented in SYBYL to describe our compounds. The molecular modeling software package SYBYL 5.5[32] and the Tripos force field[33] were used to model **48**. BUILDING menu of the program SYBYL was used to build up and optimize the geometry of **48**. The conformational analysis was then carried out in two steps. Initially, with the aim of evaluating all the possible conformations of the seven-membered ring lacking the substituents at C-6 and C-7, the structure was submitted to a random search. A maximum energy of 100 kcal/mol, a rms threshold value of 0.1 Å, a maximum number of search interactions of 1000, and a minimum rms energy gradient equal to 0.005 kcal/mol/Å were the criteria used. Four minimum energy conformations, two by two specular and equienergetic, were obtained. One of them was very close to the conformation of the benzothiazepine ring found in the crystal structure of **48**. Then the substituents at C-6 and C-7 were added to each conformer in *S,S-cis* configuration. Each of the four conformations was further submitted to a conformational analysis by using the SEARCH routine within SYBYL. The most relevant torsion angles were scanned with 15° increments in the absolute range of 0−360°. A 0.75 van der Waals scaling factor was used to "soften" steric contact in the rigid rotamers. Finally, by fixing a 5 kcal/mol energy window, about 1000 minimum energy conformers were obtained. By using the FAMILY option of the SYBYL/TABLE routine, all these conformations were clustered into 239, 229, 287, and 205 "families", respectively, according to the values of their torsional angles.

The minimum energy conformation of each family was then reminimized by using the MAXIMIN2 option of SYBYL. Four absolute minimum energy conformations were found, named **48a**−**d**, and the final absolute minimum energy values for **48a**−**d** were equal to 17.6 (**48a**), 17.8 (**48b**), 18.0 (**48c**), and 18.2 (**48d**) kcal/mol, respectively, indicating the very close stability of each minimum (in Figure 3 only **48a**,**b** are reported). The final conformations (**48a**−**d**) are two by two quite similar, showing remarkable differences only in the puckering of the heterocyclic system (Figure 3). Specifically, the conformer **48a** gave the best match (rms = 0.08 Å) with the X-ray structure of **48**. Accordingly, **48a** was used for further studies.

2. Conformational Analysis of 62. The conformational analysis of **62** was performed. The basic side chain at C-10 was added to **48a**, and the structure obtained was submitted to conformational analysis by using the SEARCH routine within SYBYL. Eighty minimum energy conformations were identified in the range of 5 kcal/mol, clustered into 39 "families". At first, in order to superimpose the investigated compound to the X-ray structure of diltiazem and the energy-minimized structure of **6**, which has been previously determined,[15c] a set of pharmacophoric elements was defined. The key pharmacophoric substructures taken into account were those identified for **6** and **4a**: (i) the centroid of the condensed benzene ring, (ii) the ether function (OMe) at C-4′, (iii) the basic side chain, and (iv) the pyrrole ring, resembling the bioisosteric amide function of **4a**, and (v) the ester group at C-7. The minimum energy conformer of each of the 39 families was then superimposed to diltiazem X-ray structure[15c] and to **6**[15c] using the FIT procedure within SYBYL. The conformation of **62** that best matched the reference structures is depicted in Figures 4 and 5. By taking into account the specific pharmacophoric groups for the matching processes, the fitting experiments between **62** and **4a** (rms = 0.55 Å) and between **62** and **6** (rms = 0.65 Å) showed good superimpositions. Figures 4 and 5 show the high similarity of the pharmacophoric features of **6** and **4a** with those of **62**.

Then, to find a correlation between pharmacological activities and structural features of our compounds, we measured the distances between the endocyclic nitrogen, the exocyclic nitrogen, and the ether function at C-4′ for the three compounds investigated. **62**, **4a**,[15c] and **6**[15c] displayed very similar distances between the selected pharmacophoric groups. Thus, the results of this

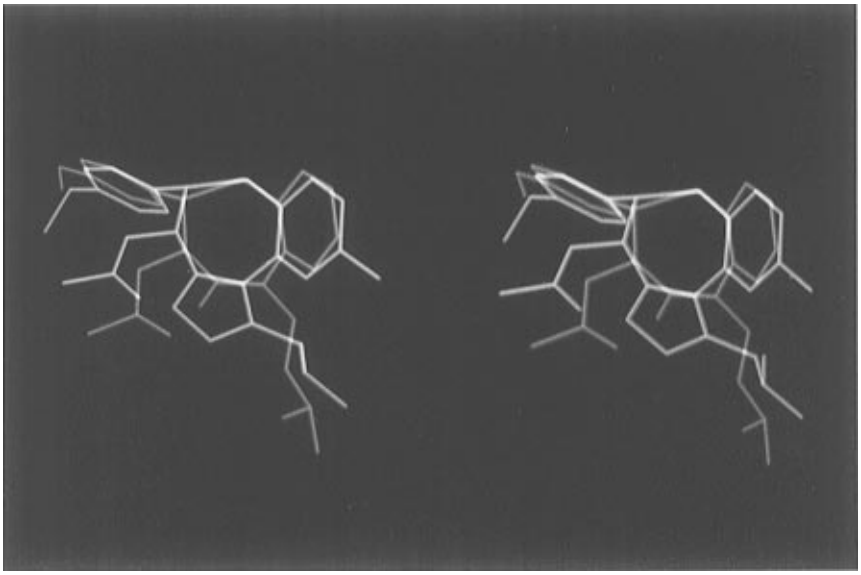


Figure 4. Superimposition of the X-ray-determined structure of diltiazem (red)[15c] and the structure of the minimum energy conformer of **62** (green).

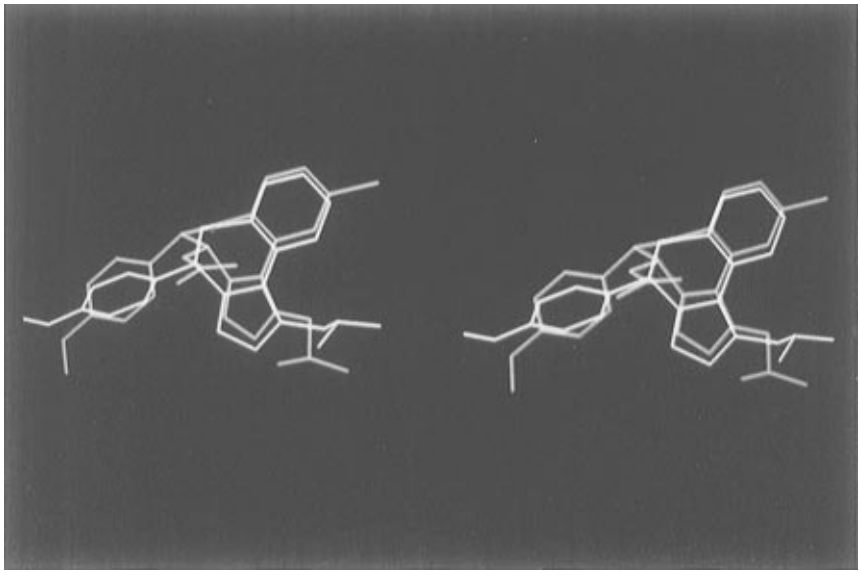


Figure 5. Superimposition of the minimum energy conformer of **62** (green) and the minimum energy conformer of **6** (magenta).[15c]

CAMM study show a high structural similarity between diltiazem, **6**, and our novel pyrrolobenzothiazepine **62**.

Furthermore, in order to explain the different pharmacological profile of the pyrrolobenzothiazepines **3** and **62**, two compounds that display a calcium channel blocking activity, we compared the minimum energy conformer of **62** with that of **3**, modeled following the same procedure as for **48**. As shown in Figure 6, the superimposition between **62** and **3** gave a poor overlapping. This fact, together with the biological data presented above, confirms that **3** and **62** might act as CEBs interacting with different binding sites. In fact, these results suggest that while **3**, through the PBR binding, modulates the L-type VOCC indirectly through an interaction with the DHP binding site (displacement of [^{3}H]PK 11195 and [^{3}H]nitrendipine, see biological section), **62** binds to a different domain of the L-type calcium channel protein, allosterically coupled to the DHP binding site and not associated to PBR (displacement of [^{3}H]nitrendipine but not of [^{3}H]PK 11195, see biological section), which is probably the same binding site of compound **6** and diltiazem.

Conclusion

The cardiovascular characterization of a recently described PBR ligand (**3**) is reported in this paper. In functional studies it showed a potent negative inotropic activity without affecting chronotropy. For compound **3** and analogous pyrrolobenzothiazepines (**7**−**20**), the PBR affinity is associated to potent calcium antagonist activity, and this property supports the hypothesis that the cardiovascular action of PBR ligands is a calcium-dependent process through DHP-sensitive voltage-dependent channels. Unlike PK 11195 and Ro 5-4864 which do not show selectivity between cardiac and vascular tissue, our prototypic pyrrolobenzothiazepine **3** proved to be selective for cardiac over vascular tissue, being about 3-fold more potent as negative inotropic agent than diltiazem, a representative "nonselective" calcium channel blocker. The selectivity shown by **3** might be due to the discrimination between two PBR binding site domains (or two PBR subpopulations) in the two tissues, associated to VOCC. Pyrrolobenzothiazepine **3** might therefore represent an effective new

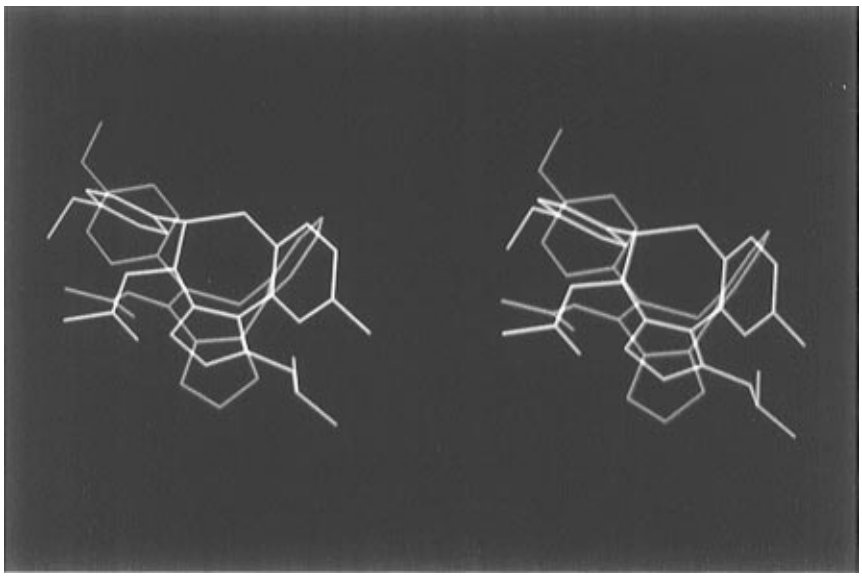


Figure 6. Superimposition of the minimum energy conformer of **62** (green) and the minimum energy conformer of **3** (red).

tool to elucidate the physiological relevance of the "peripheral-type" benzodiazepine binding sites in the cardiac tissue and might help to discriminate hypothetical subtypes of these receptors (cardiac and vascular tissues).

Furthermore, starting from our PBR ligands, we have investigated the structural dependence of PBR affinity and calcium antagonist activity. By structural and conformational modifications of our PBR ligands, we identified novel and selective calcium entry blockers based on a pyrrolobenzothiazepine skeleton with no affinity for PBR. In this case the lack of PBR affinity is probably due to the interaction with a calcium channel subunit different than that of DHPs in the calcium channel protein, not associated to PBR (DHPs displace [^{3}H]Ro 5-4864 from PBR). Among the synthesized compounds, **62** was the most promising calcium channel blocker. This compound was found to be a potent L-type calcium channel ligand, and in *in vitro* pharmacological studies, it displayed a negative inotropic activity comparable to that of diltiazem, although, unlike diltiazem, as a negative chronotropic agent it was about 13-fold less potent. Recently, several benzothiazepines and analogous structures have been described as CEBs.[23,34] Several of these compounds showed an equal or superior pharmacological profile to that of diltiazem, but none of them proved to be cardioselective. Therefore, since in functional studies **62** showed a clear-cut selectivity between cardiac and vascular tissue, we can conclude that **62** represents the first example of a CEB selective for cardiac over vascular tissue, based on a benzothiazepine structure.

Experimental Section

Melting points were determined using an Electrothermal 8103 apparatus and are uncorrected. IR spectra were taken with Perkin-Elmer 398 and FT 1600 spectrophotometers. ^{1}H NMR spectra were recorded on a Bruker 200 MHz spectrometer with TMS as internal standard; the values of chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. All reactions were carried out in an argon atmosphere. 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 $MgSO_4$, 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 purified products and are not optimized. Physical data for compounds **21**−**53** and **54**−**68** are reported in Tables 2 and 3, respectively.

General Procedure for Preparation of Alcohols 35 and 37−**42.** This procedure is illustrated for the preparation of (±)-6,7-dihydro-7-hydroxy-6-(*p*-methoxyphenyl)-2-(trifluoromethyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (**35**). To a well-stirred suspension of sodium borohydride (0.12 g, 3.19 mmol) in *i*-PrOH (3.5 mL) was added a solution of ketone **24** (0.63 g, 1.61 mmol) in *i*-PrOH (4.5 mL). The resulting mixture was stirred at room temperature for 1 h. The solvent was removed under vacuum, and the pasty residue was treated with ice−water and then extracted with EtOAc. The organic solution was washed with brine, dried, and concentrated. The residue was flash chromatographed (EtOAc) and recrystallized to afford 0.59 g of **35** as a white solid: R_f = 0.31 (EtOAc); IR (KBr) 3420 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.80 (d, 1 H, J = 7.9 Hz), 7.59 (m, 1 H), 7.47 (d, 1 H, J = 8.1 Hz), 7.20 (d, 2H, J = 8.6 Hz), 6.94 (m, 1 H), 6.80 (d, 2 H, J = 8.5 Hz), 6.29 (t, 1 H, J = 3.0 Hz), 6.01 (d, 1 H, J = 2.5 Hz), 4.93 (d, 1 H, J = 5.7 Hz), 4.77 (t, 1 H, J = 6.3 Hz), 3.74 (s, 3 H), 2.01 (d, 1 H, J = 7.2 Hz).

(±)-2-Chloro-6,7-dihydro-7-hydroxy-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (37). Starting from **26** (1.0 g, 2.81 mmol), the title compound was obtained following an identical procedure as for **35**: R_f = 0.3 (EtOAc); IR (KBr) 3410 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.67−6.95 (m, 6 H), 6.87 (d, 2 H, J = 8.6 Hz), 6.33 (t, 1 H, J = 3.4 Hz), 6.07 (m, 1 H), 5.0 (d, 1 H, J = 5.9 Hz), 4.86 (d, 1 H, J = 6.0 Hz), 3.77 (s, 3 H), 1.74 (d, 1 H, J = 7.5 Hz).

(±)-4-Chloro-6,7-dihydro-7-hydroxy-6-phenylpyrrolo-[2,1-*d*][1,5]benzothiazepine (38). Starting from **27** (1.26 g, 3.86 mmol), the title compound was obtained according to the procedure described for **35**: R_f = 0.35 (EtOAc); IR (KBr) 3400 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.50−7.10 (m, 8 H), 6.96 (d, 1 H, J = 2.2 Hz), 6.33 (m, 1 H), 6.12 (m, 1 H), 5.05 (d, 1 H, J = 5.9 Hz), 4.94 (d, 1 H, J = 5.8 Hz), 1.68 (s, 1 H).

(±)-4-Chloro-6,7-dihydro-7-hydroxy-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (39). Starting from **28** (1.43 g, 4.01 mmol), **39** was obtained according to the procedure described above for **35** (reaction time 4 h at 30 °C): R_f = 0.42 (EtOAc); IR (KBr) 3454 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.50−7.20 (m, 5 H), 6.92 (t, 1 H, J = 2.2 Hz), 6.85 (d, 2 H, J = 8.7 Hz), 6.28 (t, 1 H, J = 2.9 Hz), 6.11 (d, 1 H, J = 2.6 Hz), 5.05 (d, 1 H, J = 5.9 Hz), 4.88 (t, 1 H, J = 6.6 Hz), 3.78 (s, 3 H), 1.76 (d, 1 H, J = 7.8 Hz).

(±)-3-Chloro-6,7-dihydro-7-hydroxy-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (40). Starting from **29** (0.96 g, 2.69 mmol), **40** was obtained according to the procedure described above for **35** (reaction time 2 h at room temperature): R_f = 0.42 (EtOAc); IR ($CHCl_3$) 3460 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.73 (d, 1 H, J = 1.9 Hz), 7.50−7.20 (m, 4 H), 6.93 (t, 1 H, J = 2.2 Hz), 6.86 (d, 2 H, J = 8.8 Hz), 6.32 (t, 1 H, J = 2.9 Hz), 6.05 (d, 1 H, J = 2.5 Hz), 4.99 (d, 1 H, J = 5.8 Hz), 4.86 (t, 1 H, J = 6.6 Hz), 3.79 (s, 3 H), 1.78 (d, 1 H, J = 7.6 Hz).

(±)-6,7-Dihydro-7-hydroxy-2-methyl-6-phenylpyrrolo-[2,1-*d*][1,5]benzothiazepine (41). Starting from 0.8 g (2.62 mmol) of **30**, the title compound was obtained as colorless prisms (reaction time 3.5 h at 40 °C) according to the procedure described for **35**: R_f = 0.41 (EtOAc and $CHCl_3$, 1/1); IR ($CHCl_3$) 3360 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.62 (d, 1 H, J = 7.8 Hz), 7.58−7.20 (m, 4 H), 7.09 (d, 2 H, J = 7.8 Hz), 6.98 (t, 1 H, J = 1.7 Hz), 6.32 (t, 1 H, J = 3.3 Hz), 6.07 (m, 1 H), 6.49 (d, 1 H, J = 3.2 Hz), 5.02 (d, 1 H, J = 5.9 Hz), 4.93 (t, 1 H, J = 6.3 Hz), 2.42 (s, 3 H), 1.67 (d, 1 H, J = 7.1 Hz).

(±)-6,7-Dihydro-7-hydroxy-6-(*o*-fluorophenyl)pyrrolo-[2,1-*d*][1,5]benzothiazepine (42). Starting from 1.42 g (4.59 mmol) of **31**, the title compound was obtained (reaction time 1 h at 30 °C) as colorless plates: R_f = 0.32 ($CHCl_3$); IR (Nujol) 3450 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.48−6.84 (m, 9 H), 6.27 (t, 1 H, J = 2.9 Hz), 6.15 (t, 1 H, J = 2.7 Hz), 5.03 (d, 1 H, J = 6.0 Hz), 4.87 (d, 1 H, J = 5.9 Hz), 1.89 (br d, 1 H).

General Procedure for Preparation of Esters 46 and 48−53. This procedure is illustrated for the preparation of (±)-*cis*-7-acetoxy-6,7-dihydro-6-(*p*-methoxyphenyl)-2-(trifluoromethyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (**46**). Dry triethylamine (138 μL, 0.97 mmol) was added to a cold (0 °C) solution of **35** (0.35 g, 0.89 mmol) in anhydrous THF (7 mL), and the mixture was stirred at 0 °C for 20 min under argon. Then, acetyl bromide (68 μL, 0.92 mmol), dissolved in anhydrous THF (1 mL), was slowly added. The resulting suspension was allowed to stir at 0 °C for 1 h. The solvent was removed in vacuo, and the residue was partitioned between water and EtOAc. The organic layer was washed with brine, dried, and concentrated. The residue was flash chromatographed (toluene) and recrystallized to give 260 mg of **46** as pale yellow prisms: R_f = 0.53 ($CHCl_3$ and EtOAc, 1/1); IR ($CHCl_3$) 1746 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.90 (d, 1 H, J = 7.8 Hz), 7.64 (m, 1 H), 7.55 (d, 1 H, J = 7.7 Hz), 7.20 (d, 2 H, J = 8.8 Hz), 7.0 (m, 1 H), 6.84 (d, 2 H, J = 8.8 Hz), 6.36 (m, 1 H), 6.20 (m, 1 H), 5.94 (d, 1 H, J = 5.8 Hz), 5.16 (d, 1 H, J = 5.9 Hz), 3.81 (s, 3 H), 1.89 (s, 3 H).

(±)-*cis*-7-Acetoxy-2-chloro-6,7-dihydro-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (48). Starting from **37** (0.86 g, 2.4 mmol), the title compound was prepared following the procedure described for **46** and crystallized as colorless prisms: IR (KBr) 1736, 741 cm^{-1}; for ^1H and ^{13}C NMR, see Nuclear Magnetic Resonance Analysis section and Table 9.

(±)-*cis*-7-Acetoxy-4-chloro-6,7-dihydro-6-phenylpyrrolo-[2,1-*d*][1,5]benzothiazepine (49). Starting from 180 mg (0.55 mmol) of **38**, the title compound was obtained (reaction time 2 h at 30 °C) following the procedure as for **46**. Ester **49** was recrystallized as colorless prisms: IR (film) 1748 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.47−7.24 (m, 8 H), 6.94 (t, 1 H, J = 2.2 Hz), 6.32 (t, 1 H, J = 3.1 Hz), 6.21 (m, 1 H), 5.95 (d, 1 H, J = 6.1 Hz), 5.16 (d, 1 H, J = 5.9 Hz), 1.76 (s, 3 H).

(±)-*cis*-7-Acetoxy-4-chloro-6,7-dihydro-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (50). Similarly to **46**, the ester **50** was prepared starting from 0.63 g (1.75 mmol) of **39** (reaction time 3 h at room temperature). An analytical sample was obtained after trituration with EtOAc: IR ($CHCl_3$) 1733 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.80−7.24 (m, 4 H), 7.15 (d, 2 H, J = 8.7 Hz), 6.82 (d, 2 H, J = 8.4 Hz), 6.24 (m, 1 H), 6.05 (m, 1 H), 5.67 (d, 1 H, J = 6.4 Hz), 5.14 (d, 1 H, J = 6.2 Hz), 3.80 (s, 3 H), 2.01 (s, 3 H).

(±)-*cis*-7-Acetoxy-3-chloro-6,7-dihydro-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (51). Starting from 0.86 g (3.12 mmol) of alcohol **40**, the title compound was obtained as colorless prisms (reaction time 5 h at 30 °C) following the procedure described for **46**. **51** was purified by flash chromatography (toluene): IR (film) 1746 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.60−7.20 (m, 7 H), 6.90 (m, 1 H), 6.28 (t, 1 H, J = 3.1 Hz), 6.12 (m, 1 H), 5.78 (d, 1 H, J = 6.3 Hz), 5.38 (d, 1 H, J = 6.1 Hz), 3.78 (s, 3 H), 1.91 (s, 3 H).

Table 9. Crystal Data and Summary of Experimental Details for Compound **48**

formula	$C_{21}H_{18}ClNO_3S$
M_r	399.9
crystal size (mm^3)	0.10 × 0.25 × 0.45
crystal system	triclinic
space group	$P\bar{1}$ (no. 2)
a (Å)	8.719(2)
b (Å)	9.639(2)
c (Å)	12.326(2)
α (deg)	95.01(2)
β (deg)	93.96(2)
γ (deg)	110.74(2)
U ($Å^3$)	959.5(3)
Z	2
F(000)	416
D_{calcd} (g cm^{-3})	1.38
μ(Mo Kα) (cm^{-1})	3.29
radiation graphite monochromatized	Mo Kα (λ = 0.710 73 Å)
scan mode	ω
scan range (deg)	$2 \le 2\Theta \le 60$
scan width (deg)	1.4
scan speed (deg min^{-1})	2.70
temperature (°C)	22
independent refltns	5589 (R_{int} = 0.008)
obsd refltns ($F_o > 4\sigma(F)$)	3575
no. of parameters refined	293
R	0.049
R_w	0.055

(±)-*cis*-7-Acetoxy-6,7-dihydro-2-methyl-6-phenylpyrrolo-[2,1-*d*][1,5]benzothiazepine (52). Starting from **41** (0.75 g, 2.43 mmol), the title compound was obtained (reaction time 2 h at 40 °C) following the procedure described for **46**. After flash chromatography (5% EtOAc in benzene), **52** was obtained as colorless prisms: IR (film) 1732 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.86 (m, 2 H), 7.69 (m, 3 H), 7.65−7.00 (m, 3 H), 6.97 (m, 1 H), 6.31 (m, 1 H), 6.14 (m, 1 H), 5.96 (d, 1 H, J = 6.2 Hz), 5.13 (d, 1 H, J = 6.6 Hz), 2.43 (s, 3 H), 1.87 (s, 3 H).

(±)-*cis*-7-Acetoxy-6,7-dihydro-6-(*o*-fluorophenyl)pyrrolo-[2,1-*d*][1,5]benzothiazepine (53). Starting from **42** (0.72 g, 2.31 mmol), the title compound was obtained following the procedure described for **46**. After flash chromatography (5% EtOAc in toluene), **53** was obtained as colorless prisms: IR (film) 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.86 (m, 2 H), 7.69 (m, 3 H), 7.60−6.95 (m, 4 H), 6.42 (m, 1 H), 6.10 (m, 1 H), 6.02 (d, 1 H, J = 6.1 Hz), 5.31 (d, 1 H, J = 6.6 Hz), 1.81 (s, 3 H).

General Procedure for Preparation of Compounds 54−68. This procedure is illustrated for the preparation of (±)-*cis*-7-acetoxy-6,7-dihydro-10-[(*N*,*N*-dimethylamino)methyl]-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (**58**). To a solution of **44** (0.68 g, 1.9 mmol) in glacial acetic acid (35 mL) was added a mixture of 40% HCHO (0.64 mL) and 40% aqueous dimethylamine (1.4 mL) in glacial acetic acid (5 mL). The solution was stirred at room temperature for 12 h. The solvent was removed under vacuum, and the residue was partitioned between 10% $NaHCO_3$ and EtOAc. The organic solution was washed with brine, dried, and concentrated. The residue was flash chromatographed (EtOAc) and recrystallized to afford 0.65 g of **58** as a white solid: R_f = 0.31 (EtOAc); IR (KBr) 1745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.87−7.25 (m, 4 H), 7.12 (dd, 2 H, J = 8.7, 2.0 Hz), 6.82 (dd, 2 H, J = 7.9, 2.1 Hz), 6.25 (d, 1 H, J = 3.4 Hz), 6.03 (d, 1 H, J = 3.2 Hz), 5.71 (d, 1 H, J = 6.4 Hz), 5.15 (d, 1 H, J = 6.3 Hz), 3.80 (s, 3 H), 3.40 (d, 2 H, J = 18.3 Hz), 2.17 (s, 6 H), 1.85 (s, 3 H).

7-Acetoxy-10-[(*N*,*N*-dimethylamino)methyl]-6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepine (54). Starting from **7** (0.62 g, 1.85 mmol), the title compound was obtained as colorless prisms following an identical procedure as for **58**: R_f = 0.3 (EtOAc); IR (KBr) 1765 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.86−7.14 (m, 9 H), 6.60 (d, 1 H, J = 3.2 Hz), 6.41 (d, 1 H, J = 3.1 Hz), 3.43 (AB q, 2 H, J = 12.5 Hz), 2.18 (s, 6 H), 1.95 (s, 3 H).

7-Acetoxy-2-chloro-10-[(*N*,*N*-dimethylamino)methyl]-6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepine (55). Starting from **15** (0.52 g, 1.41 mmol), **55** was obtained as white crystals according to the procedure described above for **58** (reaction time 15 h): $R_f = 0.42$ (EtOAc); IR (KBr) 1765 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.90−7.20 (m, 8 H), 6.55 (d, 1 H, $J = 3.8$ Hz), 6.38 (d, 1 H, $J = 3.9$ Hz), 3.47 (AB q, 2 H, $J = 14.6$ Hz), 2.22 (s, 6 H), 1.96 (s, 3 H).

7-Acetoxy-2-chloro-10-[(*N*,*N*-dimethylamino)methyl]-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (56). Starting from **19** (0.73 g, 1.83 mmol), the title compound was obtained as pale yellow prisms according to the procedure described for **58**, after chromatography using $CHCl_3$ as eluant: $R_f = 0.38$ (EtOAc); IR ($CHCl_3$) 1778 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.07−7.25 (m, 5 H), 6.86 (d, 2 H, $J = 8.7$ Hz), 6.53 (d, 1 H, $J = 3.7$ Hz), 6.29 (d, 1 H, $J = 3.7$ Hz), 3.81 (s, 3 H), 3.35 (AB q, 2 H, $J = 12.8$ Hz), 2.28 (s, 6 H), 1.97 (s, 3 H).

(±)-*cis*-7-Acetoxy-6,7-dihydro-10-[(*N*,*N*-dimethylamino)methyl]-6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepine (57). Starting from **43** (1.27 g, 3.76 mmol), **57** was obtained according to the procedure described for **58**, except that the column chromatography was not necessary. **57** was collected and recrystallized as colorless prisms: IR ($CHCl_3$) 1732 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.90−7.30 (m, 9 H), 6.26 (d, 1 H, $J = 3.2$ Hz), 6.04 (d, 1 H, $J = 2.5$ Hz), 5.75 (d, 1 H, $J = 6.3$ Hz), 5.20 (d, 1 H, $J = 6.3$ Hz), 3.60 (AB q, 2 H, $J = 13.6$ Hz), 2.18 (s, 6 H), 1.83 (s, 3 H).

(±)-*cis*-7-Acetoxy-6,7-dihydro-10-[(*N*,*N*-dimethylamino)methyl]-6-phenyl-2-(trifluoromethyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (59). Starting from 0.78 g (1.89 mmol) of **45**, the title compound was obtained using an identical procedure as for **58** (reaction time 15 h). **59** recrystallized as colorless prisms: $R_f = 0.49$ (EtOAc); IR ($CHCl_3$) 1744 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.65−7.20 (m, 8 H), 6.25 (d, 1 H, $J = 3.7$ Hz), 6.04 (d, 1 H, $J = 3.5$ Hz), 5.76 (d, 1 H, $J = 6.4$ Hz), 5.23 (d, 1 H, $J = 6.5$ Hz), 3.25 (AB q, 2 H, $J = 13.7$ Hz), 2.21 (s, 6 H), 1.84 (s, 3 H).

(±)-*cis*-7-Acetoxy-6,7-dihydro-10-[(*N*,*N*-dimethylamino)methyl]-6-(*p*-methoxyphenyl)-2-(trifluoromethyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (60). The title compound was prepared according to the procedure described for **58**, starting from **46** (0.62 g, 1.26 mmol) (reaction time 24 h at room temperature). **60** was flash chromatographed ($CHCl_3$) and recrystallized as colorless prisms: $R_f = 0.26$ ($CHCl_3$); IR (film) 1744 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.60−7.30 (m, 7 H), 6.35 (d, 1 H, $J = 3.6$ Hz), 6.10 (d, 1 H, $J = 3.4$ Hz), 5.66 (d, 1 H, $J = 6.4$ Hz), 5.33 (d, 1 H, $J = 6.5$ Hz), 3.81 (s, 3 H), 3.20 (AB q, 2 H, $J = 13.1$ Hz), 2.2 (s, 6 H), 1.87 (s, 3 H).

(±)-*cis*-7-Acetoxy-2-chloro-6,7-dihydro-10-[(*N*,*N*-dimethylamino)methyl]-6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepine (61). Starting from **47** (0.56 g, 1.51 mmol), **61** was obtained as a white solid according to the procedure described above for **58** (reaction time 14 h): $R_f = 0.44$ (EtOAc); IR (KBr) 1748 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.96−7.20 (m, 8 H), 6.39 (d, 1 H, $J = 3.6$ Hz), 6.07 (d, 1 H, $J = 3.3$ Hz), 5.72 (d, 1 H, $J = 6.5$ Hz), 5.17 (d, 1 H, $J = 6.4$ Hz), 3.54 (AB q, 2 H, $J = 13.9$ Hz), 2.29 (s, 6 H), 1.84 (s, 3 H).

(±)-*cis*-7-Acetoxy-2-chloro-6,7-dihydro-10-[(*N*,*N*-dimethylamino)methyl]-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (62). The title compound was prepared according to the procedure described for **58**, starting from **48** (0.62 g, 1.55 mmol) (reaction time 20 h at room temperature). **62** was recrystallized as white prisms: $R_f = 0.32$ (EtOAc); IR (film) 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.19−7.25 (m, 3 H), 7.13 (d, 2 H, $J = 8.7$ Hz), 6.83 (d, 2 H, $J = 8.7$ Hz), 6.23 (d, 1 H, $J = 3.5$ Hz), 6.03 (d, 1 H, $J = 3.5$ Hz), 5.70 (d, 1 H, $J = 6.2$ Hz), 5.14 (d, 1 H, $J = 6.5$ Hz), 3.80 (s, 3 H), 3.28 (AB q, 2 H, $J = 13.7$ Hz), 2.22 (s, 6 H), 1.85 (s, 3 H).

(±)-*cis*-7-Acetoxy-4-chloro-6,7-dihydro-10-[(*N*,*N*-dimethylamino)methyl]-6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepine (63). The title compound was prepared according to the procedure described for **58**, starting from **49** (0.57 g, 1.42 mmol) (reaction time 20 h at room temperature). **63** was recrystallized as white prisms: $R_f = 0.38$ (EtOAc); IR (film) 1743 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.80−7.20 (m, 8 H), 6.25 (d, 1 H, $J = 3.4$ Hz), 6.05 (d, 1 H, $J = 3.5$ Hz), 5.70 (d, 1 H, $J = 5.7$ Hz), 5.18 (d, 1 H, $J = 6.3$ Hz), 3.33 (AB q, 2 H, $J = 9.1$ Hz), 2.17 (s, 6 H), 1.82 (s, 3 H).

(±)-*cis*-7-Acetoxy-4-chloro-6,7-dihydro-10-[(*N*,*N*-dimethylamino)methyl]-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (64). The title compound was prepared according to the procedure described for **58**, starting from **50** (0.52 g, 1.30 mmol) (reaction time 20 h at room temperature). **64** was recrystallized as colorless prisms: $R_f = 0.40$ (EtOAc); IR (film) 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.80−7.25 (m, 3 H), 7.14 (d, 2 H, $J = 8.6$ Hz), 6.83 (d, 2 H, $J = 8.4$ Hz), 6.25 (d, 1 H, $J = 3.4$ Hz), 6.05 (d, 1 H, $J = 3.5$ Hz), 5.66 (d, 1 H, $J = 6.4$ Hz), 5.15 (d, 1 H, $J = 6.3$ Hz), 3.81 (s, 3 H), 3.33 (AB q, 2 H, $J = 13.7$ Hz), 2.17 (s, 6 H), 1.85 (s, 3 H).

(±)-*cis*-7-Acetoxy-3-chloro-6,7-dihydro-10-[(*N*,*N*-dimethylamino)methyl]-6-(*p*-methoxyphenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (65). Starting from 0.83 g (2.07 mmol) of **51**, the title compound was obtained using an identical procedure as for **58** (reaction time 15 h). **65** recrystallized as colorless prisms: $R_f = 0.49$ (EtOAc); IR ($CHCl_3$) 1746 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.91−7.17 (m, 3 H), 7.12 (d, 2 H, $J = 8.7$ Hz), 6.83 (d, 2 H, $J = 8.7$ Hz), 6.26 (d, 1 H, $J = 3.6$ Hz), 6.04 (d, 1 H, $J = 3.4$ Hz), 5.69 (d, 1 H, $J = 6.3$ Hz), 5.16 (d, 1 H, $J = 6.2$ Hz), 3.81 (s, 3 H), 3.32 (AB q, 2 H, $J = 13.7$ Hz), 2.19 (s, 6 H), 1.85 (s, 3 H).

(±)-*cis*-7-Acetoxy-6,7-dihydro-10-[(*N*,*N*-dimethylamino)methyl]-3-methyl-6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepine (66). Starting from 0.62 g (1.77 mmol) of **52**, the title compound was obtained using a procedure identical with that for **58** (reaction time 20 h). **66** recrystallized as colorless prisms: $R_f = 0.51$ (EtOAc); IR ($CHCl_3$) 1751 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.79−7.06 (m, 8 H), 6.53 (d, 1 H, $J = 3.4$ Hz), 6.34 (d, 1 H, $J = 3.5$ Hz), 5.74 (d, 1 H, $J = 6.4$ Hz), 5.15 (d, 1 H, $J = 6.2$ Hz), 3.48 (AB q, 2 H, $J = 13.4$ Hz), 2.38 (s, 3 H), 2.20 (s, 6 H), 1.82 (s, 3 H).

(±)-*cis*-7-Acetoxy-6,7-dihydro-10-[(*N*,*N*-dimethylamino)methyl]-6-(*o*-fluorophenyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (67). Starting from **53** (0.74 g, 2.09 mmol), **67** was obtained as colorless prisms according to the procedure described for **58**, except that column chromatography was not necessary: IR ($CHCl_3$) 1755 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.95−7.11 (m, 8 H), 6.23 (d, 1 H, $J = 3.5$ Hz), 5.90 (m, 1 H), 5.87 (d, 1 H, $J = 6.2$ Hz), 5.60 (d, 1 H, $J = 6.0$ Hz), 3.38 (AB q, 2 H, $J = 13.7$ Hz), 2.17 (s, 6 H), 1.91 (s, 3 H).

(±)-*cis*-7-Acetoxy-2-chloro-6,7-dihydro-6-(*p*-methoxyphenyl)-10-(morpholinomethyl)pyrrolo[2,1-*d*][1,5]benzothiazepine (68). Starting from **48** (100 mg, 0.25 mmol) and morpholine, **68** was obtained as colorless prisms according to the procedure described for **58** (reaction time 24 h): IR ($CHCl_3$) 1748 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.38 (d, 1 H, $J = 1.81$ Hz), 7.69 (dd, 1 H, $J = 8.3, 1.4$ Hz), 7.30 (dd, 1 H, $J = 8.1, 2.2$ Hz), 7.14 (d, 2 H, $J = 8.7$ Hz), 6.84 (d, 2 H, $J = 8.7$ Hz), 6.22 (d, 1 H, $J = 3.3$ Hz), 6.03 (d, 1 H, $J = 3.6$ Hz), 5.72 (d, 1 H, $J = 6.3$ Hz), 5.14 (d, 1 H, $J = 6.5$ Hz), 3.81 (s, 3 H), 3.73 (m, 4 H), 3.33 (s, 2 H), 2.59−2.37 (m, 4 H), 1.86 (s, 3 H).

Radioligand Binding Assays. 1. Mitochondrial Benzodiazepine Receptor Binding Assay. Male CRL:CD(SD)-BR rats (Charles River Italia, Calco, CO, Italy), weighing about 150 g, were used in this experiment. The rats were housed in groups of five in plastic cages, kept under standard conditions (21 ± 1 °C, relative humidity 55 ± 10%, 12−12 h light−dark cycle), and given tap water and food 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 times more by resuspension in fresh buffer and centrifuged as before. The last pellet was resuspended just before the binding assay. For mitochondrial benzodiazepine binding,[35] 10 mg of original wet tissue weight was incubated with 1 nM [^3H]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, Wallac) 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 fluid (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[36] fitting of binding inhibition curves, using the Allifit program running on an IBM AT personal computer. Each point was the mean of triplicate samples.

2. Calcium Channel Receptor Binding Assay. Tissue homogenate of cerebral cortex and heart containing calcium channel receptors was prepared according to Ehlert *et al.*[17a] Cerebral cortices of male Sprangue−Dawley rats were homogenized (100 mg/mL in 50 mM Na-HEPES buffer, pH 7.4). Hearts were also removed, perfused through the aorta with ice-cold saline solution, and homogenized (100 mg/mL in 50 mM Na-Hepes buffer, pH 7.4). Subsequentely, the cardiac homogenates were filtered through four layers of cheese cloth. Both cortical and cardiac homogenates were washed five times by centrifugation for 10 min at 48000*g*. The final pellet was resuspended to a concentration of 50 mg of original wet tissue wt/mL of buffer and stored at −70 °C for the assay. The receptor binding assay was determined as follows: 200 μL of tissue homogenate was incubated for 90 min in a dark room at 0 °C with 100 μL of [^{3}H]nitrendipine (3×10^{-10} M, 87 Ci/mmol; NEN) and 100 μL of the test compound (dissolved in 5% DMSO) in 50 mM Na/HEPES buffer, pH 7.4 (total vol 2 mL). The incubations were stopped by adding 4 mL of cold buffer followed by rapid filtration through glass fiber filter disks. The samples were subsequentely washed three times with 4.5 mL of the same buffer and placed into scintillation vials; 10 mL of Filter-Count (Packard) liquid scintillation cocktail was then added to each vial, and counting was carried out by a scintillation spectrometer (Packard T.-C. 300C). Nonspecific binding was defined in the presence of 1×10^{-4} M unlabeled diltiazem and verapamil and specific binding as the difference between total and nonspecific binding. Inhibition of [^{3}H]nitrendipine by diltiazem and verapamil is 70% approximately, in agreement with ref 17. Blank experiments were carried out to determine the effect of the solvent DMSO (5%) on the binding. The concentration of the test compounds that inhibited [^{3}H]nitrendipine binding by 50% (IC_{50}) was determined by log−probit analysis with six concentrations of the displacers, each performed in triplicate. The IC_{50} values obtained were used to calculate apparent inhibition constants (K_i) by Prusoff's method[37] according to the following equation: $K_i = IC_{50}/(1 + S/K_d)$, where S represents the concentration of the ligand used and K_d is its receptor dissociation constant, obtained by Scatchard analysis (K_d value of [^{3}H]nitrendipine was 0.25 nM).

Pharmacology. The pharmacological profile of the compounds was assessed on guinea pig isolated left and right atria to evaluate their negative inotropic and chronotropic effects and K^+-depolarized guinea pig artery strips to test their calcium antagonist activity. All the compounds were first checked at increasing doses to examine the dose-dependent decrease both of the developed tension in the left atria driven at 1 Hz and of the frequency in spontaneously beating right atria and then to measure the inhibitory effect on K^+-evoked contractions in guinea pig helicoidal aortic strips. ED_{50}, ED_{30}, and IC_{50} values were evaluated from log concentration−response curves in the appropriate pharmacological preparation. Guinea pigs (300−400 g, male and female) were sacrificed by cervical dislocation. After thoracotomy, the hearts were immediately removed and washed by perfusion through the aorta with oxygenated Tyrode solution of the following composition (mmol/L): 136.9 NaCl, 5.4 KCl, 2.5 $CaCl_2$, 1.0 $MgCl_2$, 0.4 $NaH_2PO_4 \cdot H_2O$, 11.9 $NaHCO_3$, 5.5 glucose. The physiological salt solution (PSS) was buffered to pH 7.4 by saturation with 95% O_2−5% CO_2 gas, and the temperature was maintained at 35 °C. Isolated guinea pig heart preparations were used, spontaneously beating right and left atria driven at 1 Hz.

For each preparation the entire left and right atria were dissected from ventricles, cleaned of excess tissue, and hung vertically in a 15 mL organ bath containing the PSS continuously bubbled with 95% O_2−5% CO_2 gas at 35 °C, pH 7.4. The contractile activity was recorded isometrically by means of a force transducer (FT 0.3, Grass Instruments, Quincy, MA) connected to a pen recorder (KV 380, Battaglia-Rangoni, Bologna, Italy). The left atria were stimulated by rectangular pulse of 0.6−0.8 ms duration and ca. 50% threshold voltage through two platinum contact electrodes in the lower holding clamp (Grass S88 stimulator). After the tissue was beating for several minutes, a length−tension curve was determined, and the muscle length was maintained at that which elicited 90% of maximum contractile force observed at the optimal length. A stabilization period of 45−60 min was allowed before the atria were challenged by various agents. During this equilibration period, the bathing solution was changed every 15 min and the threshold voltage was ascertained for the left atria.

Atrial muscle preparations were used to examine the inotropic and chronotropic activity of the compounds (0.1, 0.5, 1, 5, 10, and 50 μmol/L) dissolved in DMSO. According to this procedure the concentration of DMSO in the bath solution never exceeded 0.3%, a concentration which did not produce appreciable inotropic and chronotropic effects. During generation of cumulative dose−response curves, the next higher concentration of the compounds was added only after the preparation reached a steady state. The thoracic aorta was removed and placed in Tyrode solution of the following composition (mmol/L): 118 NaCl, 4.75 KCl, 2.54 $CaCl_2$, 1.20 $MgSO_4$, 1.19 KH_2PO_4, 25 $NaHCO_3$, 11 glucose, equilibrated with 95% O_2 and 5% CO_2 gas at pH 7.4. Vessel was cleaned of extraneous connective tissue. Two helicoidal strips (15 mm × 3 mm) were cut from each aorta beginning from the end most proximal to the heart. Vascular strips were then tied with surgical thread and suspended in a jacketed tissue bath (15 mL) containing aerated PSS at 35 °C. Strips were secured at one end to Plexiglass hooks and connected via the surgical thread to a force displacement (FT. 0.3, Grass) transducer for monitoring changes in isometric contraction. Aortic strips were subjected to a resting force of 1 g and washed every 20 min with fresh PSS for 1 h. After the equilibration period guinea pig aortic strips were contracted by washing in PSS containing 80 mmol/L KCl (equimolar substitution of K^+ for Na^+). After the contraction reached a plateau (approximately 30 min), the compounds (0.1, 0.5, 1, 5, 10, and 50 μmol/L) were added cumulatively to the bath allowing for any relaxation to obtain an equilibrate level of force. Addition of the drug vehicle had no appreciable effect on the K^+-induced level of force (DMSO for all compounds). Data were analyzed by Student's *t*-test. The criterion for significance was a p value less than 0.05. ED_{50}, ED_{30}, and IC_{50} values were calculated from log concentration−response curves (probit analysis by Litchfield and Wilcoxon, $n = 6-8$). All data are presented as mean ± SEM.[38]

X-ray Crystallography. Single crystals of **48** were obtained by dissolving 100 mg of powder in 50 mL of ethanol and allowing the solution to concentrate at room temperature. A colorless prism of approximate dimensions 0.10 × 0.25 × 0.45 mm was used. Crystal data: $C_{21}H_{18}ClNO_3S$, triclinic, $P\bar{1}$ (no. 2); $a = 8.719(2)$, $b = 9.639(2)$, and $c = 12.326(2)$ Å; $\alpha = 95.01(2)°$, $\beta = 93.96(2)°$, $\gamma = 110.74(2)°$; $U = 959.5(3)$ Å^3, $Z = 2$.

Lattice parameters were determined by least-squares refinement on 26 randomly selected and automatically centered reflections. Full crystal data are reported in Table 9. The data were collected on a Siemens P4 four-circle diffractometer with graphite monochromated Mo Kα radiation, in the range $0 \le h \le 12$, $-13 \le k \le 12$, $-17 \le l \le 17$, ω scan mode for $2 \le 2\Theta \le 60°$ scan range, scan width 1.4°, constant scan speed 2.70° min^{-1}. Three standard reflections (223, 033, 222) measured every 60 min showed no variations; 5589 independent reflections ($R_{int} = 0.008$) were collected at 22 °C and 3575 observed reflections with $F_o > 4(F)$. No absorption correction was applied. The structure was solved by direct methods of the SHELXTL PC package.[39] The refinement was carried out by full-matrix anisotropic least-squares on F for all non-H atoms. The hydrogen atoms were located on Fourier difference maps and included in the structure-factor calculations with an

isotropic temperature factor. The two methyl groups were refined as rigid groups with the thermal parameter for the hydrogen atoms fixed at 80 Å^2 × 10^3. Final refinement of 293 parameters gave $R = 0.049$ and $R_w = 0.055$, minimized function $\sum w(|F_o| - |F_c|)^2$ with weighing scheme $w = 1/[\sigma^2(F) + 0.0002F^2]$, min and max heights in last map of −0.30 and 0.34 e Å^{-3}. Atomic scattering factors including f' and f'' were taken from ref 40. The final fractional atomic coordinates and equivalent isotropic thermal parameters are given in Table 9.

Nonbonded Potential Energy Calculations. Atom−atom nonbonded potential energy calculations were carried out by using the function: $E_{jk} = B_{jk} \exp(-C_{jk}r_{jk}) - A_{jk} r_{jk}^{-6}$ implemented in the program ROTENER.[41] The X-ray structure of **48** and those of **4a**,[22]**b**[23] were used. The torsion angles C(7)−C(6)−C(1′)−C(2′) and C(7a)−C(7)−O(7)−C(13) were rotated by a step of 10° in the range 0−360°. All calculations were performed on a personal computer equipped with a 80486/50 processor and ISA/IDE cache controller.

Nuclear Magnetic Resonance Study. ^{1}H NMR spectra were recorded for $CDCl_3$ solutions with a Bruker AC 200 spectrometer (200 MHz). Chemical shifts (J in Hz) are reported downfield from internal tetramethylsilane. Proton−proton nuclear Overhauser effects (NOEs) were measured with gated decoupling techniques using NOE difference pulse sequences. ^{1}H−^{13}C heterocorrelation and homonuclear correlation (COSY) experiments were performed according to standard sequences.

Molecular Mechanics Calculations. Molecular modeling calculations were performed by using the packages SYBYL 5.5.[32] SYBYL was run on a DEC VAX 6610 computer tethered to an Evans & Sutherland PS390 graphics system.

Acknowledgment. This work was supported by a grant from MURST (60%) and CNR, Rome, and by CNR-progetto Speciale "Meccanismi della Trasduzione del Segnale", Rome.

Supporting Information Available: X-ray crystal coordinates of compound **48** (5 pages). Ordering information is given on any current masthead page.

References

(1) (a) Marangos, P. J.; Patel, J.; Boulenger, J. P.; Clark-Rosemberg, R. Characterization of Peripheral-Type Benzodiazepine Binding Sites in Brain Using [^{3}H]Ro 5-4864. *Mol. Pharmacol.* **1982**, *22*, 26−32. (b) Shoemaker, H.; Boles, R. G.; Horst, W. D.; Yamamura, H. I. Specific High-Affinity Binding Sites for [^{3}H]Ro 5-4864 in Rat Brain and Kidney. *J. Pharmacol. Exp. Ther.* **1983**, *225*, 61−69.

(2) (a) Zavala, F.; Haumont, J.; Lenfant, M. Interaction of Benzodiazepines with Mouse Macrophages. *Eur. J. Pharmacol.* **1984**, *106*, 561−566. (b) Mestre, M.; Carriot, T.; Belin, C.; Uzan, A.; Renault, C.; Dubroeucq, M. C.; Gueremy, C.; Le Fur, G. Electrophysiological and Pharmacological Characterization of Peripheral Benzodiazepine Receptors in Guinea Pig Heart Preparation. *Life Sci.* **1984**, *35*, 953−962. (c) Grupp, I. L.; French, J. F.; Matlib, M. A. Benzodiazepine RO 5-4864 Increases Coronary Flow. *Eur. J. Pharmacol.* **1987**, *143*, 143−147. (d) Basile, A. S.; Lueddens, H. W. M.; Skolnick, P. Regulation of Renal Peripheral Benzodiazepine Receptors by Anion Transport Inhibitors. *Life Sci.* **1988**, *42*, 715−726. (e) Anholt, R. R. H.; De Souza, E. B.; Oster-Granite, M. L.; Snyder, S. H. Peripheral-Type Benzodiazepine Receptors: Autoradiographic Localization in Whole-Body Sections of Neonatal Rats. *J. Pharmacol. Exp. Ther.* **1985**, *233*, 517−526. (f) Ruff, M. R.; Pert, C. B.; Weber, R. J.; Wahl, L. M.; Paul, S. M. Benzodiazepine Receptors-Mediated Chemotaxis of Human Monocytes. *Science* **1985**, *229*, 1281−1283.

(3) Wang, J. K. T.; Morgan, J. I.; Spector, S. Benzodiazepines that Bind at Peripheral Sites Inhibit Cell Proliferation. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 3770−3772.

(4) (a) Newman, A. H.; Hogue, B. A.; Basile, A. S.; Hansford, R. G.; Chiang, P. K.; Moreno-Sanchez, R. Inhibition of Oxidative Phosphorylation by Peripheral-Type Benzodiazepines. *FASEB J.* **1989**, *3*, A703. (b) Moreno-Sanchez, R.; Hogue, B. A.; Bravo, C.; Newman, A. H.; Basile, A. S.; Chiang, P. K. Inhibition of Substrate Oxidation in Mitochondria by Peripheral-Type Benzodiazepine Receptor Ligand AHN 086. *Biochem. Pharmacol.* **1990**, *41*, 1479−1484. (c) Larcher, J.-C.; Vayssiere, J.-L.; LeMarquer, F. J.; Cordeau, L. R.; Keane, P. E.; Bachy, A.; Grus, F.; Croizat, B. P. Effect of Peripheral Benzodiazepines Upon the O_2 Consumption of Neuroblastoma Cells. *Eur. J. Pharmacol.* **1989**, *161*, 197−202.

(5) (a) Besman, M. J.; Yanagibashi, K.; Lee, T. D.; Kawamura, M.; Hall, P. F.; Shively, J. E. Identification of Des-(Gly-Ile)-Endozepine as an Effector of Corticotropin-Dependent Adrenal Steroidogenesis: Stimulation of Cholesterol Delivery is Mediated by the Peripheral Benzodiazepine Receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 4897−4901. (b) Amsterdam, A.; Sun Suh, B. An Inducible Functional Peripheral Benzodiazepine Receptor in Mitochondria of Steroidogenic Granulosa Cells. *Endocrinology* **1991**, *129*, 503−510. (c) McCauley, L. D.; Park, C. H.; Lan, N. C.; Tomich, J. M.; Shively, J. E.; Gee, K. W. Benzodiazepines and Peptides Stimulate Pregnenolone Synthesis in Brain Mitochondria. *Eur. J. Pharmacol.* **1995**, *276*, 145−153.

(6) (a) Le Fur, G.; Mestre, M.; Carriot, T.; Belin, C.; Renault, C.; Dubroeucq, M. C.; Gueremy, C.; Uzan, A. Pharmacology of Peripheral-Type Benzodiazepine Receptors in the Heart. In *Endocoids*; Lal, H., Labella, F., Lane, J., Eds.; Alan R. Liss Inc.: New York, 1985; pp 175−186. (b) Mestre, M.; Bouefard, G.; Uzan, A.; Gueremy, C.; Renault, C.; Dubroeucq, M.-C.; Le Fur, G. PK 11195, an Antagonist of Peripheral Benzodiazepine Receptors, Reduces Ventricular Arrhythmias During Myocardial Ischemia and Reperfusion in the Dog. *Eur. J. Pharmacol.* **1985**, *112*, 257−260.

(7) (a) Bender, A. S.; Hertz, L. Pharmacological Evidence that the Non-Neuronal Diazepam Binding Site in Primary Cultures of Glial Cells is Associated to Calcium Channel. *Eur. J. Pharmacol.* **1985**, *110*, 287−288. (b) Cantor, E. H.; Kenessey, A.; Semenuk, G.; Spector, S. Interaction of Calcium Channel Blockers with Non-Neuronal Benzodiazepine Binding Sites. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1549−1552.

(8) Bolger, G. T.; Weissman, B. A.; Lueddens, H.; Barret, J. E.; Witkin, J.; Paul, S. M.; Skolnick, P. Dihydropyridine Calcium Channel Antagonist Binding in Non-Mammalian Vertebrates: Characterization and Relationship to "Peripheral-Type" Binding Sites for Benzodiazepines. *Brain Res.* **1986**, *368*, 351−356.

(9) (a) Mestre, M.; Belin, C.; Uzan, A.; Renault, C.; Dubroeucq, M.-C.; Gueremy, C.; Le Fur, G. Modulation of Voltage-operated, but Not Receptor-Operated, Calcium Channels in Rabbit Aorta by PK 11195, an Antagonist of Peripheral-Type Benzodiazepine Receptors. *J. Cardiovasc. Pharmacol.* **1986**, *8*, 729−734. (b) Mestre, M.; Carriot, T.; Neliat, G.; Uzan, A.; Renault, C.; Dubroeucq, M.-C.; Gueremy, C.; Doble, A.; LeFur, G. PK 11195, an Antagonist of Peripheral Benzodiazepine Receptors, Modulates BAY K 8644 Sensitive but Not β- or H_2-Receptor Sensitive Voltage-Operated Calcium Channels in the Guinea Pig Heart. *Life Sci.* **1986**, *39*, 329−339. (c) Elgoyhen, B.; Adler-Graschinsky, E. Diminution by Benzodiazepines of the Chronotropic Responses to Noradrenaline in Rat Isolated Atria. *Eur. J. Pharmacol.* **1989**, *164*, 467−478. (d) Holck, M.; Osterrieder, W. The Peripheral, High Affinity Benzodiazepine Binding Site is Not Coupled to the Cardiac Ca^{2+} Channel. *Eur. J. Pharmacol.* **1985**, *118*, 293−301.

(10) Gavish, M.; Katz, Y.; Bar-Ami, S.; Weizman, R. Biochemical, Physiological, and Pathological Aspects of the Peripheral Benzodiazepine Receptor. *J. Neurochem.* **1992**, *58*, 1589−1601.

(11) Doble, A.; Benavides, J.; Ferris, O.; Bertrand, P.; Menager, J.; Vaucher, N.; Burgevin, M.-C.; Uzan, A.; Gueremy, C.; Le Fur, G. Dihydropyridine and Peripheral-Type Benzodiazepine Binding Sites: Subcellular Distribution and Molecular Size Determination. *Eur. J. Pharmacol.* **1985**, *119*, 153−167.

(12) (a) Saano, V.; Raty, M.; MacDonald, E. Effect of Peripheral Benzodiazepine Receptor Ligands on the Contraction of Isolated Heart Atrium and Papillary Muscle of Rats. *Pharmacol. Toxicol.* **1989**, *64*, 147−149. (b) Shany, E.; Hochhauser, E.; Halpern, P.; Vidne, B.; Gavish, M.; Geller, E.; Hasharoni, A.; Barak, Y.; Yakirevich, V. Ro 5-4864 has a Negative Inotropic Effect on Human Atrial Muscle Strips that is Not Antagonized by PK 11195. *Eur. J. Pharmacol.* **1994**, *253*, 231−236.

(13) Thuillez, J.; Loueslati, H.; Duhaze, P.; Giudicelli, J. F. Functional Antagonism Between PK 11195, a Peripheral Benzodiazepine Receptor Antagonist, and Nicardipine at the Vascular Level in Healthy Subjects: a Peripheral Hemodinamic Study. *J. Cardiovasc. Pharmacol.* **1989**, *13*, 307−313.

(14) (a) Nacci, V.; Fiorini, I. Ricerche su Composti ad Attivita′ Psicotropa. Nota VII. Sintesi e Stereochimica della cis-4,5-Diidro-4-idrossi-5-fenilpirrolo[2,1-d][1,5]benzotiazepina. (Studies on Compounds with Psycotropic Activity. VII. Synthesis and Stereochemistry of cis-4,5-Dihydro-4-hydroxy-5-phenylpyrrolo[2,1-d][1,5]benzothiazepine.) *Farmaco Ed. Sci.* **1983**, *38*, 112−120. (b) Nacci, V.; Fiorini, I.; Garofalo, A.; Cagnotto, A. Research on Compounds with Psycotropic Activity. IX. Synthesis of 6-p-Methoxyphenylpyrrolo[2,1-d][1,5]benzothiazepines and Evaluation of their Affinity for BDZ and GABA Receptor Subtypes. *Farmaco Ed. Sci.* **1990**, *45*, 545−557. (c) Nacci, V.; Fiorini, I.; Vomero, S.; Taddei, I.; Taddei, E. Ricerche su Composti ad Attivita' Psicotropa. Nota VIII. Sintesi ed Attivita′ Sedativa di Alcuni Derivati 9-Sostituiti della 5-Fenilpirrolo[2,1-d][1,5]-benzotiazepina e della *cis*-4,5-Diidro-4-idrossi-5-fenilpirrolo[2,1-d][1,5]benzotiazepina. (Studies on Compounds with Psycotropic Activity. VIII. Synthesis and Sedative Activity of Some 9-Substituted Derivatives of 5-Phenylpyrrolo[2,1-d][1,5]benzothiazepine

and *cis*-4,5-Dihydro-4-hydroxy-5-phenylpyrrolo[2,1-d][1,5]benzothiazepine.) *Farmaco Ed. Sci.* **1984**, *39*, 289−304. (d) Fiorini, I.; Nacci, V.; Ciani, S. M.; Garofalo, A.; Campiani, G.; Savini, L.; Novellino, E.; Greco, G.; Bernasconi, P.; Mennini, T. Novel Ligands for Mitochondrial Benzodiazepine Receptors: 6-Arylpyrrolo[2,1-d][1,5]benzothiazepine Derivatives. Synthesis, Structure-Activity Relationships, and Molecular Modeling Studies. *J. Med. Chem.* **1994**, *37*, 1427−1438. (e) Greco, G.; Novellino, E.; Fiorini, I.; Nacci, V.; Campiani, G.; Ciani, S. M.; Garofalo, A.; Bernasconi, P.; Mennini, T. A Comparative Molecular Field Analysis Model for 6-Arylpyrrolo[2,1-d][1,5]benzothiazepines Binding Selectively to the Mitochondrial Benzodiazepine Receptor. *J. Med. Chem.* **1994**, *37*, 4100−4108.

(15) (a) Campiani, G.; Nacci, V.; Garofalo, A.; Botta, M.; Fiorini, I.; Tafi, A.; Bruni, G.; Romeo, M. R.; Peres, A.; Bertollini, L. Synthesis and Preliminary Biological Evaluation of 1-Aminomethyl-4-substituted-4*H*-pyrrolo[2,1-c][1,4]benzothiazines, a New Class of Calcium Antagonists. *BioMed. Chem. Lett.* **1992**, *2*, 1193−1198. (b) Campiani, G.; Garofalo, A.; Fiorini, I.; Nacci, V.; Botta, M.; Tafi, A.; Chiarini, A.; Budriesi, R.; Bruni, G.; Romeo, M. R. Synthesis and "In Vitro" Cardiovascular Activity of 4-Aryl-2,3,3a,4-tetrahydropyrrolo[2,1-c][1,4]benzothiazin-1-ones and 7-Acetoxy-6-phenyl-7a,8,9,10-tetrahydropyrrolo[2,1-d][1,5]benzothiazepin-10-one. *BioMed. Chem. Lett.* **1994**, *4*, 1235−1240. (c) Campiani, G.; Garofalo, A.; Fiorini, I.; Botta, M.; Nacci, V.; Tafi, A.; Chiarini, A.; Budriesi, R.; Bruni, G.; Romeo, M. R. Pyrrolo-[2,1-c][1,4]benzothiazines: Synthesis, Structure-Activity Relationships, Molecular Modeling, and Cardiovascular Activity. *J. Med. Chem.* **1995**, *38*, 4393−4410.

(16) Gould, R. J.; Murphy, M. M. K.; Snyder, S. H. Tissue Heterogeneity of Calcium Channel Antagonist Binding Sites Labeled by [^{3}H]Nitrendipine. *Mol. Pharmacol.* **1983**, *25*, 235−241.

(17) (a) Ehlert, F. J.; Itoga, E.; Roeske, W. R.; Yamamura, H. I. The Interaction of [^{3}H]Nitrendipine with Receptor for Calcium Antagonists in the Cerebral Cortex and Heart of Rats. *Biochem. Biophys. Res. Commun.* **1982**, *104*, 937−943. (b) Boles, R. J.; Yamamura, H. I.; Schoemaker, H.; Roeske, W. R. Temperature-Dependent Modulation of [^{3}H]Nitrendipine Binding by Calcium Channel Antagonists Verapamil and Diltiazem in Rat Brain Synaptosomes. *J. Pharmacol. Exp. Ther.* **1984**, *229*, 333−339.

(18) Hall, H. K. Correlation of the Base Strengths of Amines. *J. Am. Chem. Soc.* **1957**, *79*, 5441−5444.

(19) Janis, R. A.; Triggle, D. J. New Developments in Ca^{2+} Channel Antagonists. *J. Med. Chem.* **1983**, *26*, 775−785 and references therein.

(20) (a) Catteral, W. A.; Seagar, M. J.; Takahashi, M. Molecular Properties of Dihydropyridine-Sensitive Calcium Channels in Skeletal Muscle. *J. Biol. Chem.* **1988**, *263*, 3533−3538. (b) Striessnig, J.; Scheffauer, F.; Mitterdorfer, J.; Schirmer, M.; Glossmann, H. Identification of the Benzothiazepine-Binding Polypeptide of Skeletal Muscle Calcium Channels with (+)-*cis*-Azidodilthiazem and Anti-Ligand Antibodies. *J. Biol. Chem.* **1990**, *265*, 363−370. (c) Tsien, R. W.; Ellinor, P. T.; Horne, V. A. Molecular Diversity of Voltage-Dependent Ca^{2+} Channels. *Trends Pharmacol. Sci.* **1991**, *12*, 349−354.

(21) (a) Ellis, S. B.; Williams, M. E.; Ways, N. R.; Brenner, R.; Sharp, A. H.; Leung, A. T.; Campbell, K. P.; McKenna, E.; Koch, W. J.; Hui, A.; Schwartz, A.; Harpold, M. M. Sequence and Expression of mRNAs Encoding the α1 and α2 Subunits of a DHP-Sensitive Calcium Channel. *Science* **1988**, *241*, 1661−1664. (b) Hofmann, F.; Flockerzi, V.; Nastainczyk, W. The Molecular Structure and Regulation of Muscular Calcium Channels. *Curr. Top. Cell. Regul.* **1990**, *31*, 223−239. (c) Hofmann, F.; Biel, M.; Flockerzi, V. Molecular Basis for Ca^{2+} Channel Diversity. *Annu. Rev. Neurosci.* **1994**, *17*, 399−418.

(22) Kojic-Prodic, B.; Ruzic-Toros, Z.; Sunjic, V.; Decorte, E.; Moimas, F. Absolute Conformation of (2S,3S)-3-Acetoxy-5-(dimethylaminoethyl)-2-(4-methoxyphenyl)-2,3-dihydro-1,5-benzothiazepin-4(5H)-one Chloride (Dilthiazem Hydrochloride). *Helv. Chim. Acta* **1984**, *67*, 916−926.

(23) Inoue, H.; Mikihiko, K.; Hashiyama, T.; Otsuka, H.; Takahashi, K.; Gaino, M.; Date, T.; Aoe, K.; Takeda, M.; Murata, S.; Narita, H.; Nagao, T. Synthesis of Halogen-Substituted 1,5-Benzothiazepine Derivatives and Their Vasodilating and Hypotensive Activities. *J. Med. Chem.* **1991**, *34*, 675−687.

(24) Sbit, P. M.; Dupont, L.; Dideberg, O.; Liegeois, J.; Delarge, J. Structure de la Clothiapine. *Acta Crystallogr. Sect. C* **1987**, *43*, 720−722.

(25) Sbit, P. M.; Dupont, L.; Dideberg, O.; Liegeois, J.; Delarge, J. Structure du 5-(1-Methyl-4-piperazinyl)pyrido[2,3-b][1,5]benzothiazepine. *Acta Crystallogr. Sect. C* **1988**, *44*, 319−321.

(26) Dung, N. H.; Viossat, B.; Lancelot, J. C.; Robba, M. 2,3-Dihydro-2-(3-thienyl)-5H-[1,5]benzothiazepin-4-one. *Acta Crystallogr. Sect. C* **1990**, *46*, 1560−1562.

(27) Allen, F. H.; Kennard, O.; Watson, D. G.; Brammer, L.; Orpen, A. G.; Taylor, R. Tables of Bond Lengths Determined by X-Ray and Neutron Diffraction. Part 1. Bond Lenghts in Organic Compounds. *J. Chem. Soc., Perkin Trans. 2* **1987**, S1.

(28) Ane, W. P.; Bartholdi, E.; Ernst, R. R. Two-dimensional Spectroscopy. Application to Nuclear Magnetic Resonance. *J. Chem. Phys.* **1976**, *64*, 2229−2232.

(29) Wilde, J. A.; Bolton, P. H. Suppression of Homonuclear Couplings in Heteronuclear Two-Dimensional Spectroscopy. *J. Magn. Reson.* **1984**, *59*, 343−348.

(30) Kugita, H.; Inoue, H.; Ikezaki, M.; Konda, M.; Takeo, S. Synthesis of 1,5-Benzothiazepine Derivatives. III. *Chem. Pharm. Bull.* **1971**, *19*, 595−602.

(31) Noggle, J. H.; Shirner, R. E. *The Nuclear Overhauser Effect*; Academic Press: New York, 1971.

(32) *SYBYL Molecular Modeling System*, version 5.41; Tripos Assoc., St. Louis, MO.

(33) Vinter, J. G.; Davis, A.; Saunder, M. R. Strategic Approaches to Drug Design. I. An Integrated Software Framework for Molecular Modeling. *J. Comput.-Aided Mol. Des.* **1987**, *1*, 31−51.

(34) (a) Floyd, D. M.; Kimball, S. D.; Krapcho, J.; Das, J.; Turk, C. F.; Moquin, R. V.; Lago, M. W.; Duff, K. J.; Lee, V. G.; White, R. E.; Ridgewell, R. E.; Moreland, S.; Brittain, R. J.; Normandin, D. E.; Hedberg, S. A.; Cucinotta, G. G. Benzazepinone Calcium Channel Blockers. 2. Structure-Activity and Drug Metabolism Studies Leading to Potent Antihypertensive Agents. Comparison with Benzothiazepinones. *J. Med. Chem.* **1992**, *35*, 756−772. (b) Das, J.; Floyd, D. M.; Kimball, S. D.; Duff, K. J.; Vu, T. C.; Lago, M. W.; Moquin, R. V.; Lee, V. G.; Gougoutas, Z. J.; Malley, M. F.; Moreland, S.; Brittain, R. J.; Hedberg, S. A.; Cucinotta, G. G. Benzazepinone Calcium Channel Blockers. 3. Synthesis and Structure-Activity Studies of 3-Alkylbenzazepinones. *J. Med. Chem.* **1992**, *35*, 773−780. (c) Kimball, S. D.; Floyd, D. M.; Das, J.; Hunt, J. T.; Krapcho, J.; Rovnyak, G.; Duff, K. J.; Lee, V. G.; Moquin, R. V.; Turk, C. F.; Hedberg, S. A.; Moreland, S.; Brittain, R. J.; McMullen, D. M.; Normandin, D. E.; Cucinotta, G. G. Benzazepinone Calcium Channel Blockers. 4. Structure-Activity Overview and Intracellular Binding Site. *J. Med. Chem.* **1992**, *35*, 780−793.

(35) Cantoni, L.; Rizzardini, M.; Skorupska, M.; Cagnotto, A.; Categoni, A.; Pecora, N.; Frigo, L.; Ferrarese, C.; Mennini, T. Hepatic Protoporphyria is Associated with a Decrease in Ligand Binding for Mitochondrial Benzodiazepine Receptors in Liver. *Biochem. Pharmacol.* **1992**, *44*, 1159−1164.

(36) Munson, P. G.; Roadbard, D. *Computers in Endocrinology*; Raven Press: New York, 1984; pp 117−145.

(37) Cheng, Y.; Prusoff, W. Relation between the Inhibition Constant Ki and the Concentration of Inhibitor which Causes Fifty Per Cent Inhibition (I_{50}) of an Enzymic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.

(38) (a) Valenti, P.; Chiarini, A.; Gasperi, F.; Budriesi, R. Xantone 1,4-Dihydropyridine Derivatives with Potent Selective Bradicardic Effect. *Arzneim.-Forsch./Drug Res.* **1990**, *40* (I), 122−125. (b) Tallarida, R. J.; Murray, R. B. *Manual of Pharmacologic Calculations with Computer Programs*, version 4.2; Springer-Verlag: New York, 1991.

(39) *SHELXTL PC‰*; Siemens Analytical X-Ray Instruments, Inc.: Madison, WI, 1990; Rel. 4.1.

(40) *International Tables for X-ray Crystallography*; Kynoch Press: Birmingham, England, 1974; Vol. IV.

(41) Nardelli, M.; Rotener, A. *Fortran Routine for Calculating Nonbonded Potential Energy*; University of Parma: Parma, 1988.

JM960162Z