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New pyrrolobenzothiazepine derivatives as molecular probes of the 'peripheral-type' benzodiazepine receptor (PBR) binding site

G Campiani[1], V Nacci[1]*, I Fiorini[1], MP De Filippis[1], A Garofalo[1], SM Ciani[1], G Greco[2], E Novellino[2], C Manzoni[3], T Mennini[3]

[1]*Dipartimento Farmaco Chimico Tecnologico, Università di Siena, Banchi di Sotto 55, 53100 Siena;*
[2]*Dipartimento di Chimica Farmaceutica e Tossicologica, Università di Napoli 'Federico II', via D Montesano 49, 80131 Naples;*
[3]*Istituto di Ricerche Farmacologiche 'Mario Negri', via Eritrea 62, 40126 Milan, Italy*

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Summary — A number of new pyrrolobenzothiazepine derivatives and a pyrrolobenzothiazocine derivative have been synthesized and evaluated for their affinity towards the 'peripheral-type' benzodiazepine receptor (PBR). The new compounds were tested in rat cortex, a tissue expressing a high density of mitochondrial PBR. Some of the pyrrolobenzothiazepines exhibited IC_{50} values in the low nanomolar range as measured by the displacement of [^{3}H]PK 11195 binding. Compound **4i** was found to be the most potent ligand for this receptor in the pyrrolobenzothiazepine subgroup with an IC_{50} practically identical to that determined for PK 11195. Structure–affinity relationships (SARs) have been developed to elucidate the topology of the PBR binding site.

'peripheral-type' benzodiazepine receptor / PBR ligand / pyrrolo[2,1-*d*][1,5]benzothiazepine / pyrrolo[2,1-*d*][1,5]benzothiazocine

Introduction

The 'peripheral-type' benzodiazepine receptor (PBR) is pharmacologically distinct from the central-type benzodiazepine receptor (CBR), and in the last decade has been the object of extensive studies in order to elucidate its biological and pharmacological role. This receptor protein, expressed in central and peripheral tissues, is located in the outer mitochondrial membrane [1–8], and is characterized by high affinity binding for different classes of structurally unrelated compounds (isoquinolines, such as PK 11195 **1** [9, 10], benzodiazepines, such as Ro 5-4864 **2** [11], and benzothiazepines **3** [12–14]) (chart 1). A variety of biological effects arising from binding to the PBR have been described [15–28]. Among these the steroidogenic effect in endocrine cells consequent to stimulation of PBR [24–26] appears to be particularly intriguing with respect to possible therapeutical application.

Recently [12–14] we have reported a comprehensive study using X-ray diffraction and computational tecniques on a set of pyrrolobenzothiazepine derivatives that bind selectively the PBR. Chart 1 lists some of the previously investigated compounds. The structure–affinity relationships (SARs) outlined that the presence of an alkanoyloxy (OCOR) or mesyloxy (OSO_2Me) group at the position 7 of the pyrrolobenzothiazepine tricyclic system is crucial to display high PBR affinity. The oxygen of the C=O or S=O fragment has in fact been hypothesized to accept a hydrogen bond from a protic function of the PBR binding site. In addition, the fused benzene and the pendant phenyl rings were thought to be necessary for a tight binding to the receptor protein. The three-dimensional disposition of the above pharmacophoric elements was identified by applying the Marshall's active analogue approach [29] in the set of structures shown in chart 1: PK 11195 **1**, Ro 5-4864 **2**, and the pyrrolobenzothiazepines **3a–d**. This study led us to a pharmacophore scheme that includes two lipophilic regions (L1 and L3 sites) and a hydrogen-donating group of the receptor (H1 site) located 2 Å away from the carbonyl oxygen in the plane of the >C=O fragment. The distances among H1, L1 and L3, in the receptor-recognized conformations of the investigated molecules, are comprised in the following intervals: 4.9 Å < L1-L3 < 6.3 Å, 6.0 Å < H1-L1 < 6.5 Å and 7.8 Å < H1-L3 < 8.2 Å.

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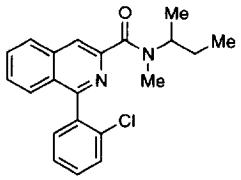
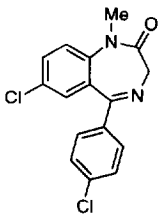
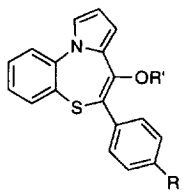


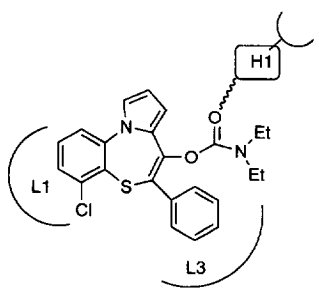
1 PK 11195



2 Ro 5-4864



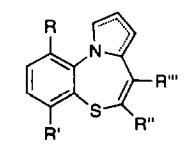
3a R = H, R' = COMe
b R = H, R' = $CON(Me)_2$
c R = H, R' = SO_2Me
d R = OMe, R' = nicotinoyl



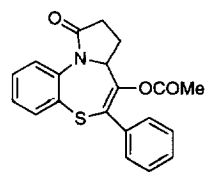
H1 is a hydrogen bond donor site on the receptor protein. L1 and 3 are lipophilic pockets in the protein at the binding site.

Chart 1.

To gain further insights into the topology of the PBR binding site we have prepared new pyrrolobenzothiazepine derivatives **4a–k** and **5**, and their larger homologous based on a novel pyrrolobenzothiazocine system (**7**). Several of these new compounds bind the PBR with high affinity, and one of these (**4i**) was found to be the most potent ligand in the subgroup of the benzothiazepine ligands. We detail herein the synthesis of these compounds (chart 2) and their SARs for PBR affinity associated with variation of the substituents on the heterocyclic system. The SARs developed in the present study are discussed in the light of the aforementioned pharmacophore model [12–14].



4 General Structure for Title Compounds



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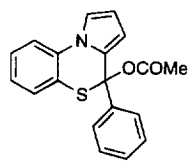

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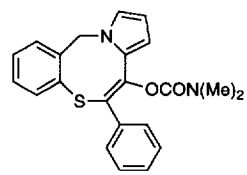
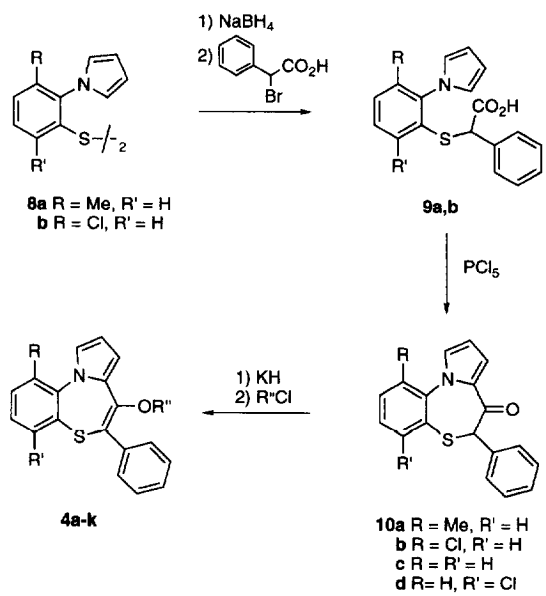


7

Chart 2.

Chemistry

The new pyrrolobenzothiazepinone intermediates **10a,b** were synthesized as shown in scheme 1, according to our previously reported route for **10c,d** [12]. Thus, starting from the bis(2-*N*-pyrrolylphenyl)disulfides **8a,b**, in turn prepared by the appropriate precursors



8a R = Me, R' = H
b R = Cl, R' = H

9a,b

4a-k

10a R = Me, R' = H
b R = Cl, R' = H
c R = R' = H
d R= H, R' = Cl

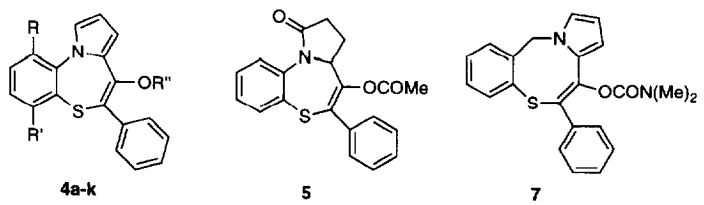
Scheme 1.

[30] by standard procedure [12], the acids **9a,b** were obtained by a reductive alkylation by means of sodium borohydride and α-bromophenylacetic acid. Intramolecular cyclization by exposure of the acids **9a,b** to phosphorus pentachloride gave the ketones **10a,b**. Treatment of the corresponding potassium enolates of compounds **10a–d** with selected acyl chlorides finally yielded the desired pyrrolobenzothiazepines **4a–k** (see table I).

The tetrahydropyrrolobenzothiazepinone analogue **5** was synthesized following the route outlined in scheme 2 [31]. The Dieckman reaction of diester **12**, in turn prepared by a reductive alkylation of the previously described disulfide **11** [32], afforded the cyclic β-ketoester **13**, which after saponification and decarboxylation (**14**) and subsequent halogenation was transformed into compound **15**. Aluminium chloride catalyzed Friedel–Crafts reaction of **15** in benzene provided the ketone **16**. Finally, compound **16** was enolized (KH) and acetylated to the ester **5**.

The pyrrolobenzothiazocine derivative **7** was prepared according to scheme 3, starting from thiophenol **17** [33] which after alkylation with α-bromophenylacetic acid (**18**), and intramolecular cyclization was transformed to the ketone **19**. Enolization with potassium hydride and subsequent acylation with dimethylcarbamoyl chloride yielded the final pyrrolobenzothiazocine **7**.

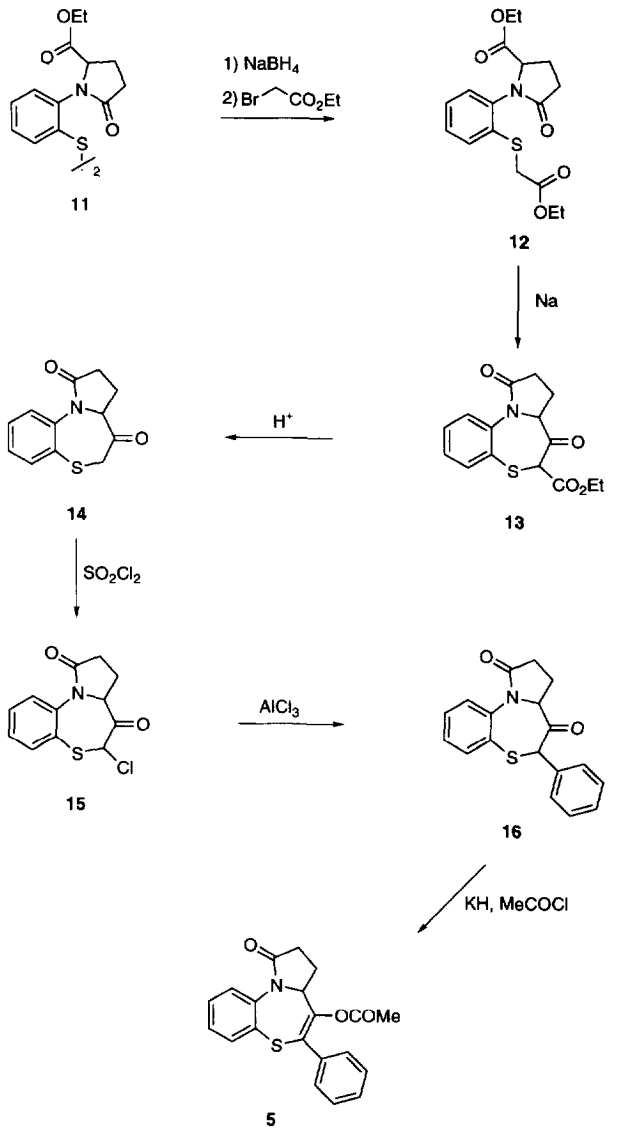
Table I. Physical data for new compounds.



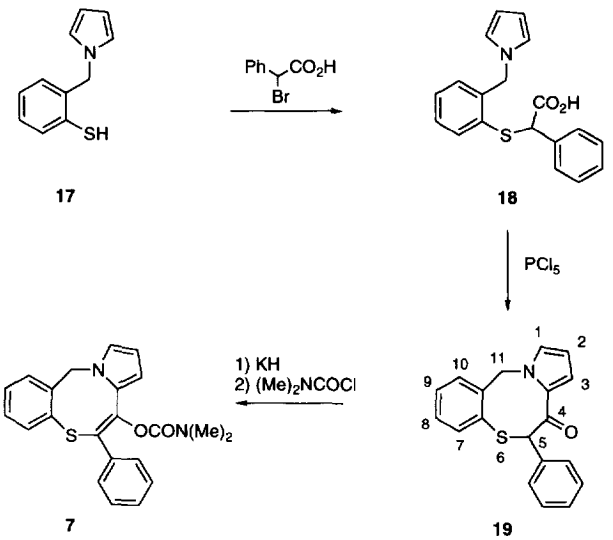
4a-k **5** **7**

Compound	*R*	*R'*	*R''*	*Yield (%)*	*Mp (°C)*	*Recrystallization solvent*	*Molecular formula*[a]
4a	H	H	$CON(Et)_2$	69	113–115	EtOH	$C_{23}H_{22}N_2O_2S$
4b	H	H	$CO(CH_2)_5Me$	50			$C_{25}H_{25}NO_2S$
4c	H	H	$CO(CH_2)_6Me$	86			$C_{26}H_{27}NO_2S$
4d	H	H	COC_6H_{11}	60	130–131	EtOH	$C_{25}H_{23}NO_2S$
4e	Me	H	COMe	59	133–134	MeOH	$C_{21}H_{17}NO_2S$
4f	Me	H	$CON(Me)_2$	58	176–178	Hexanes	$C_{22}H_{20}N_2O_2S$
4g	Cl	H	COMe	53	146–147	EtOH	$C_{20}H_{14}ClNO_2S$
4h	Cl	H	$CON(Me)_2$	66	175–176	EtOH	$C_{21}H_{17}ClN_2O_2S$
4i	H	Cl	$CON(Et)_2$	61	163–164	Hexanes	$C_{23}H_{21}ClN_2O_2S$
4j	H	Cl	$CON(i\text{-}Pr)_2$	70	142–143	Hexanes	$C_{25}H_{25}ClN_2O_2S$
4k	H	Cl	$CON(CH_2CH_2)_2O$	48	202–203	EtOH	$C_{23}H_{19}ClN_2O_3S$
5				89	176–177	EtOAc	$C_{20}H_{17}NO_3S$
7				20	177–179	EtOH	$C_{22}H_{20}NO_2S$
8a	Me	H		75	130–131	EtOH	$C_{22}H_{20}N_2S_2$
8b	Cl	H		50	88–89	Cyclohexane	$C_{20}H_{14}Cl_2N_2S_2$
9a	Me	H		70	148–150	Cyclohexane	$C_{19}H_{17}NO_2S$
9b	Cl	H		76	108–110	Hexanes	$C_{18}H_{14}ClNO_2S$
10a	Me	H		40	135–136	EtOAc	$C_{19}H_{15}NOS$
10b	Cl	H		53	125–127	EtOH	$C_{18}H_{12}ClNOS$
10c[b]	H	H					
10d[b]	H	Cl					
12				76			$C_{17}H_{21}NO_5S$
13				59	98–99	Cyclohexane	$C_{15}H_{15}NO_4S$
14				90	173–174	EtOAc	$C_{12}H_{11}NO_3S$
15				89	118–119		
16				51	65–67	EtOH	$C_{18}H_{15}NO_2S$
18				73	95–96	EtOAc	$C_{19}H_{17}NO_2S$
19				41	242–243	EtOAc/hexanes	$C_{19}H_{15}NOS$

[a]Anal C, H, N; [b]reference [12].



Scheme 2.



Scheme 3.

Results and discussion

In vitro SAR study

The affinities of the new pyrrolobenzothiazepine derivatives **4a–k** and **5**, and of the pyrrolobenzothiazocine derivative **7** for the PBR in rat cortex homogenate are illustrated in table II. The binding data represent the ability of the tested compounds to displace [^{3}H]PK 11195 from the receptor protein. Binding data of PK 11195 are also included.

To identify the structural requirements capable of improving the inhibitory potency of the [^{3}H]PK 11195 binding, the SAR study was carried out as a function of: (i) the nature and position of the substituents in the benzo-fused ring; (ii) the nature and hindrance of the alkyl chain at position 7; (iii) the replacement of the pyrrole ring; and (iv) the modification of the fused heterocyclic 7-membered ring.

The poor affinities of the compounds bearing a chlorine atom or a methyl group at position 1 (**4e–h** vs **4a** and **4i**) indicate that the receptor subsite complementary to this position of the benzothiazepine system is rather limited in its dimension since substituents bulkier than a hydrogen (chlorine or methyl) are not tolerated. Electrostatic factors are not likely to be involved in this unfavourable interaction because the two substituents exert different electronic effects on the fused benzene ring: the methyl group and the chlorine atom are classified as an electron-donating and electron-withdrawing group, respectively [34]. It is worth noting that 2- and 3-substituted pyrrolobenzothiazepine analogues have been found to possess affinities for PBR in the submicromolar range [12, 13], while the insertion of a chlorine atom at position 4 increases the affinity to a nanomolar range [12, 13]. Taken together these findings suggest that the 1-, 2- and 3-positions of the fused benzene ring are in close contact with the steric boundaries of the binding site and that the 4-chlorine fits into a relatively large lipophilic receptor domain (**4i** vs **4e–h**).

In previous studies [12, 13] we have observed that an aroyloxy side chain at position 7 damages the affi-

Table II. Receptor binding affinity of compounds **4a–k**, **5** and **7**.

Compound	*R*	*R'*	*R''*	IC_{50} *(nM)*[a] *(± SEM)*
4a	H	H	$CON(Et)_2$	4.7 ± 0.3
4b	H	H	$CO(CH_2)_5Me$	5.6 ± 1.2
4c	H	H	$CO(CH_2)_6Me$	55 ± 6.0
4d	H	H	COC_6H_{11}	377 ± 62
4e	Me	H	COMe	>5000
4f	Me	H	$CON(Me)_2$	2390 ± 180
4g	Cl	H	COMe	NA
4h	Cl	H	$CON(Me)_2$	>5000
4i	H	Cl	$CON(Et)_2$	2.0 ± 0.3
4j	H	Cl	$CON(i\text{-}Pr)_2$	11.0 ± 4.0
4k	H	Cl	$CON(CH_2CH_2)_2O$	3.9 ± 0.9
5				NA
7				1340 ± 21
6[b]				NA
PK11195				2.0 ± 0.1

[a]The concentration of the tested compounds that inhibited [^{3}H]PK 11195 binding to rat cortex homogenate by 50% (IC_{50}) was determined 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 at the highest concentration tested (10^{-5} M); [b]reference [39].

nity to a considerable extent (eg, **3d**, compounds with R = isonicotinoyl and COC_6H_2-3,4,5-$(OMe)_3$). Molecular mechanics calculations on **3d** demonstrated that the very low affinities exhibited by the above-mentioned compounds could not be related to conformational effects [14]. Steric and/or electrostatic unfavourable interactions occurring between the aryl moieties and the receptor were assumed to disfavour the binding. It was difficult to single out which of these two factors was responsible for the inactivity because of the lack of compounds sufficiently varied in the steric and electronic properties at position 7. Compounds **4d** and **4k** featuring encumbering non-aromatic side chains were purposely synthesized and tested. While **4k** surprisingly binds the receptor with a nanomolar affinity, compound **4d**, although less potent, binds the PBR with an affinity that is about 100-fold higher than that of previously described 7-aroyloxy derivatives (eg, **3d**, IC_{50} = 35 μM). These data confirm that the unfavourable effect of the 7-aroyloxy group depends essentially on electrostatic repulsive interactions occurring between the π-conjugated system of the aromatic rings and an electron-rich subsite of the PBR. The different potencies of **4d** and **4k** may arise from the favourable effect of the 4-chlorine and the better hydrogen-bond-acceptor ability of the amide-type carbonyl oxygen [35]. In order to compare the steric and electronic properties of the considered substituents at position 7, we generated molecular models of **4d** and **4k** using the PM3 semi-empirical quantomechanics method [36] available in the MOPAC program [37]. A model of the inactive compound **3d**, in a conformation matching the pharmacophoric constraints, was already available from a previous study [14]. Figure 1 shows the structures of **3d**, **4d** and **4k** overlapped about the non-hydrogen atoms of the common tricyclic system and of the 7-OCO fragment. Figure 2 shows the molecular electrostatic potentials (MEPs) of the same compounds calculated with the PM3 Hamiltonian and contoured at –2.5 kcal/mol (see *Experimental protocols*). It can be seen that the three substituents are quite similar in terms of shape (fig 1) while larger differences exist in their MEPs (see the locus highlighted by an arrow in fig 2). These calculations confirm the hypothesis that the inactivity of the 7-aroyloxy derivatives should be ascribed mainly to unfavourable electronic factors.

Compounds **4a**, **4i** and **4j** bear C-7 substituents that are bulkier than those of **4d** and **4k**, as they are not constrained in a ring system. On the other hand, they show an affinity for PBR in the low nanomolar range, with **4i** being the most potent PBR ligand based on a benzothiazepine skeleton. A substructure search performed in the Cambridge Structural Database [38] showed, in fact, that the ethyl and the isopropyl groups attached to carbamoyl nitrogen lie apart in

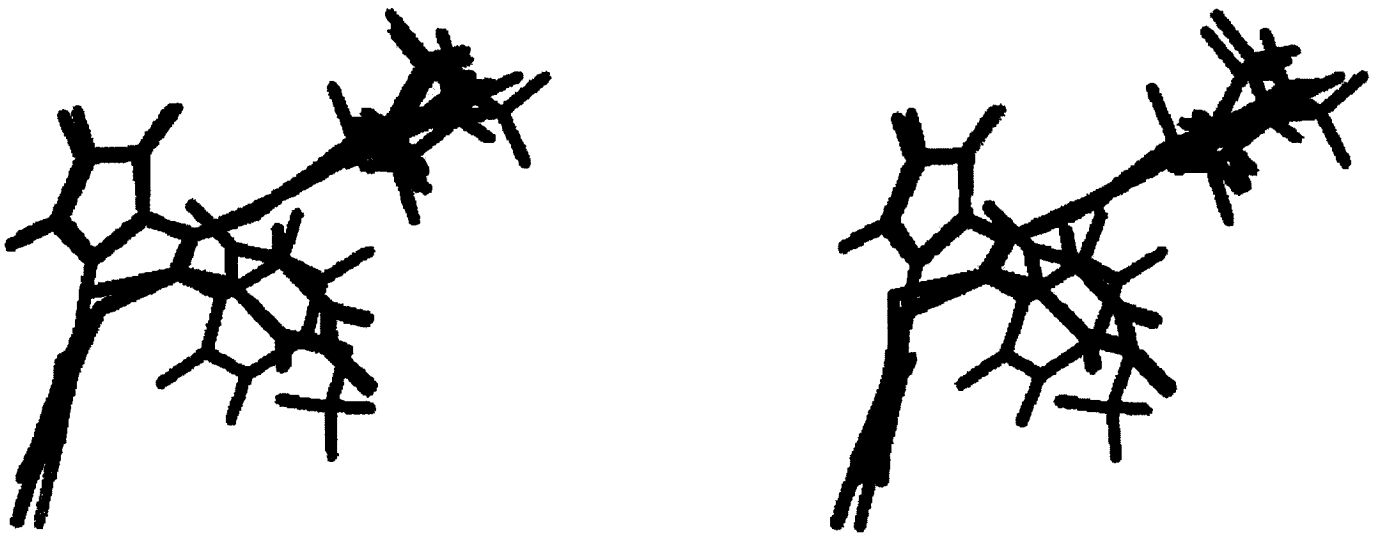


Fig 1. Stereopair picture of the overlapped pharmacophore-consistent conformations **3d** (green), **4d** (black) and **4k** (red).

order to minimize intramolecular steric repulsion. The two compounds **4a** and **4i** bear a diethylcarbamoyloxy group at position 7 and are thus more potent than the corresponding compound substituted with a dimethylcarbamoyloxy group, described in previous articles [12, 13]. This increased binding affinity might be explained hypothesizing that at least one of the *N*-ethyl fragments is accommodated into a lipophilic subsite of the PBR. The drop of affinity observed passing from **4i** to **4j** is probably related to the excessive steric bulk of the isopropyl groups.

Previously we have reported [12] the synthesis of benzothiazepine PBR ligands substituted at position 7 with the ester moiety $OCO(CH_2)_nMe$ with n comprised between 0 and 4. The aforementioned compounds exhibited IC_{50} values > 20 nM. In the present paper, we have synthesized compounds **4b** and **4c** with n equal to 5 and 6, respectively. The analogue **4b** characterized by the lengthy 7-eptanoyloxy substituent is by far more potent than its inferior homologue and about ten fold more potent than **4c**, its superior homologue. The extra-affinity showed by **4b** is probably linked to the possibility for the heptanoyloxy chain to assume an arrangement corresponding to an optimum of ligand–receptor shape complementarity. The side chain of **4b** in a folded arrangement would be thus capable of giving rise to a favourable lipophilic interaction with the receptor.

Replacement of the pyrrole portion with a pyrrolidinone ring yielded compound **5**, which turned out to be devoid of affinity. The inactivity of compound **5** is difficult to explain since the investigated structural modification involves a combination of steric and electronic factors (**5** vs **3a** (IC_{50} = 20 nM [26])).

Previously [39] we have reported that the reduction of the seven-membered thiazepine ring to a six-membered ring of benzothiazine derivatives strongly lowered the affinity to PBR (**6** (IC_{50} > 1000 nM) vs **3a** (IC_{50} = 20 nM, ref [26])). It was also found that the expansion of the thiazepine ring of **3a** to the benzothiazocine ring of **7** caused a drop in affinity. Evidently, the insertion of a methylene group between the benzene and the pyrrole rings to give **7** causes too drastic a change in the overall three-dimensional arrangement of this ligand.

Conclusion

Novel optimized ligands based on a benzothiazepine structure have been synthesized specific for 'peripheral-type' benzodiazepine receptors. Some of these compounds showed high affinity for rat brain PBR and compound **4i** was found to be the most potent PBR ligand in the benzothiazepine subgroup. The SAR study outlined the structural requirements for optimal activity: (i) the [1,5]thiazepine ring; (ii) a chlorine atom at position 4; (iii) the pyrrole ring; and (iv) a diethylcarbamoyloxy group at the C-7 position. Replacement of the pyrrole ring and the transformation of the thiazepine ring to thiazine or thiazocine rings strongly lowered the affinity.

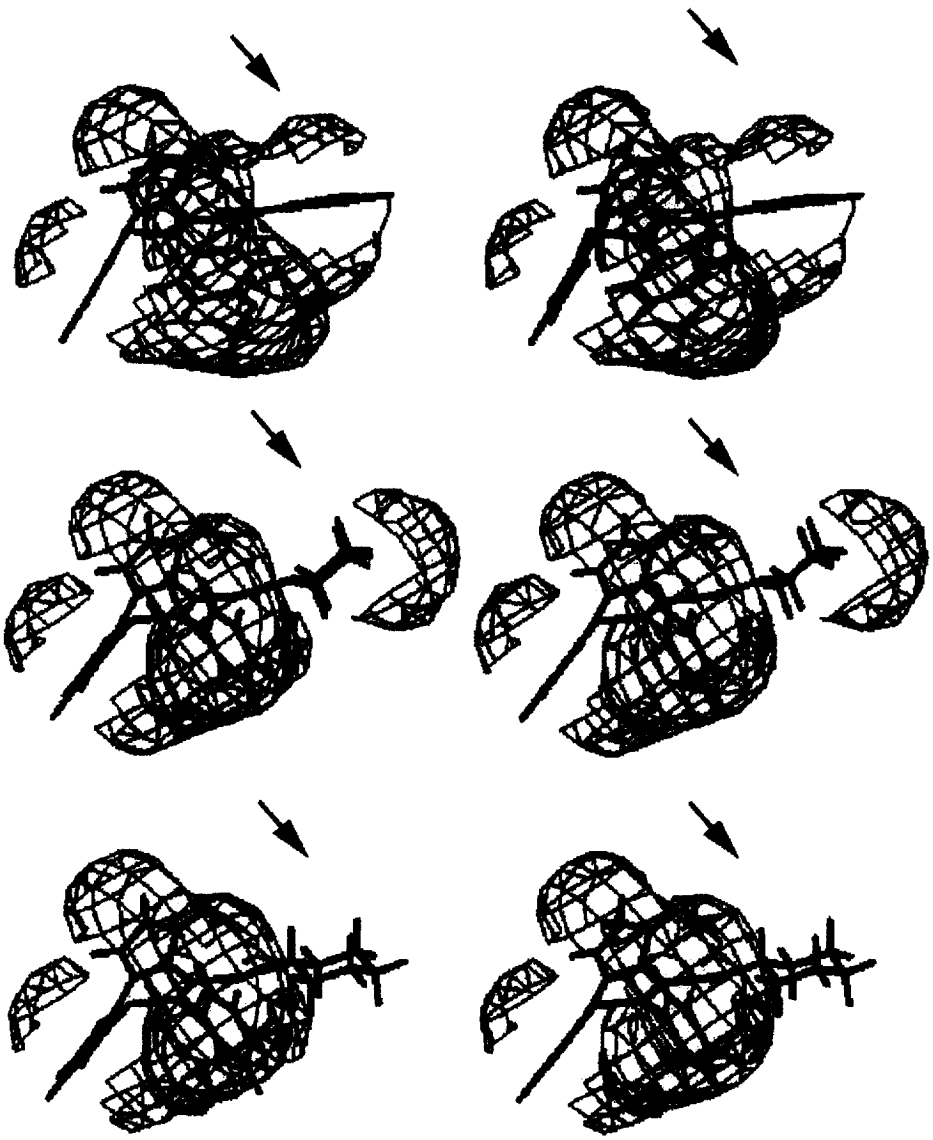


Fig 2. Stereopair picture of the molecular electrostatic potentials (MEPs) of **3d** (green), **4d** (black) and **4k** (red) contoured at the –2.5 kcal/mol. An arrow points to the region where the MEP of **3d** is most dissimilar to those of **4d** and **4k**.

Experimental protocols

Chemistry

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. Analyses indicated by the elemental symbols were within ±0.4% of the theoretical values and were performed on a Perkin-Elmer 240C elemental analyzer. Yields refer to purified products and are not optimized. Physical data for the new compounds are reported in table I.

Bis(3-methyl-2-N-pyrrolylphenyl)disulfide ***8a***
A suspension of 2-amino-4-methylbenzothiazole (3.0 g, 18.2 mmol) and potassium hydroxide (9.0 g) in 100 mL of water was refluxed for 6 h, until ammonia was no longer evolved. The solution was then neutralized with glacial acetic acid, and was extracted with ethyl acetate. The organic layers were washed with brine, dried and concentrated. Evaporation of the solvent afforded a residue which was used in the next step without further purification.

A solution of bis(2-amino-3-methylphenyl)disulfide (0.86 g, 3.1 mmol) and 2,5-dimethoxytetrahydrofurane (0.40 mL, 3.1 mmol) in 3 mL of glacial acetic acid was refluxed for 30 min under argon. The solvent was removed under reduced pressure, and the residue was taken up in chloroform. The organic phase was washed with brine, dried and concentrated. The residue was purified by chromatography (chloroform) to give **8a** as pale yellow prisms (0.87 g); IR ($CHCl_3$): 1625 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.03 (s, 6H), 6.37 (t, 4 H, J = 2.1 Hz), 6.63 (t, 4H, J = 2.2 Hz), 7.10–7.42 (m, 6 H). Anal $C_{22}H_{20}N_2S_2$ (C, H, N).

Bis(3-chloro-2-N-pyrrolylphenyl)disulfide ***8b***
Starting from 2-amino-4-chlorobenzothiazole (2.5 g, 13.5 mmol), the title compound was obtained following the procedure as for **8a**. After purification by flash chromatography (chloroform), **8b** crystallized as colourless prisms; IR (Nujol): 1605 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 6.36 (t, 4H, J = 2.0 Hz), 6.79 (t, 4H, J = 2.0 Hz), 7.16–7.27 (m, 4H), 7.51 (d, 2H, J = 2.2 Hz). Anal $C_{20}H_{14}Cl_2N_2S_2$ (C, H, N).

(±)-α-[[3-Methyl-2-(1H-pyrrol-1-yl)phenyl]thio]phenylacetic acid ***9a***
A suspension of disulfide **8a** (1.12 g, 3.43 mmol) in 18 mL of anhydrous EtOH was heated to reflux under argon. Sodium borohydride (0.26 g, 6.86 mmol) was then added portionwise over 30 min. The reaction mixture was then allowed to cool to room temperature and a solution of α-bromophenylacetic acid (1.48 g, 6.86 mmol) in 5 mL of anhydrous EtOH was slowly added. The suspension was stirred at room temperature for 12 h, concentrated, and the pH was adjusted to 5 with 10% HCl. The mixture was extracted with EtOAc and the organic layers were washed with brine, dried and concentrated. The residue was triturated with petroleum ether (bp 40–60 °C) to afford a solid residue which was purified by crystallization to give **9a** as colourless prisms (0.68 g); IR ($CHCl_3$): 1710 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.99 (s, 3H), 4.62 (s, 1H), 6.30 (d, 2H, J = 1.9 Hz), 6.52 (d, 2H, J = 1.8 Hz), 6.62 (d, 1H, J = 1.6 Hz), 7.05–7.60 (m, 6H), 7.97 (d, 1H, J = 7.4 Hz). Anal $C_{19}H_{17}NO_2S$ (C, H, N).

(±)-α-[[3-Chloro-2-(1H-pyrrol-1-yl)phenyl]thio]phenylacetic acid ***9b***
Starting from **8b** (1.2 g, 2.87 mmol), the title compound was obtained (reaction time 18 h) following the procedure as for **9a**. After purification by flash chromatography (EtOAc), **9b** crystallized as colourless prisms; IR (Nujol): 1726 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 4.54 (s, 1H), 6.36 (d, 2H, J = 2.1 Hz), 6.69 (d, 2H, J = 2.0 Hz), 7.15–7.40 (m, 8H). Anal $C_{18}H_{14}ClNO_2S$ (C, H, N).

(±)-1-Methyl-6-phenylpyrrolo[2,1-d][1,5]benzothiazepin-7-(6H)-one ***10a***
To a well-stirred solution of acid **9a** (0.73 g, 2.27 mmol) in 9 mL of dry 1,2-dichloroethane a suspension of phosphorus pentachloride (0.4 g, 1.92 mmol) in 5 mL of the same solvent was slowly added. The resulting mixture was heated at 70 °C for 5 h under argon.The solvent was removed under vacuum and the residue was taken up in dichloromethane. The organic solution was washed with 5% aqueous NaOH, brine, dried and concentrated. The crude residue was purified by chromatography ($CHCl_3$) to give **10a** as colourless prisms (0.42 g); IR (Nujol) 1658 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.88 (s, 3H), 4.50 (s, 1H), 6.45 (m, 1H), 7.08 (m, 1H), 7.15–7.50 (m, 9H). Anal $C_{19}H_{15}NOS$ (C, H, N).

(±)-1-Chloro-6-phenylpyrrolo[2,1-d][1,5]benzothiazepin-7(6H)-one ***10b***
Starting from **9b** (1.47 g, 4.27 mmol), the title compound was obtained (reaction time 10 h) following the procedure as for **10a**. After purification by flash chromatography ($CHCl_3$), **10b** crystallized as colourless prisms; IR (Nujol): 1650 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 4.54 (s, 1H), 6.36 (m, 1H), 6.69 (m, 1H), 7.15–7.40 (m, 9H). Anal $C_{18}H_{12}ClNOS$ (C, H, N).

General procedure for the preparation of compounds ***4a–k***
This procedure is illustrated for the preparation of 7-[(diethylcarbamoyl)oxy]-6-phenylpyrrolo[2,1-*d*][1,5]benzothiazepine **4a**. A solution of ketone **10c** (0.2 g, 0.68 mmol) in 4 mL of anhydrous THF was slowly added to a suspension of potassium hydride (77.7 mg, 0.68 mmol, 35% in oil) in 4 mL of anhydrous THF. After stirring at room temperature for 2 h, a solution of diethylcarbamoyl chloride (93 mg, 0.68 mmol) in 2 mL of anhydrous THF was added and the mixture was allowed to stir for 2 h. The reaction mixture was poured into crushed ice and extracted with dichloromethane. The combined organic layers were washed with brine, dried and concentrated. The residue was purified by flash chromatography ($CHCl_3$) to afford **4a** as colourless prisms (187 mg); IR ($CHCl_3$) 1706 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.02–1.29 (m, 6H), 3.27–3.49 (m, 4H), 6.40 (m, 1H), 7.06–7.50 (m, 10H), 7.80 (m, 1H). Anal $C_{23}H_{22}N_2O_2S$ (C, H, N).

7-Heptanoyloxy-6-phenylpyrrolo[2,1-d][1,5]benzothiazepine ***4b***
Similarly to **4a**, the thiazepine **4b** was prepared starting from 0.5 g (1.7 mmol) of **10c** (reaction time 8 h at room temperature), using heptanoyl chloride. **4b** was obtained as a colorless oil; IR ($CHCl_3$): 1761 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.79 (t, 3H, J = 6.2 Hz), 1.18–1.72 (m, 8H), 2.33 (m, 2H), 6.42 (m, 1H), 6.51 (m, 1H), 7.20–7.70 (m, 10H). Anal $C_{25}H_{25}NO_2S$ (C, H, N).

7-Octanoyloxy-6-phenylpyrrolo[2,1-d][1,5]benzothiazepine ***4c***
Similarly to **4a**, the thiazepine **4c** was prepared starting from 1.0 g (3.4 mmol) of **10c** (reaction time 8 h at 30 °C), using octanoyl chloride. **4c** was obtained as a colourless oil; IR ($CHCl_3$): 1761 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.86 (t, 3H, J = 6.0 Hz), 1.16–1.60 (m, 10H), 2.21 (m, 2H), 6.38 (m, 1H), 6.54 (m, 1H), 7.20–7.80 (m, 10H). Anal $C_{26}H_{27}NO_2S$ (C, H, N).

7-[(Cyclohexanecarbonyl)oxy]-6-phenylpyrrolo[2,1-d][1,5]-benzothiazepine ***4d***
Starting from **10c** (0.3 g, 1.02 mmol), the title compound was obtained following the procedure as for **4a**, using cyclohexanecarbonyl chloride. **4d** was recrystallized as pale yellow prisms; IR (Nujol) 1750 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.11–1.98 (m, 10H), 2.45 (m, 1H), 6.39 (m, 1H), 6.57 (m, 1H), 7.13 (m, 1H), 7.21–7.75 (m, 9H). Anal $C_{25}H_{23}NO_2S$ (C, H, N).

7-Acetoxy-1-methyl-6-phenylpyrrolo[2,1-d][1,5]benzothiazepine ***4e***
Starting from **10a** (0.5 g, 1.63 mmol), the title compound was obtained (reaction time 10 h) following the procedure as for **4a**. After purification by flash chromatography (EtOAc), **4e** crystallized as colourless prisms; IR (Nujol): 1780 cm^{-1}; ^{1}H-NMR

($CDCl_3$) δ 1.97 (s, 3H), 2.28 (s, 3H), 6.36 (m, 1H), 6.56 (m, 1H), 6.97 (m, 1H), 7.15–7.60 (m, 8H). Anal $C_{21}H_{17}NO_2S$ (C, H, N).

7-[(Dimethylcarbamoyl)oxy]-1-methyl-6-phenylpyrrolo[2,1-d]-[1,5]benzothiazepine ***4f***
Starting from **10a** (0.5 g, 1.63 mmol), the title compound was obtained (reaction time 10 h) following the procedure as for **4a**. After purification by flash chromatography (EtOAc), **4f** crystallized as colourless prisms; IR (Nujol): 1726 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 2.30 (s, 3H), 2.77 (s, 3H), 2.90 (s, 3H), 6.36 (m, 1H), 6.55 (m, 1H), 6.97 (m, 1H), 7.15–7.60 (m, 8H). Anal $C_{22}H_{20}N_2O_2S$ (C, H, N).

7-Acetoxy-1-chloro-6-phenylpyrrolo[2,1-d][1,5]benzothiazepine ***4g***
Starting from **10b** (0.38 g, 1.16 mmol), the title compound was obtained (reaction time 8 h) following the procedure as for **4a**, using acetyl chloride. After purification by flash chromatography (dichloromethane), **4g** crystallized as white prisms; IR (Nujol): 1778 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 1.97 (s, 3H), 6.38 (m, 1H), 6.61 (m, 1H), 7.18–7.67 (m, 9H). Anal $C_{20}H_{14}ClNO_2S$ (C, H, N).

1-Chloro-7-[(dimethylcarbamoyl)oxy]-6-phenylpyrrolo[2,1-d][1,5]benzothiazepine ***4h***
Starting from **10b** (0.25 g, 0.76 mmol), the title compound was obtained (reaction time 10 h) following the procedure as for **4a**, using dimethylcarbamoyl chloride. After purification by flash chromatography (EtOAc), **4h** crystallized as colourless prisms; IR (Nujol): 1743 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 2.78 (s, 3H), 2.89 (s, 3H), 6.38 (m, 1H), 6.60 (m, 1H), 7.15–7.65 (m, 9H). Anal $C_{21}H_{17}ClN_2O_2S$ (C, H, N).

4-Chloro-7-[(diethylcarbamoyl)oxy]-6-phenylpyrrolo[2,1-d]-[1,5]benzothiazepine ***4i***
Starting from **10d** (0.25 g, 0.76 mmol), the title compound was obtained (reaction time 8 h) following the procedure as for **4a**, using diethylcarbamoyl chloride. After purification by flash chromatography ($CHCl_3$), **4i** crystallized as colourless prisms; IR (Nujol): 1728 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 0.99–1.32 (m, 6H), 3.20–3.42 (m, 4H), 6.40 (m, 1H), 6.89 (m, 1H), 7.16–7.50 (m, 9H). Anal $C_{23}H_{21}ClN_2O_2S$ (C, H, N).

4-Chloro-7-[(diisopropylcarbamoyl)oxy]-6-phenylpyrrolo[2,1-d]-[1,5]benzothiazepine ***4j***
Similarly to **4a**, the thiazepine **4j** was prepared starting from 0.2 g (0.6 mmol) of **10d** (reaction time 12 h), using diisopropylcarbamoyl chloride. **4j** was obtained as white prisms; IR ($CHCl_3$): 1711 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 1.10 (m, 12H), 3.83 (d, 2H, J = 6.2 Hz), 6.41 (m, 1H), 6.62 (m, 1H), 7.20–7.60 (m, 9H). Anal $C_{25}H_{25}ClN_2O_2S$ (C, H, N).

4-Chloro-7-[(4-morpholinecarbonyl)oxy]-6-phenylpyrrolo[2,1-d]-[1,5]benzothiazepine ***4k***
Similarly to **4a**, the thiazepine **4k** was prepared starting from 0.25 g (0.75 mmol) of **10d** (reaction time 20 h), using 4-morpholinecarbonyl chloride. **4k** was obtained as colourless prisms; IR ($CHCl_3$): 1724 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 3.10–3.80 (m, 8H), 6.43 (m, 1H), 6.61 (m, 1H), 7.12 (m, 1H), 7.19–7.46 (m, 6H), 7.68 (m, 2H). Anal $C_{23}H_{19}ClN_2O_3S$ (C, H, N).

(±)-1-[[(2-Ethoxycarbonylmethyl)thio]phenyl]pyrrolidin-5-one-2-carboxylic acid ethyl ester ***12***
To a refluxing solution of disulfide **11** (2.5 g, 4.8 mmol) in 20 mL of anhydrous EtOH was added sodium borohydride (0.37 g, 9.7 mmol) portionwise, within 20 min. After 20 min the reaction mixture was cooled to room temperature and a solution of ethyl α-bromoacetate (1.09 mL, 9.8 mmol) in 10 mL of anhydrous EtOH was slowly added. The suspension was stirred for 15 h under argon, and concentrated. The oily residue was poured into crushed ice and extracted with ethyl ether. The organic layers were washed with 10% aqueous potassium carbonate and brine, dried and concentrated. The residue was chromatographed ($CHCl_3$) to give the ester **12** (2.6 g) as a colourless oil; IR (neat) 1730, 1695 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 1.23 (m, 6H), 2.20–2.80 (m, 4H), 3.63 (m, 2H), 4.00–4.30 (m, 4H), 4.81 (m, 1H), 7.30–7.50 (m, 4H). Anal $C_{17}H_{21}NO_5S$ (C, H, N).

(±)-6-Ethoxycarbonyl-7,10-dioxo-6,7,7a,8,9,10-hexahydropyrrolo[2,1-d][1,5]benzothiazepine ***13***
To a vigorously stirred suspension of sodium (45 mg, 0.002 g-atom) in 12 mL of dry toluene at 110 °C under argon, were added a solution of ester **12** (0.7 g, 2 mmol) in 5 mL of dry toluene, and 20 μL of anhydrous EtOH. The reaction mixture was heated at reflux for 5 h, then cooled at 0 °C, and quenched with 5 mL of 0.5 N HCl. The organic phase was separated, washed with brine, dried and concentrated. The residue was purified by column chromatography ($CHCl_3$) to give the benzothiazepine **13** (0.35 g) as colourless prisms; IR ($CHCl_3$) 1750, 1715 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 1.07 (t, 3H, J = 6.2 Hz), 2.20–2.80 (m, 4H), 4.12 (q, 2H, J = 7.0 Hz), 4.73 (m, 1H), 4.93 (m, 1H), 7.10–7.70 (m, 4H). Anal $C_{15}H_{15}NO_4S$ (C, H, N).

(±)-7,10-Dioxo-6,7,7a,8,9,10-hexahydropyrrolo[2,1-d][1,5]-benzothiazepine ***14***
A mixture of ester **13** (1.32 g, 4.3 mmol), 6 N HCl (15 mL), and glacial acetic acid (15 mL) was heated at reflux for 5 h. After cooling the pH was adjusted to 7 with 20% potassium carbonate solution, and the mixture was extracted with EtOAc. The organic layers were washed with brine, dried and concentrated. The residue was chromatographed ($CHCl_3$) to give 0.91 g of ketone **14** as a white solid; IR ($CHCl_3$) 1715 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 2.20–2.80 (m, 7H), 7.05–7.48 (m, 4H). Anal $C_{12}H_{11}NO_3S$ (C,H,N).

(±)-6-Chloro-7,10-dioxo-6,7,7a,8,9,10-hexahydropyrrolo[2,1-d][1,5] benzothiazepine ***15***
To a solution of **14** (1.1 g, 4.2 mmol) in 20 mL of dry dichloromethane was added a solution of freshly distilled sulfuryl chloride (0.21 mL, 2.6 mmol) in 4 mL of dry dichloromethane. The reaction mixture was stirred at room temperature for 30 min, then was concentrated and the residue was directly purified by column chromatography (EtOAc) to give **15** (1.02 g) as a brownish solid; IR (Nujol) 1712 cm^{-1}; ^1H-NMR (DMSO-d_6) δ 2.45 (m, 4H), 5.09 (s, 1H), 6.56 (s, 1H), 7.45 (m, 4H); MS *m/z* 267 (M^+, 45%), 239, 204 (100%), 149, 136.

(±)-7,10-Dioxo-6-phenyl-6,7,7a,8,9,10-hexahydropyrrolo[2,1-d]-[1,5] benzothiazepine ***16***
Aluminum chloride (0.33 g, 2.5 mmol) was added to a solution of **15** (0.58 g, 2.1 mmol) in 15 mL of dry benzene at 0 °C under argon. The resulting mixture was stirred at 0 °C for 1 h and then heated at 80 °C for 5 h. The reaction mixture was cooled at 0 °C, and quenched with water (2 mL). The organic phase was separated, washed with brine, dried and concentrated. Removal of the solvent afforded a residue which was chromatographed (EtOAc) to give 0.28 g of ketone **16** as a white solid; IR ($CHCl_3$) 1700 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 2.53 (m, 4H), 5.06 (m, 1H), 5.62 (s, 1H), 7.37–7.70 (m, 9H). Anal $C_{18}H_{15}NO_2S$ (C, H, N).

(±)-7-Acetoxy-10-oxo-6-phenyl-7a,8,9,10-tetrahydropyrrolo-[2,1-d][1,5] benzothiazepine ***5***
Starting from ketone **16** (0.35 g, 1 mmol), the title compound was obtained (reaction time 4 h) following the procedure as for **4a**. After purification by flash chromatography (EtOAc and hexanes 1:1), **5** crystallized as colourless prisms; IR (Nujol): 1770, 1708 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.75 (s, 3 H), 2.64 (m, 4H), 4.58 (m, 1H), 7.30–7.45 (m, 9H). Anal $C_{20}H_{17}NO_3S$ (C, H, N).

α-[2-[[(1H-Pyrrol-1-yl)methyl]phenyl]thio]phenylacetic acid ***18***
To a stirred solution of sodium ethoxide (1.63 g, 24 mmol) in 50 mL of absolute EtOH, was added dropwise a solution of thiol **17** (3.56 g, 20 mmol) in 20 mL of absolute EtOH. After stirring an additional 15 min, α-bromophenylacetic acid (4.3 g, 20 mmol) in 40 mL of absolute EtOH was added dropwise. The resulting suspension was stirred overnight at room temperature, then concentrated in vacuo. The semisolid residue was partitioned between water and EtOAc, and the organic layers were washed with water and dried. Evaporation of the solvent gave the crude acid **18** (4.5 g) which was purified by crystallization. IR (Nujol): 1708 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 5.05 (0.5 AB q, 1H, J = 16.1 Hz), 5.20 (0.5 AB q, 1H, J = 15.8 Hz), 6.05 (s, 1H), 6.15 (m, 2H), 6.70 (m, 3H), 7.35 (m, 8H). Anal $C_{19}H_{17}NO_2S$ (C, H, N).

(±)-4-Oxo-5-phenyl-4,5-dihydro-11H-pyrrolo[2,1-d][1,5] benzothiazocine ***19***
Starting from **18** (0.1 g, 0.33 mmol), the title compound was obtained following the procedure as for **10a**. After purification by flash chromatography ($CHCl_3$), **19** crystallized as pale yellow prisms; IR (Nujol): 1656 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 5.45 (0.5 AB q, 1H, J = 15.2 Hz), 6.10 (0.5 AB q, 1H, J = 15.3 Hz), 6.15 (m, 1H), 6.45 (s, 1H), 6.75 (m, 1H), 7.25 (m, 2H), 7.40 (m, 5H), 7.55 (m, 1H), 7.73 (m, 2H). Anal $C_{19}H_{15}NOS$ (C, H, N).

(±)-4-[(Dimethylcarbamoyl)oxy]-5-phenyl-11H-pyrrolo[2,1-d]-[1,5] benzothiazocine ***7***
Starting from **19** (0.1 g, 0.33 mmol), the title compound was obtained (reaction time 10 h) following the procedure as for **4a**. After purification by flash chromatography (EtOAc), **7** crystallized as colourless prisms; IR (Nujol): 1728 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.85 (s, 3H), 2.98 (s, 3H), 5.66 (s, 2H), 6.40 (m, 2H), 6.75 (m, 1H), 7.20–7.30 (m, 7H), 6.68 (m, 2H). Anal $C_{22}H_{20}NO_2S$ (C, H, N).

Molecular modelling

All molecular modelling was performed with use of the software package SYBYL [40] running on a Silicon Graphics Indigo XS24 workstation. Geometry optimizations were performed with the semiempirical quanto-mechanics method PM3 [36] available in the MOPAC program [37]. MOPAC was run using default settings and the keyword 'MMOK' for compounds containing an amide bond.

Starting from the crystal structure of the benzothiazepine **3d**, molecular models of **4d** and **4k** were built through Tripos atom types and standard geometries and energy-minimized by the PM3 method. Provided that the solid-state conformation of **3d** has an internal energy near that of the gas-phase global minimum conformer, we assumed that the PM3 geometries of **4d** and **4k** were also low-energy conformers.

The side chain torsion angles C6-C7-O1'-C1' and C7-O1'-C1' = O" in **4d** and **4k** were set to the same values characterizing the pharmacophore-consistent conformation of **3d** [14] (160° and −13°, respectively). Owing to mesomeric effects, in **4k** and **3d** the morpholine and pyridine rings lay approximately in the plane of the carbonyl group. Concerning **4d**, the torsional angle O1'-C1'-C2'-C3' was adjusted manually so as to bring the cyclohexane moiety (fixed in a chair conformation) in the same region of space occupied by the morpholine ring of **4k**. The modification of the above torsion angles did not increase significantly the steric energy of **4d** and **4k** (less than 3 kcal/mol according to PM3 partial geometry-optimizations performed by holding fixed the modified torsional angles).

Compounds **4d** and **4k** were superimposed on **3d** by minimizing the root-mean-squared distance calculated over the non-hydrogen atoms of the common tricyclic system and of the 7-OCO fragment. This was accomplished using the SYBYL/FIT command.

Molecular electrostatic potentials for **4d**, **4k** and **3d**, based on PM3 partial atomic charges were contoured at −2.5 kcal/mol through the SYBYL/POTENTIAL routine.

Mitochondrial benzodiazepine receptor binding assay

Male CRL:CD(SD)BR (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 (room temperature 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 x 20 s) instrument and centrifuged at 50 000 *g* for 10 min. The pellet was washed three more times by resuspension in fresh buffer and centrifuged as before. The last pellet was resuspended just before the binding assay. For mitochondrial benzodiazepine binding 10 mg of original wet tissue weight was incubated with 1 nM [^{3}H]PK 11195 (specific activity 85.8 Ci/mmol; NEN) in 1 mL final volume for 120 min at 4 °C in the presence of eight to twelve increasing concentrations of drugs. Non-specific 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 (LKB) and counted in a 1204 BS Betaplate liquid scintillation counter, with a counting efficiency of about 45%. IC_{50} values were determined by nonlinear [41] fitting of binding inhibition curves, using the Allifit program running on an IBM AT personal computer. Each point was the mean of triplicate samples.

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