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Studies on annulated 1,4-benzothiazines and 1,5-benzothiazepines. IX. Imidazo[2,1-*d*][1,5]benzothiazepines: synthesis and *in vitro* benzodiazepine receptor affinity

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Summary — The synthesis of three series of 1- and 2-substituted imidazo[2,1-*d*][1,5]benzothiazepines is accomplished starting from 1,5-benzothiazepin-4-ones. All the synthesized compounds were evaluated for their affinity for the benzodiazepine receptor, testing their ability to displace [^{3}H]Flunitrazepam from bovine brain membrane protein. A few of the tested compounds showed good affinity, in particular compound **9a** (K_i = 43.00 nM). The GABA-ratio of the active compounds suggests an antagonist or partial agonist activity. The data obtained allow us to draw some comments on the structure–activity relationships.

imidazo[2,1-*d*][1,5]benzothiazepine / benzodiazepine receptor affinity / structure–activity relationship

Introduction

Since the discovery of benzodiazepine receptor a number of ligands with different structures have been found. Recently, we have synthesized some triazolo[3,4-*d*][1,5]benzothiazepines, which showed moderate affinity for the benzodiazepine receptor [1, 2]. With the aim of expanding available structure–activity relationship (SAR) correlations, we describe here the synthesis and the biological evaluation of some 1- or 2-substituted derivatives of imidazo[2,1-*d*]-[1,5]benzothiazepine, a heterocyclic ring system of which only a few derivatives are already known [3, 4].

Considering the biological importance of various imidazo derivatives [5–11] all the compounds described here were also screened as antiinflammatory and CNS agents. These results will be discussed in a forthcoming paper.

Chemistry

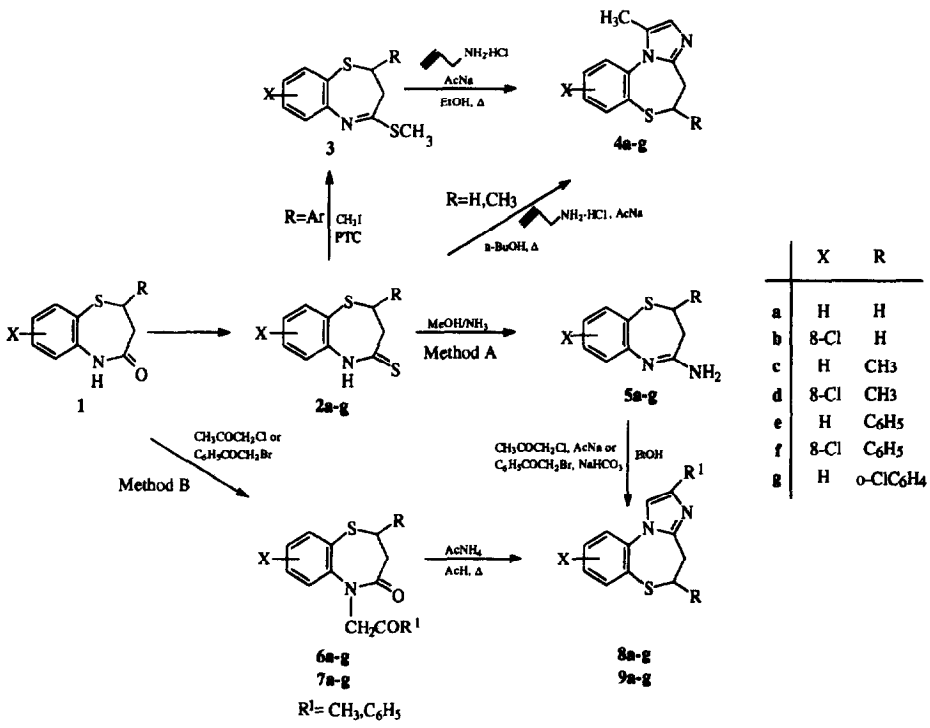
The synthesis of 1-methyl derivatives **4a–g** was achieved starting from the known 1,5-benzothiazepinthiones **2** [12, 13]. Compounds **4a–d** were directly obtained by refluxing the corresponding thiones **2** with propargylamine hydrochloride, whereas the 5-aryl-substituted derivatives **4e–g** and **12** were obtained through intermediates **3** [13] and **11** [1]. The direct reaction between 2-phenyl-2,3-dihydro-1,5-benzothiazepin-4-(5*H*)-thione and propargylamine hydrochloride gave rise to 2-styrylbenzothiazole in agreement with our previous observations [1] (schemes 1 and 2).

2-Methyl or 2-phenyl derivatives **8a–g** and **9a–g** could be synthesized by two methods (scheme 1). According to the known method [3] (*Method A*), 4-amino-1,5-benzothiazepines **5** were used as intermediates for the synthesis of **8** and **9**. Compounds **5** were prepared in a different manner to that indicated in the literature [3], starting from benzothiazepinones **1**, which, after conversion into thiones **2**, were reacted with ammonia in methanol. The final compounds were obtained by refluxing 4-aminobenzothiazepines **5** with either chloroacetone or phenacylbromide.

Alternatively (*Method B*), benzothiazepinones **1** were reacted with chloroacetone or phenacylbromide using the phase-transfer catalysis conditions (PTC). The N-substituted derivatives **6** and **7**, obtained in good yields, were then refluxed with ammonium acetate in glacial acetic acid to give the desired compounds **8** and **9**.

Considering the low yields obtained by *Method A*, the instability of compounds **5a–g** and the procedure length, we preferred to use *Method B* to obtain the compounds **8a–g** and **9a–g** and prepare the dehydro derivatives **15** and **16** (scheme 2).

The physical, spectral and analytical data for the synthesized compounds are given in the *Experimental protocols* and in tables I–V.



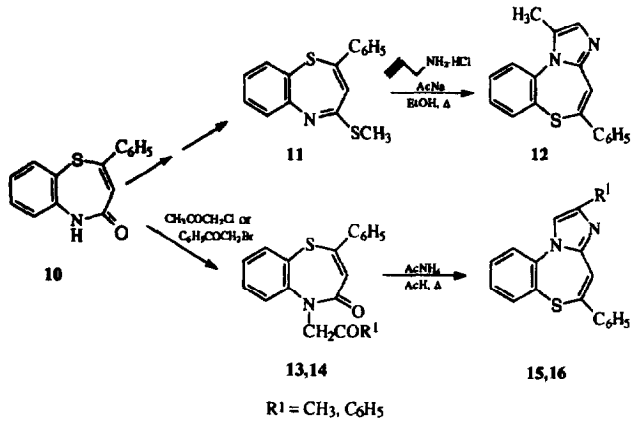
Scheme 1.

Pharmacology

In two previous reports [1, 2] we reported the synthesis and the benzodiazepine receptor affinity of 1,5-benzothiazepines annulated with either a triazole or tetrazole ring, and 1,4-benzothiazines or 1,5-benzothiazepines annulated with the triazine ring. Continuing our interest in this field we have now studied some new derivatives of imidazo[2,1-*d*][1,5]benzothiazepines.

The benzodiazepine receptor affinity of these compounds was measured by their ability to displace [^{3}H]Flunitrazepam (at 0.2 nM, K_d = 1.8 nM) from its specific binding in bovine brain membranes. First a single concentration (10 μM) of the tested compounds was examined and then, for the most active ones, the IC_{50} values were calculated by log-probit plots.

From the IC_{50} values the K_i were calculated (table VI). These were used to define the benzodiazepine receptor affinity, and the GABA-ratio, which according to various authors [14–18] generally predicts the expected behaviour properties of a benzodiazepine receptor ligand.

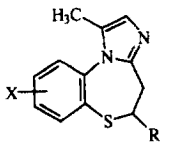


Scheme 2.

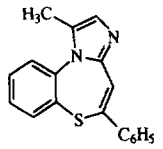
Results and discussion

The binding results showed the importance of the presence of the lipophilic phenyl substituent at the 2-position on the imidazole nucleus (compound **9a**, K_i = 43 nM). The replacement of the phenyl ring with a methyl group actually caused a decrease of two

Table I. Physical and chemical data of compounds **4a–g** and **12**.



4a-g



12

Compound	*X*	*R*	*Yield (%)*	*Mp (°C)*	*Crystallization solvent*	*Colour, crystal form*	*Formula*	*^{1}H-NMR, δ*[a]
4a	H	H	33	114–115	–[b]	–	$C_{12}H_{12}N_2S$	2.21 (s, 3H, CH_3), 2.33–3.70 (m, 4H, CH_2CH_2), 6.82 (s, 1H, H-2), 7.16–7.83 (m, 4H, Ar)
4b	8-Cl	H	56	47–48	–[b]	–	$C_{12}H_{11}ClN_2S$	2.19 (d, J = 2 Hz, 3H, CH_3), 2.58, 3.27, 3.54 (AXY system, 4H, CH_2CH_2), 6.82 (d, J = 2 Hz, 1H, H-2), 7.17–7.79 (m, 3H, Ar)
4c	H	CH_3	42	oil	–	–	$C_{13}H_{14}N_2S$	1.41 (d, J = 6 Hz, 3H, $SCHCH_3$), 2.20 (d, J = 2 Hz, 3H, CH_3), 2.70–4.13 (m, 3H, $CHCH_2$), 6.80 (d, J = 2 Hz, 1H, H-2), 7.14–7.86 (m, 4H, Ar)
4d	8-Cl	CH_3	37	120–121	EtAc	white prisms	$C_{13}H_{13}ClN_2S$	1.39 (d, J = 6 Hz, 3H, $SCHCH_3$), 2.20 (d, J = 2 Hz, 3H, CH_3), 2.56–4.16 (m, 3H, $CHCH_2$), 6.77 (d, J = 2 Hz, 1H, H-2), 7.06–7.80 (m, 3H, Ar)
4e	H	C_6H_5	71	152–153	Acetone	white prisms	$C_{18}H_{16}N_2S$	2.25 (d, J = 2 Hz, 3H, CH_3), 2.83, 3.40, 4.83 (ABX system, 3H, $CHCH_2$), 6.80 (d, J = 2 Hz, 1H, C2-H), 7.06–7.93 (m, 9H, Ar)
4f	8-Cl	C_6H_5	47	166–167	EtAc	white prisms	$C_{18}H_{15}ClN_2S$	2.23 (d, J = 2 Hz, 3H, CH_3), 2.86, 3.46, 4.86 (ABX system, 3H, $CHCH_2$), 6.85 (d, J = 2 Hz, 1H, H-2), 7.06–7.96 (m, 8H, Ar)
4g	H	*o*-ClC_6H_4	58	143–147	–[b]	–	$C_{18}H_{15}ClN_2S$	2.26 (d, J = 2 Hz, 3H, CH_3), 2.83, 3.34, 5.43 (ABX system, 3H, $CHCH_2$), 6.89 (d, J = 2 Hz, 1H, H-2), 7.06–7.97 (m, 8H, Ar)
12	–	–	95	105–106	EtAc	white prisms	$C_{18}H_{14}N_2S$	2.36 (d, J = 2 Hz, 3H, CH_3), 6.96 (d, J = 2 Hz, 1H, H-2), 7.03–7.82 (m, 10H, 9Ar + 1H-4)

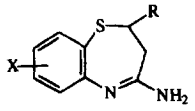
[a]The ^{1}H-NMR spectra of compounds **4c–g** showed signals attributable to mixtures of isomers (60:40), as previously observed in 1-substituted-5-methyl- or 5-phenyl-4,5-dihydro-*s*-triazolo[3,4-*d*]-1,5-benzothiazepines [25]. [b]Purified by flash chromatography and not crystallized.

orders of magnitude in receptor affinity as observed for compound **8a** (K_i = 4.24 μM) and the absence of the 2-position substituent (compound **4a**) caused a loss of affinity. This is in agreement with the hypothesis that a lipophilic region of steric attraction is required at the 2-position [18].

The introduction of a phenyl ring at the 5-position in the benzothiazepine system resulted in a loss of affinity, as shown for compounds **4e**, **4f**, **4g**, **9e**, **9f**, **9g**, **12**, **15** and **16**. This suggests that a phenyl group in this position may introduce a steric hindrance which would interfere with a hypothetical region of repulsive interaction for the benzodiazepine receptor [18].

The same paper [18] also suggests that antagonist/inverse agonist activities depend on the presence of a lipophilic group, a proton acceptor and a proton donor

Table II. Physical and chemical data of compounds **5a–g**.



5a-g

Compound	*X*	*R*	*Yield (%)*	*Mp (°C)*	*Formula*	*^{1}H-NMR, δ*
5a[a]	H	H	35	186	$C_9H_{10}N_2S$	2.55, 3.50 (2t, A_2B_2 system, 4H, CH_2CH_2), 5.02 (br s, 2H, NH_2), 6.70–7.53 (m, 4H, Ar)
5b	8-Cl	H	32	131–135	$C_9H_9ClN_2S$	2.52, 3.48 (2t, A_2B_2 system, 4H, CH_2CH_2), 5.20 (br s, 2H, NH_2), 6.90–7.50 (m, 3H, Ar)
5c	H	CH_3	41	134–138	$C_{10}H_{12}N_2S$	1.40 (d, J = 7 Hz, 3H, CH_3), 2.00–2.56 (m, 2H, CH_2), 3.70–4.16 (m, 1H, CH)
5d	8-Cl	CH_3	49	134–137	$C_{10}H_{11}ClN_2S$	1.33 (d, J = 6 Hz, 3H, CH_3), 1.73–2.60 (m, 2H, CH_2), 3.20, 4.20 (superimposed br s and m, 3H, NH_2 and CH), 6.74–7.43 (m, 3H, Ar) (DMSO-d_6)
5e	H	C_6H_5	37	121–123	$C_{15}H_{14}N_2S$	2.58, 2.77, 4.93 (ABX system, 3H, $CHCH_2$), 4.93 (vbr s, 2H, NH_2), 6.93–7.59 (m, 9H, Ar)
5f	8-Cl	C_6H_5	27	131–133	$C_{15}H_{13}ClN_2S$	2.50–2.85 (m, 2H, CH_2), 4.56–5.23 (superimposed br s and m, 3H, NH_2 and CH), 6.73–7.86 (m, 8H, Ar) (DMSO-d_6)
5g	H	*o*-ClC_6H_5	50	125–127	$C_{15}H_{13}ClN_2S$	2.76–3.08 (m, 2H, CH_2), 4.23 (vbr s, 2H, NH_2), 5.20–5.53 (m, 1H, CH), 7.00–7.60 (m, 8H, Ar) (DMSO-d_6)

[a]Known [3], mp 175–177°C, yield 77%.

group. Another recent article [19] reported the synthesis of a new benzodiazepine ligand which had no proton donor group. Both this benzodiazepine ligand and **9a** have a lipophilic group (phenyl) and a proton acceptor (nitrogen at 3-position) in the five-membered ring and are devoid of proton donor group.

These remarks suggest that the presence of a proton donor group is not an essential requirement for the antagonist/inverse agonist activities. The GABA-ratio value of **9a** and its structural requirement analogy with other compounds [18, 19] suggest that it could act as an antagonist or an inverse agonist. This compound will be submitted to further investigations to define its pharmacological profile.

Experimental protocols

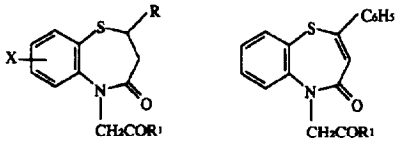
Chemistry

Melting points were taken on a Kofler hot-stage apparatus and are uncorrected. ^{1}H-NMR spectra were recorded in $CDCl_3$ solution, unless otherwise indicated, using a Varian Em-390 (90 MHz) spectrometer. The chemical shift values are reported in δ (ppm) relative to tetramethylsilane as an internal standard. Mass spectra were recorded on a Varian MAT 311A spectrometer. Elemental analyses were performed for C, H and N on a Carlo Erba Elemental Analyzer model 1106 and results were within ±0.4% of the theoretical values. The purity of the compounds was checked by TLC (pre-coated silica-gel plates, Merck Kieselgel 60 F_{254}). Flash chromatographies were performed on columns packed with Merck silica gel, 230–400 mesh.

General procedure for 1-methylimidazo[2,1-d][1,5]benzothiazepines 4a–g and 12

A mixture of **2a–d** or **3** (1 mmol), propargylamine hydrochloride (1.1 mmol) and sodium acetate (1.1 mmol) in *n*-butanol (10 ml) for **2a–d** or anhydrous ethanol (10 ml) for **3** was refluxed under a nitrogen current for 15 h for **4a**, **4c** and **4d**, 5 h for **4b** and **4e**, and 10 h for **4f**, **4g** and **12**. The reaction mixture was then evaporated *in vacuo* and the residue was dissolved in chloroform and extracted with diluted hydrochloric acid solution. The acidic layer was neutralized with 30% ammonium hydroxide solution and extracted with chloroform. Finally, the chloroform phase was washed with water, dried over anhydrous Na_2SO_4, filtered and evaporated *in vacuo*. The residue was either induced to crystallize by adding small amounts of hexane/ethyl acetate 1:1 for **4a**, ethyl acetate for **4d** and acetone for **4e**, or purified by flash chromatography (chloroform) (table I).

Table III. Physical and chemical data of compounds **6a–g**, **7a–g**, **13** and **14**.



6a-g, 7a-g **13,14**

Compound	*X*	*R*	*R¹*	*Yield (%)*	*Mp (°C)*	*Crystallization solvent*	*Formula*	*¹H-NMR, δ*
6a	H	H	CH_3	70	103–105 dec	EtOH	$C_{12}H_{13}NO_2S$	2.29 (s, 3H, CH_3), 2.63 (t, 2H, CH_2), 3.38 (br t, 2H, CH_2), 4.06, 4.95 (2br d, 2H, NCH_2), 7.13–7.68 (m, 4H, Ar)
6b	8-Cl	H	CH_3	26	94–96	EtAc	$C_{12}H_{12}ClNO_2S$	2.28 (s, 3H, $COCH_3$), 2.65 (t, 2H, CH_2), 3.40 (br t, 2H, CH_2), 3.97, 4.96 (2 br d, 2H, NCH_2), 7.10–7.64 (m, 3H, Ar)
6c	H	CH_3	CH_3	81	50–55	–[a]	$C_{13}H_{15}NO_2S$	1.43 (br d, 3H, $CHCH_3$), 2.32 (s, 3H, $COCH_3$), 2.40–2.95 (m, 2H, $CHCH_2$), 3.65–4.05 (m, 1H, $CHCH_2$), 4.25, 4.85 (2 br d, 2H, NCH_2), 7.10–7.73 (m, 4H, Ar)
6d	8-Cl	CH_3	CH_3	100	129–132	EtAc	$C_{13}H_{14}ClNO_2S$	1.40 (br d, 3H, $CHCH_3$), 2.29 (s, 3H, $COCH_3$), 2.38–2.90 (m, 2H, $CHCH_2$), 3.53–4.10 (m, 1H, $CHCH_2$), 4.06, 4.90 (2 br d, 2H, NCH_2), 7.03–7.63 (m, 3H, Ar)
6e	H	C_6H_5	CH_3	75	89–90	EtAc	$C_{18}H_{17}NO_2S$	2.32 (s, 3H, $COCH_3$), 2.70–3.05 (m, 2H, $CHCH_2$), 4.06, 5.00 (AX system, J = 18 Hz, 2H, NCH_2), 4.56–5.06 (m, 1H, $CHCH_2$), 7.03–7.70 (m, 9H, Ar)
6f	8-Cl	C_6H_5	CH_3	51	152–154	EtAc	$C_{18}H_{16}ClNO_2S$	2.30 (s, 3H, $COCH_3$), 2.74–3.04 (m, 2H, $CHCH_2$), 4.03, 5.02 (AX system, J = 17.6 Hz, 2H, NCH_2), 4.74–4.90 (m, 1H, $CHCH_2$), 7.06–7.78 (m, 8H, Ar)
6g	H	*o*-ClC_6H_4	CH_3	90	103–105	EtAc	$C_{18}H_{16}ClNO_2S$	2.55 (s, 3H, $COCH_3$), 2.96–3.25 (m, 2H, $CHCH_2$), 4.36, 5.24 (AX system, J = 17.6 Hz, NCH_2), 5.53–5.68 (m, 1H, $CHCH_2$), 7.25–7.82 (m, 8H, Ar)
7a	H	H	C_6H_5	57	85–87	EtAc	$C_{17}H_{15}NO_2S$	2.66, 3.36 (2t, A_2B_2 system, J= 6 Hz, 4H, CH_2CH_2), 4.60, 5.63 (2br d, 2H, CH_2), 6.90–8.00 (m, 9H, Ar)
7b	8-Cl	H	C_6H_5	80	134–135	EtAc	$C_{17}H_{14}ClNO_2S$	2.70 (br t, 2H, CH_2), 3.42 (br t, 2H, CH_2), 4.48, 5.76 (2 vbr d, 2H, NCH_2), 7.13–8.10 (m, 8H, Ar)
7c	H	CH_3	C_6H_5	70	119–121	EtOH	$C_{18}H_{17}NO_2S$	1.36 (br d, 3H, CH_3), 1.93–2.86 (m, 2H, $CHCH_2$), 3.60–4.06 (m, 1H, $CHCH_2$), 4.57, 5.76 (AX system, J = 17 Hz, 2H, NCH_2), 6.60–8.50 (m, 9H, Ar)
7d	8-Cl	CH_3	C_6H_5	75	109–110	EtOH	$C_{18}H_{16}ClNO_2S$	1.35 (d, J= 6 Hz, 3H, CH_3), 2.30–2.75 (m, 2H, $CHCH_2$), 3.67–4.04 (m, 1H, $CHCH_2$), 4.50, 5.28 (AX system, J = 17.5 Hz, 2H, NCH_2), 7.12–8.10 (m, 8H, Ar)

Table III. *(Continued.)*

Compound	*X*	*R*	*R¹*	*Yield (%)*	*Mp (°C)*	*Crystallization solvent*	*Formula*	*¹H-NMR, δ*
7e	H	C_6H_5	C_6H_5	43	138–139	EtOH	$C_{23}H_{19}NO_2S$	2.80–3.15 (m, 2H, CHC*H*$_2$), 4.16, 5.87 (AX system, J = 17 Hz, 2H, NCH_2), 4.78–4.95 (m, 1H, C*H*CH_2), 7.13–8.12 (m, 14H, Ar)
7f	8-Cl	C_6H_5	C_6H_5	28	128–130	EtAc/ cyclohexane 1:1	$C_{23}H_{18}ClNO_2S$	2.80–3.15 (m, 2H, CHC*H*$_2$), 4.60, 5.80 (AX system, *J* = 17.5 Hz, 2H, NCH_2), 4.75–4.95 (m, 1H, C*H*CH_2), 7.12–8.15 (m, 13H, Ar)
7g	H	*o*-ClC_6H_4	C_6H_5	64	128–129	EtOH	$C_{23}H_{18}ClNO_2S$	2.77–3.14 (m, 2H, CHC*H*$_2$), 4.65, 5.88 (AX system, *J* = 17.6 Hz, 2H, NCH_2), 5.34–5.50 (m, 1H, C*H*CH_2), 7.05–8.14 (m, 13H, Ar)
13	–	–	CH_3	90	119–120	EtOH	$C_{18}H_{15}NO_2S$	2.33 (s, 3H, $COCH_3$), 4.66 (1br d, 2H, NCH_2), 6.45 (s, 1H, H-3), 7.03–7.86 (m, 9H, Ar)
14	–	–	C_6H_5	90	138-140	EtAc	$C_{23}H_{17}NO_2S$	4.94, 5.73 (AX system, *J* = 17.2 Hz, 2H, NCH_2), 6.53 (s, 1H, H-3), 7.10–8.20 (m, 14H, Ar)

[a]Purified by flash chromatography and not crystallized.

General procedure for 2-methyl- and 2-phenylimidazo[2,1-d]-[1,5]benzothiazepines 8 and 9
Compounds **8** and **9** were synthesized according to two methods. *Method A* starting from 2,3-dihydrobenzothiazepin-3(5*H*)-thiones **2** and *Method B* starting from 2,3-dihydro-1,5-benzothiazepin-3(5*H*)-ones **1** and from 2-phenyl-1,5-benzothiazepin-3(5*H*)-one **10**.

General procedure for 4-amino-2,3-dihydro-1,5-benzothiazepines 5a–g. Method A. A mixture of 2,3-dihydrobenzothiazepin-3(5*H*)-thione **2a–g** (10 mmol) in methanol saturated with ammonia (50 ml) was stirred at room temperature for 20 h. The mixture was then filtered and the filtrate was concentrated to half its volume, cooled in ice and the resulting precipitate filtered to give **5a** and **5e–g**. In the other cases, the filtrate was brought to dryness *in vacuo* and induced to crystallize by adding small amounts of ethyl acetate or methanol (**5c, d**) or was purified by flash chromatography (chloroform/methanol 95:5) (**5b**). These compounds were used without further purification (table II).

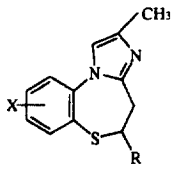
2-Methyl-imidazo[2,1-d][1,5]henzothiazepines 8c and 8d. Method A. A mixture of 4-aminobenzothiazepine **5c** and **5d** (1 mmol), chloroacetone (1 mmol) and sodium acetate (1 mmol) in anhydrous ethanol (10 ml) was refluxed for 16 h (**8c**) or 20 h (**8d**). After cooling the mixture was filtered and the filtrate evaporated *in vacuo*. The residue was dissolved with chloroform, washed with water, dried over anhydrous Na_2SO_4, filtered and dried. The residue was purified by flash chromatography (chloroform) (table IV).

General procedure for 2-phenyl-imidazo[2,1-d][1,5]benzothiazepines 9c–e and 9g. Method A. A mixture of 4-aminobenzothiazepines **5c–e** and **5g** (1 mmol), phenacyl bromide (1 mmol) and sodium bicarbonate (1 mmol) in anhydrous ethanol (10 ml) was stirred for 45 min at room temperature and was then refluxed for 1.5 h. The reaction mixture was evaporated *in vacuo* and the residue was dissolved with acetone and filtered. The filtrate was brought to dryness and the residue was either crystallized by adding small amounts of hexane (**9c**), or ethyl acetate (**9d** and **9g**) or purified by flash chromatography (methylene chloride) (**9e**) (table V).

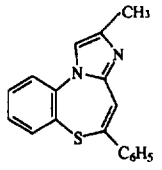
General procedure for N-(propan-2'-one)-1,5-benzothiazepin-2(5H)-ones 6a–g and 13 and N-(phenacyl)-1,5-benzothiazepin-2(5H)-ones 7a–g and 14. Method B. To a stirred solution of the benzothiazepine derivatives **1** or **10** [1] (1 mmol), tetrabutylammonium bromide (0.1 mmol) and chloroacetone (1.5 mmol) or phenacylbromide (1.5 mmol) in tetrahydrofuran (*ca* 10 ml), finely powdered potassium hydroxide (1.5 mmol) was added. The reaction mixture was kept at room temperature under stirring for 24–72 h for compounds **6a–g** and **13**, 6 h for **5b** and 16–40 h for compounds **5a**, **5c–g** and **14** and then filtered. The filtrate was evaporated *in vacuo* and the residue taken up with chloroform, the chloroform extract washed with water, dried over Na_2SO_4 and brought to dryness *in vacuo*. The crude product was then either crystallized by adding small amounts of ethanol or ethyl acetate (**6d, 6f, 6g, 7c–e, 7g** and **13**), or purified by flash chromatography (chloroform) (**6a–c, 6e, 7a, 7b, 7f** and **14**) (table III).

General procedure for 2-methyl- and 2-phenylimidazol[2,1-d]-[1,5]benzothiazepines 8a–g, 9a–c, 9f, 15 and 16. Method B. A mixture of *N*-alkyl derivative **6a–g, 7a–c, 7f, 13** and **14** (1 mmol) and ammonium acetate (10 mmol) in glacial acetic acid (10 ml), was refluxed for 3 h for **8a, 8b** and **16**, for 6 h for **8c, 8e, 9b, 9c, 9f** and **15** and 10 h for **8d, 8f, 8g** and **9a**. After cooling the solution was poured into ice-water and neutralized

Table IV. Physical and chemical data of compounds **8a–g** and **15**.



8a-g



15

Compound	*X*	*R*	*Yield (%)*		*Mp (°C)*	*Crystal-lization solvent*	*Formula*	*^{1}H-NMR, δ*
			Method A	*Method B*				
8a	H	H		37	119–121	EtOH	$C_{12}H_{12}N_2S$	2.26 (s, 3H, CH_3), 2.93, 3.42 (A_2B_2 system, 4H, CH_2CH_2), 6.76 (s, 1H, H-1), 7.03–7.73 (m, 4H, Ar)
8b	8-Cl	H		62	145–147	EtAc	$C_{12}H_{11}ClN_2S$	2.30 (s, 3H, CH_3), 3.00, 3.50 (A_2B_2 system, 4H, CH_2CH_2), 6.80 (s, 1H, H-1), 7.42–7.73 (m, 4H, Ar)
8c	H	CH_3	46	65	47–49	EtAc	$C_{13}H_{14}N_2S$	1.43 (d, J = 5 Hz, 3H, SCHCH_3), 2.29 (s, 3H, CH_3), 2.56, 3.04, 3.85 (ABC system, 3H, $CHCH_2$), 6.76 (s, 1H, H-1), 6.84–7.73 (m, 4H, Ar)
8d	8-Cl	CH_3	57	60	50–52	–[a]	$C_{13}H_{13}ClN_2S$	1.40 (d, J = 5 Hz, 3H, SCHCH_3), 2.30 (s, 3H, CH_3), 2.60, 3.03, 3.91 (ABC system, 3H, $CHCH_2$), 6.80 (s, 1H, H-1), 7.10–7.75 (m, 3H, Ar)
8e	H	C_6H_5		30	115–116	EtAc	$C_{18}H_{16}N_2S$	2.32 (s, 3H, CH_3), 3.00, 3.40, 4.93 (ABX system, 3H, $CHCH_2$), 6.90 (s, 1H, C_1-H), 7.00–7.86 (m, 9H, Ar)
8f	8-Cl	C_6H_5		46	168	Acetone	$C_{18}H_{15}ClN_2S$	2.36 (s, 3H, CH_3), 3.03, 3.36, 4.91 (ABX system, 3H, $CHCH_2$), 6.89 (s, 1H, H-1), 7.20–7.87 (m, 8H, Ar)
8g	H	*o*-ClC_6H_4		31	102–104	EtAc	$C_{18}H_{15}ClN_2S$	2.31 (s, 3H, CH_3), 2.89, 3.37, 5.41 (ABX system, 3H, $CHCH_2$), 6.84 (s, 1H, H-1), 6.95–7.75 (m, 8H, Ar)
15	–	–		37	108–110	EtAc/ cyclo-hexane 1:1	$C_{18}H_{14}N_2S$	2.38 (s, 3H, CH_3), 7.11 (s, 1H, H-1), 7.22–7.88 (m, 10 H, Ar)

[a]Purified by flash chromatography and not crystallized.

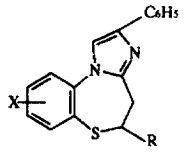
with 30% ammonium hydroxide solution. In the case of **8f**, **9a**, **9c** and **9f**, the resulting precipitate was filtered and recrystallized from a suitable solvent. In the other cases, the mixture was extracted with chloroform. The chloroform solution was washed with water, dried over Na_2SO_4, filtered and brought to dryness *in vacuo*. The oily residue was either induced to crystallize by adding small amounts of ethyl acetate or ethanol, or purified by flash chromatography (chloroform) (tables IV and V).

Receptor-binding assay

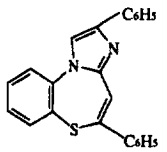
Tritiated flunitrazepam was obtained from Du Pont de Nemours New England Nuclear Division (Dreieichenhaim, Germany) and had a specific activity of 83.5 Ci/mmol and a radiochemical purity > 99%. All other chemicals were reagent grade and obtained from commercial suppliers.

The [^{3}H]Flunitrazepam binding assay to bovine cerebral cortex membrane was carried out essentially as described previously [20]. Cortices were rapidly isolated and homogenized in 10 volumes of ice-cold 0.32 M sucrose containing protease inhibitor [21]. The homogenate was centrifuged at 1000 *g* for 15 min at 4°C. The pellet was then osmotically shocked by suspension in 10 vol of 50 mM Tris-HCl buffer at pH 7.4 containing protease inhibitors and recentrifuged at 5000 *g* for 15 min at 4°C. The resulting membranes

Table V. Physical and chemica data of compounds **9a–g** and **16**.



9a-g



16

Compound	*X*	*R*	*Yield (%)*		*Mp (°C)*	*Crystal-lization solvent*	*Formula*	*^{1}H-NMR, δ*
			Method A	*Method B*				
9a[a]	H	H		67	118–119	Acetone	$C_{17}H_{14}N_2S$	3.08, 3.53 (A_2B_2 system, 4H, CH_2-CH_2), 6.76–8.00 (m, 10H, Ar + H-1)
9b	8-Cl	H		46	88–90	EtAc	$C_{17}H_{13}ClN_2S$	3.09, 3.53 (A_2B_2 system, 4H, CH_2-CH_2), 7.20–7.88 (m, 9H, Ar + H-1)
9c	H	CH_3	61	90	170–171	Acetone	$C_{18}H_{16}N_2S$	1.46 (d, J = 6 Hz, 3H, CH_3), 2.63, 3.20, 3.95 (ABC system, 3H, CH-CH_2), 7.06–7.92 (m, 9H, Ar + H-1)
9d	8-Cl	CH_3	70		151–152	EtAc	$C_{18}H_{15}ClN_2S$	1.46 (d, J = 6 Hz, 3H, CH_3), 2.63, 3.16, 3.96 (ABC system, 3H, CH-CH_2), 7.10–7.93 (m, 9H, Ar + H-1)
9e	H	C_6H_5	47		117–120	–[b]	$C_{23}H_{18}N_2S$	3.10, 3.50, 4.93 (ABX system, 3H, $CHCH_2$), 6.80–7.96 (m, 15H, Ar + H-1)
9f	8-Cl	C_6H_5		80	164–166	Acetone	$C_{23}H_{17}ClN_2S$	2.97, 3.48, 4.93 (ABX system, 3H, $CHCH_2$), 7.13–7.90 (m, 14H, Ar + H-1)
9g	H	o-ClC_6H_4	23		176–185	EtAc	$C_{23}H_{17}ClN_2S$	2.80, 3.23, 5.26 (ABX system, 3H, $CHCH_2$), 6.90–7.64 (m, 14H, Ar)
16				51	85–86	EtAc	$C_{23}H_{16}N_2S$	7.20–8.10 (m, 16H, 15Ar + H-1)

[a]Known [3], mp 119–120°C, yield 57%. [b]Purified by flash chromatography and not crystallized.

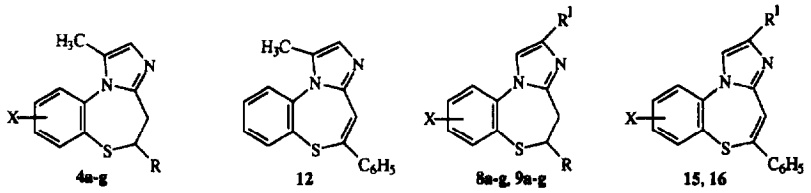
were frozen and washed using a procedure previously described for removing endogenous GABA from rat cerebral cortex [22]. Finally, the pellet was suspended in 10 volumes of 50 mM Tris-HCl buffer at pH 7.4 and used in the binding assay.

Protein concentration was assayed by the method of Lowry *et al* [23].

Routine [^{3}H]Flunitrazepam binding assays were run by incubating 0.4 mg protein of crude bovine brain membrane suspension at 0°C for 90 min with [^{3}H]Flunitrazepam (0.2 nM) in a total volume of 0.5 ml of Tris-HCl buffer. After incubation, samples were diluted at 0°C with 5 ml of the assay buffer and immediately filtered under reduced pressure through glass-fiber filter disks (Whatman GF/B). The filters were washed with 5 ml of the buffer, dried and added to 8 ml of Ready Protein Beckman scintillation counter and radioactivity was counted on a LS 1800 scintillation counter. Non-specific binding was determined by the radioactivity bound in the presence of 10 μM non-radioactive diazepam in parallel assays. Water-insoluble derivatives were dissolved in EtOH and added to the assay mixture. Blank experiments were carried out to determine the effect of EtOH (2%) or DMSO (1%) on binding. Values of inhibition of specific binding by 50% (IC_{50}) were calculated from the displacement curves by log-probit analysis with four to six concentrations of the displacers, each performed in triplicate.

Dissociation constants K_i were derived according to the equation of Chen and Prusoff [24]. The ligand affinity K_d of [^{3}H]Flunitrazepam was 1.8 nM.

Table VI. Inhibition of [^{3}H]Flunitrazepam binding by compounds **4a–g**, **8a–g**, **12**, **15** and **16**.



Compound	*X*	*R*	R_1	*Inhibition %[a] (10 μM)*	K_i[b] *(μM)*	*Compound*	*X*	*R*	R_1	*Inhibition %[a] (10 μM)*	K_i[b] *(μM)*	*GABA-ratio*
4a	H	H	–	34.4	–	**8e**	H	C_6H_5	CH_3	16.9	–	–
4b	8-Cl	H	–	16.4	–	**8f**	8-Cl	C_6H_5	CH_3	20.97	–	–
4c	H	CH_3	–	33.2	–	**8g**	H	*o*-ClC_6H_4	CH_3	30.65	–	–
4d	8-Cl	CH_3	–	33.2	–	**15**	–	–	CH_3	16.8	–	–
4e	H	C_6H_5	–	45.34	–	**9a**	H	H	C_6H_5	98.9	0.043 ±0.0021	1.09
4f	8-Cl	C_6H_5	–	45.34	–	**9b**	8-Cl	H	C_6H_5	52	–	–
4g	H	*o*-ClC_6H_4	–	42.9	–	**9c**	H	CH_3	C_6H_5	94.79	0.097 ± 0.0073	0.8
12	–	–	–	2.2	–	**9d**	8-Cl	CH_3	C_6H_5	81.3	1.97 ± 0.15	–
8a	H	H	CH_3	63.1	4.24 ± 0.32	**9e**	H	C_6H_5	C_6H_5	0	–	–
8b	8-Cl	H	CH_3	83.47	1.33 ± 0.098	**9f**	8-Cl	C_6H_5	C_6H_5	0	–	–
8c	H	CH_3	CH_3	59.04	5.34 ± 0.43	**9g**	H	*o*-ClC_6H_4	C_6H_5	0	–	–
8d	8-Cl	CH_3	CH_3	32.23	–	**16**	–	–	C_6H_5	0	–	–
Chlordiazepoxide					0.72 ± 0.07							1.73

[a]Percent of inhibition of specific [^{3}H]Flunitrazepam binding at 10 μM compound concentration; results are the means of three separate experiments with SEM less than 10%. [b]Means ± SEM of three determinations.

Acknowledgments

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