Eur J Med Chem (1995) 30, 859–868

Synthesis and structure–activity analysis of 2-(4-chlorophenyl)-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-*c*]pyridazin-3(2*H*)-ones as ligands for benzodiazepine receptors

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(Received 28 March 1995; accepted 30 June 1995)

Summary — A series of 2-(4-chlorophenyl)-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-*c*]pyridazin-3(2*H*)-ones were synthesized and evaluated *in vitro* for their affinity toward benzodiazepine receptors (BZRs) in rats and for their intrinsic efficacy in the augmentation of the γ-aminobutyric acid (GABA)-induced chloride currents in the dissociated frog sensory neurons. Compounds in which the 9-position of the condensed-ring system was substituted with alkyl group or bromine had a high affinity toward BZRs. The substituents at the same position also influenced significantly the GABA-induced chloride currents. As the result, 9-alkyl and 9-bromo substituents would interact with the lipophilic area of BZRs. A series of 2-(4-chlorophenyl)-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-*c*]pyridazin-3(2*H*)-ones exhibited partial and full agonistic activities toward BZRs.

benzodiazepine receptor / γ-aminobutyric acid / partial agonist / full agonist / structure–activity relationship

Introduction

Since the discovery of benzodiazepine receptors (BZRs) in the central nervous system [1, 2], the mechanism of the action of benzodiazepine has been rapidly clarified. BZRs act as an allosteric regulatory site of γ-aminobutyric acid (GABA) receptors and are considered to mediate two opposite effects: one to amplify or facilitate the action of GABA and the other to reduce it. In respect of these effects, ligands for BZRs have been classified into at least four categories: full agonists, partial agonists, antagonists, and inverse agonists [3, 4]. Most of the drugs in clinical use, such as diazepam **1**, are full agonist-type ligands at BZRs and exhibit anticonvulsant, sedative, and muscle relaxant effects in addition to anxiolytic properties. By contrast, the BZR partial agonists were reported as candidates that could maintain the therapeutic potential with fewer side effects than BZR full agonists in the treatment of anxiety [5]. For several years, we have been involved in the design and synthesis of BZR ligands containing a condensed-ring system of pyridazinone as a common molecular element [6–10]. The structure–activity analysis of synthesized compounds verified pharmacologically favorable characteristics of 2-(4-chlorophenyl)-5,6-dihydro[1]benzothiepino[5,4-*c*]pyridazin-3(2*H*)-one 7-oxide (Y-23684, **2b**) as a BZR partial agonist, namely, an anxioselective anxiolytic agent (fig 1) [9, 11]. Thus, **2b** is now under clinical study in the treatment of anxiety. This finding then led us to an isosteric replacement of the condensed-benzene ring of **2b** with thiophene ring. We herein report the synthesis and evaluation of receptor binding profile of 2-(4-chlorophenyl)-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-*c*]-pyridazin-3(2*H*)-ones **A** as ligands for BZRs.

Chemistry

We chose a synthetic route to compounds **A** (fig 1) that focused on the introduction of various substituents

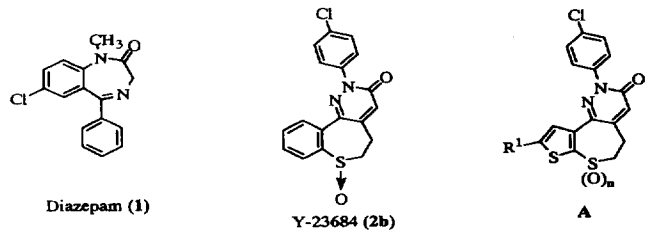
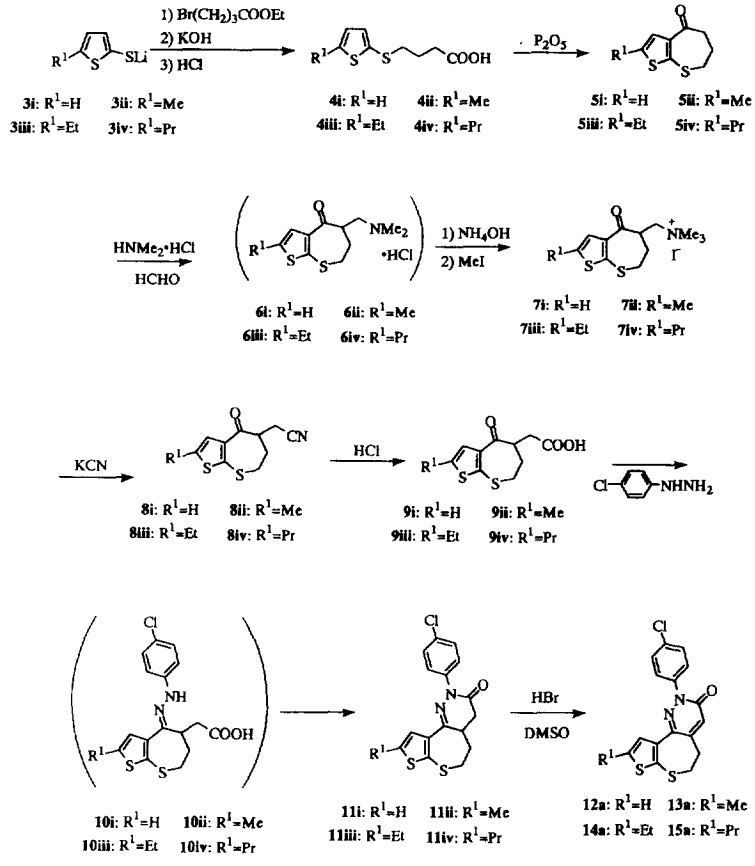


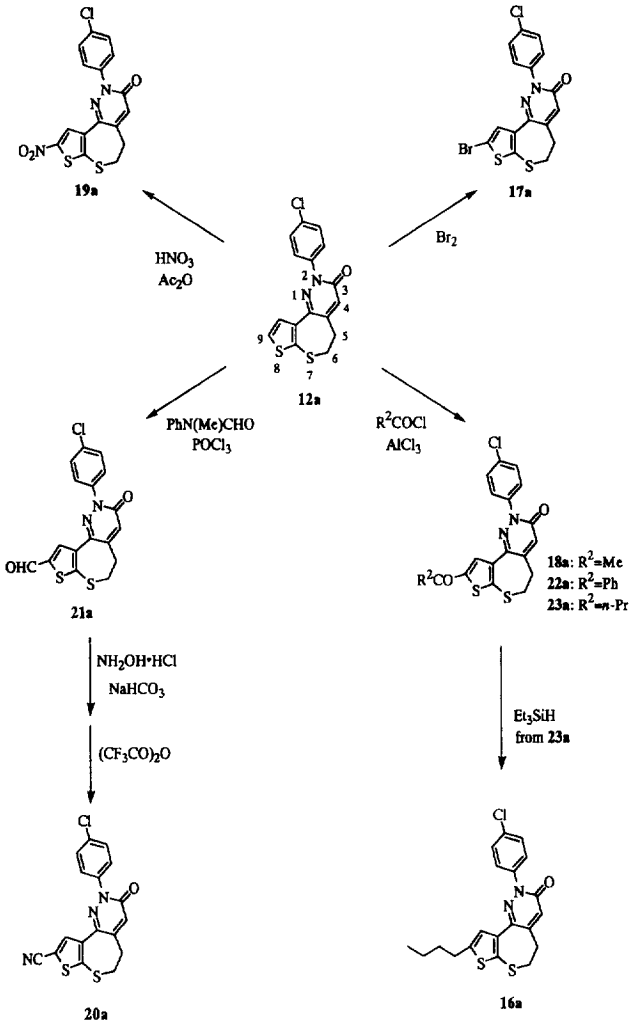
Fig 1. Chemical structures of diazepam **1**, Y-23684 and 2-(4-chlorophenyl)-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-*c*]-pyridazin-3(2*H*)-ones **A**.

into the 9-position of the condensed-ring system and on the oxidation level of sulfide function at the 7-position of the same system. Four kinds of compounds (**12a**, **13a**, **14a** and **15a**), in which the R^1-substituent at the 9-position of **A** (n = 0) were hydrogen, methyl, ethyl and propyl groups, respectively, were derived from 6,7-dihydro-5*H*-thieno[2,3-*b*]thiepin-4-ones (**5i–iv**) [12] in a similar manner to in a previous paper of ours [9] (scheme 1). The ketones **5i–iv** were prepared by intramolecular cyclization of 4-(2-thienythio)butyric acids (**4i–iv**), which were obtainable from 2-(lithiomercapto)thiophenes (**3i–iv**). Compounds **6i–iv** were prepared from **5i–iv** by the Mannich reaction, and treatment of **6i–iv** with excess ammonia, followed by iodomethane to afford corresponding quaternary ammonium salts (**7i–iv**). Cyanation of **7i–iv** and consequent hydrolysis of the products (**8i–iv**) gave carboxylic acids (**9i–iv**). These carboxylic acids were then cyclocondensed with 4-chlorophenylhydrazine to produce 2-(4-chlorophenyl)-4,4a,5,6-tetrahydrothieno[2',3':2,3]-thiepino[4,5-*c*]pyridazin-3(2*H*)-ones (**11i–iv**) *via* intermediate hydrazones (**10i–iv**). Dehydrogenation [13, 14] with bromine was inappropriate for thiophene derivatives because of the lability of these aromatic rings to electrophiles. We therefore applied a combined treatment [15] with dimethylsulfoxide and hydrogen bromide to the oxidative elimination of hydrogen at the 4- and 4a-positions of **11i**, **11ii**, **11iii** and **11iv**, and obtained **12a**, **13a**, **14a** and **15a**, respectively.

Some compounds analogous to **12a** were synthesized by introducing appropriate substituents into the 9-position of **12a** (scheme 2). Thus, 9-bromo compound **17a** was prepared by bromination, 9-acyl compounds **18a**, **22a**, and **23a** by Friedel–Crafts reaction, and 9-nitro compound **19a** by nitration of **12a**. 9-Butyl compound **16a** was produced by reduction of **23a** with triethylsilane. 9-Formyl compound **21a** was obtained by Vilsmeier–Haack reaction of **12a**, and 9-cyano compound **20a** was prepared by treatment of **21a** with hydroxylamine and by subsequent dehydra-



Scheme 1. Synthetic route to 2-(4-chlorophenyl)-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-*c*]pyridazin-3(2*H*)-ones.

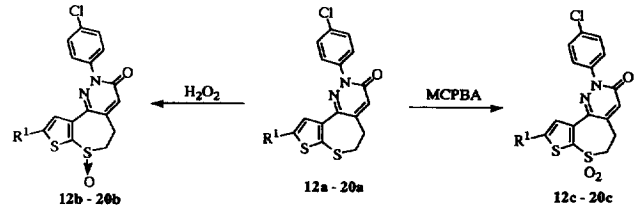


Scheme 2. Modification of the 9-position.

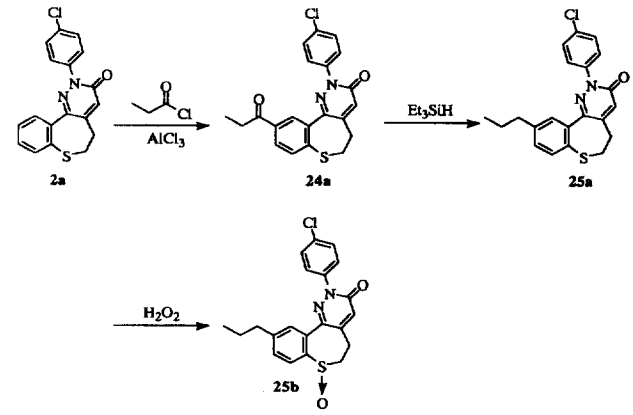
tion of the oxime with trifluoroacetic anhydride. Sulfoxide **12b–20b** and sulfone **12c–20c** were prepared by oxidation of the corresponding sulfides (scheme 3). Oxidation of the sulfide moiety in the compounds belong to the **a** series produced corresponding sulfoxide and sulfone derivatives, categorized as **b** (*eg*, compound **12b**) and **c** (*eg*, compound **12c**). The 10-propyl derivative of Y-23684 (**25b**) was prepared from **2a** [9] by Friedel–Crafts acylation, reduction of ketone, followed by oxidation of the sulfur atom (scheme 4).

Pharmacological results and discussion

2-(4-Chlorophenyl)-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-*c*]pyridazin-3(2*H*)-ones were evaluated for their affinity toward BZRs by an assay on their ability to displace [^{3}H]diazepam from the cerebral cortex of rats [2]. Here, we indicate the BZR affinity of these compounds as their K_i value (nM) in their competition with [^{3}H]diazepam at the binding site. The efficacy of these compounds on BZRs was evaluated in the dissociated frog dorsal root ganglion neurons by use of a concentration–jump (termed 'concentration–clamp') technique, under single-electrode voltage-clamp conditions [16]. Such experiments have demonstrated that all the full agonists for BZRs increase the peak amplitude of chloride currents (I_{Cl}) induced by GABA, and that partial agonists dose-dependently augment this GABA response with an amplitude significantly smaller than diazepam, a typical full agonist [16]. The relative I_{Cl} value (r-I_{Cl}), which is the ratio of the I_{Cl} induced by GABA in the presence of the test compound to that induced by GABA itself, has been used for the BZR ligands as a means of predicting the type of activity observed in whole animal models. The chemical structures of synthesized compounds and their *in vitro* pharmacological data are summarized in table I.

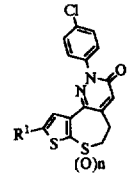


Scheme 3. Oxidation of sulfur atom.



Scheme 4. Synthesis of 10-propyl derivative of Y-23684 (**25b**).

Table I. Physicochemical and biological data of 2-(4-chlorophenyl)-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-*c*]pyridazin-3(2*H*)-ones.



Compound	*R*[1a]	*n*	*Yield*[b] *(%)*	*Mp (°C)*	*Recrystallization solvent*[c]	*Formula*	*[³H]Diazepam*[d] *K_i (nM)*	*Relative*[d] *I_{Cl}*
12a	H	0	82	140–142	AcOEt-IPE	$C_{16}H_{11}ClN_2OS_2$	6.8 ± 1.0	2.20
12b	H	1	70	184–186/dec	EtOH-$CHCl_3$	$C_{16}H_{11}ClN_2O_2S_2$	54 ± 4	1.34
12c	H	2	82	277–279	EtOH-$CHCl_3$	$C_{16}H_{11}ClN_2O_3S_2$	11 ± 1	1.90
13a	Me	0	62	150–151	EtOH-$CHCl_3$	$C_{17}H_{13}ClN_2OS_2$	3.2 ± 0.4	3.64
13b	Me	1	96	174–176/dec	EtOH-$CHCl_3$	$C_{17}H_{13}ClN_2O_2S_2$	9.7 ± 0.3	NT[e]
13c	Me	2	78	241–243	EtOH-$CHCl_3$	$C_{17}H_{13}ClN_2O_3S_2$	2.4 ± 0.7	3.35
14a	Et	0	80	127–128	EtOH-$CHCl_3$	$C_{18}H_{15}ClN_2OS_2$	1.9 ± 0.3	3.63
14b	Et	1	87	173–174/dec	EtOH-IPE	$C_{18}H_{15}ClN_2O_2S_2$	1.3 ± 0.3	2.18
14c	Et	2	81	229–231	EtOH-$CHCl_3$	$C_{18}H_{15}ClN_2O_3S_2$	1.1 ± 0.7	3.69
15a	Pr	0	82	136–137	EtOH-$CHCl_3$	$C_{19}H_{17}ClN_2OS_2$	3.6 ± 1.2	4.37
15b	Pr	1	77	149–151	EtOH-$CHCl_3$	$C_{19}H_{17}ClN_2O_2S_2$	1.7 ± 0.4	2.99
15c	Pr	2	65	222–224	EtOH-$CHCl_3$	$C_{19}H_{17}ClN_2O_3S_2$	0.84 ± 0.20	4.52
16a	Bu	0	62	125–130	EtOH	$C_{20}H_{19}ClN_2OS_2$	19 ± 5	NT[e]
16b	Bu	1	59	124–125	EtOH-IPE	$C_{20}H_{19}ClN_2O_2S_2$	8.7 ± 0.3	2.58
16c	Bu	2	83	184–186	EtOH-IPE	$C_{20}H_{19}ClN_2O_3S_2$	4.8 ± 1.1	2.25
17a	Br	0	77	148–151	EtOH-$CHCl_3$	$C_{16}H_{10}BrClN_2OS_2$	2.1 ± 0.7	3.92
17b	Br	1	72	184–185/dec	EtOH-$CHCl_3$	$C_{16}H_{10}BrClN_2O_2S_2$	0.98 ± 0.23	1.61
17c	Br	2	89	235–237	EtOH-$CHCl_3$	$C_{16}H_{10}BrClN_2O_3S_2$	0.61 ± 0.17	1.96
18a	Ac	0	80	251–253	EtOH-$CHCl_3$	$C_{18}H_{13}ClN_2O_2S_2$	7.8 ± 1.1	3.94
18b	Ac	1	70	189/dec	EtOH-$CHCl_3$	$C_{18}H_{13}ClN_2O_3S_2$	54 ± 5	1.61
18c	Ac	2	80	272–273/dec	EtOH-$CHCl_3$	$C_{18}H_{13}ClN_2O_4S_2$	9.9 ± 2.2	2.25
19a	NO_2	0	40	181–182	EtOH-$CHCl_3$	$C_{16}H_{10}ClN_3O_3S_2$	11 ± 2	2.46
19b	NO_2	1	78	174–175/dec	EtOH-$CHCl_3$	$C_{16}H_{10}ClN_3O_4S_2$	17 ± 2	1.68
19c	NO_2	2	71	280–281/dec	EtOH-$CHCl_3$	$C_{16}H_{10}ClN_3O_5S_2$	18 ± 2	1.56
20a	CN	0	60	128–129/dec	EtOH-$CHCl_3$	$C_{17}H_{10}ClN_3OS_2$	2.9 ± 0.5	3.66
20b	CN	1	78	197–199/dec	EtOH-$CHCl_3$	$C_{17}H_{10}ClN_3O_2S_2$	14 ± 1	1.39
20c	CN	2	87	292–293	EtOH-$CHCl_3$	$C_{17}H_{10}ClN_3O_3S_2$	5.3 ± 0.9	2.12
21a	CHO	0	81	182–183	EtOH-$CHCl_3$	$C_{17}H_{11}ClN_2O_2S_2$	1.9 ± 0.1	2.24
22a	PhCO	0	85	180–182	AcOEt	$C_{23}H_{15}ClN_2O_2S_2$	610 ± 20	NT[e]
1							5.3 ± 1.0	2.54
2b (Y-23684)							42 ± 2	1.64

[a]Pr, *n*-propyl; Bu, *n*-butyl; [b]isolated yield; [c]AcOEt, ethyl acetate; IPE, isopropyl ether; [d]values represent the average of three or more experiments. See *Experimental protocols* for details. [e]NT, not tested.

Compound **12b** is a simple isostere of **2b** at the condensed-ring system, where the benzene moiety was merely replaced with a thiophene ring. These two compounds exhibited a similar BZR affinity. The affinity of **12b** was enhanced by reduction or oxidation of its sulfoxide function. The K_i value was 6.8 nM for **12a**, 54 nM for **12b**, and 11 nM for **12c**, but such an order was affected by the existence of the R^1-substituent in **A**. The BZR affinity of **A** was independent of their partial structure, such as sulfide, sulfoxide and sulfone in the condensed thiophene ring, but was dependent on their structural and the electrostatic properties of the R^1-substituent. In comparison with **12a–c**, the affinity was commonly enhanced in a series of compounds where the R^1 was an alkyl group or bromine, but other compounds, where the R^1 was an electronegative function such as acyl, nitro and cyano, exhibited low binding affinity compared with alkyl compounds. The reduction of binding affinity in benzoyl compound **22a** may be attributed to the steric effects caused by the bulky phenyl substituent.

The 9-unsubstituted compounds (**12a–c**) exhibited smaller r-I_{Cl} values compared with diazepam, as well as **2b**. Introduction of alkyl substituents at the 9-position (**13a–c–16a–c**) remarkably increased the r-I_{Cl} value. Notably, 9-propyl compounds (**15a** and **15c**) possessed an r-I_{Cl} value of more than four. In the case of sulfide compounds, the 9-bromo, 9-acetyl and 9-cyano compounds (**17a**, **18a** and **20a**) also exhibited large r-I_{Cl} values. On the other hand, introduction of nitro or formyl substituent at the 9-position (**19a** and **21a**) did not give much alteration of the r-I_{Cl} value compared with **12a**. Moreover, in the case of 9-bromo, 9-acetyl, 9-nitro and 9-cyano compounds, the sulfoxide and sulfone compounds showed smaller r-I_{Cl} values than the sulfide compounds (**17a** *vs* **17b** and **17c**; **18a** *vs* **18b** and **18c**; **19a** *vs* **19b** and **19c**; **20a** *vs* **20b** and **20c**). These results indicated that the 9-substituents would interact with BZRs and affect the intrinsic efficacy of compounds **A**, and that the oxidation level of the sulfur atom also affected the intrinsic efficacy.

As mentioned above, the 9-substituent was confirmed to be a very important factor in determining not only the affinity of compounds **A** toward BZRs but also their intrinsic efficacy. This finding prompted us to prepare the 10-propyl derivative of Y-23684 (**25b**). Our interest was to check whether 10-alkyl substituent in 5,6-dihydro[1]benzothiepino[5,4-*c*]-pyridazin-3(2*H*)-ones could cause the same alteration. The binding affinity of **25b** (K_i = 7.0 nM) was six times higher than that of **2b**, but four times lower than that of **15b**. An augmentation of the r-I_{Cl} value (the values of **2b** and **25b** were 1.64 and 2.16, respectively) was smaller than the case of **12b** and **15b**. Thus, in 5,6-dihydro[1]benzothiepino[5,4-*c*]pyridazin-3(2*H*)-ones, an influence of introduction of alkyl substituent

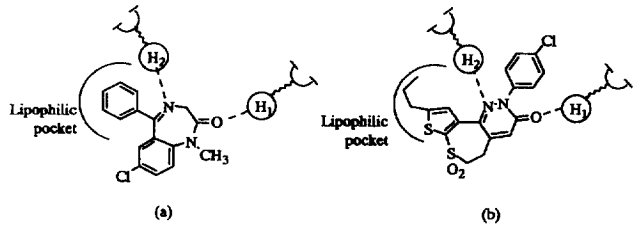


Fig 2. Binding interactions of diazepam **1** (**a**), 9-propyl-compound **15c** (**b**) with the pharmacophore model [22, 23] for the BZR agonist site. H_1, H_2 are the hydrogen bond donor sites on the receptor protein.

at the 10-position on binding affinity and the r-I_{Cl} value was smaller than the case of thieno compounds **A**. These results suggest that there is a hydrophobic interaction between alkyl substituent at this position and BZRs, and that π-excessive thiophene ring is necessary to possess high affinity and high intrinsic efficacy.

Based on the studies of SAR and molecular geometry of various ligands, several pharmacophore models [17–23] have been proposed, both agonists and inverse agonists/antagonists. Cook JM *et al* [22, 23] have reported that BZR agonists interact with two hydrogen bond donating groups termed H_1 and H_2 and with a lipophilic region whose occupation leads to a full agonist. Full occupation of the lipophilic region by phenyl ring (C-5) of diazepam (fig 2a) resulted in a full agonist, while partial occupation of this same region resulted in a partial agonist [24]. In the case of 9-propyl compound **15c**, which was classified as a full agonist, this interaction could be mediated by the imino nitrogen atom at the 1-position and carbonyl oxygen atom at 3-position, forming hydrogen bonds with two donor sites (fig 2b). Furthermore, **15c** can occupy the proposed lipophilic area with fused thiophene ring and 9-propyl substituent (fig 2b). The partial agonistic properties of 9-bromo, 9-acetyl, 9-nitro, 9-cyano and 9-formyl compounds (**17b,c–20b,c** and **21a**) can be explained by partial occupation of the lipophilic area, or by a negative interaction with the lipophilic area because of their electronegative characters. The oxidation level of sulfur atom should affect the interaction between 9-substituents and BZRs.

To confirm their *in vitro* pharmacological properties we chose representative compounds (**14c** and **17c**) for the *in vivo* tests. Compounds **14c** and **17c** have the sulfone function as a common structural unit, but are distinguishable only at the R^1-moiety (**14c**: R^1 = ethyl, **17c**: R^1 = Br). These compounds exhibited significantly higher BZR affinity than diazepam. In spite of such a large terminal difference, **14c** showed a larger r-I_{Cl} value than diazepam and was classified as a full

agonist, but **17c** with a smaller r-I_{Cl} value was classified as a partial agonist. Such a classification was confirmed *in vitro* by assessing pharmacological properties *in vivo* as follows: the ability to prevent bicuculline-induced convulsion in mice (anti-BCL test) [25], the anxiolytic activity determined by the water-lick conflict paradigm in rats (anticonflict test) [26], and the effect on motor coordination in rats (rotarod test). Diazepam **1** was used as a reference drug in these experiments. The pharmacological data are summarized in table II. Compounds **14c**, **17c** and **1** exhibited a high level of potency in both the anti-BCL and anticonflict tests. In the rotarod test, **14c** and **1** were active at doses comparable to the minimum effective doses in the anticonflict test, whereas **17c** was inactive at a dose up to 300 mg/kg. These results successfully reflect the *in vitro* classification of **14c** and **17c**.

In conclusion, we confirmed that replacement of the fused benzene ring in **2b** with a thiophene ring caused a significant change in the affinity and intrinsic efficacy. Introduction of an alkyl or bromo substituent at the 9-position produced a high affinity toward BZRs, and introduction of an electronegative group at the same position caused the r-I_{Cl} value to be smaller. This result indicates that the 9-alkyl and 9-bromo substituents would interact with the lipophilic area of BZRs, and that the electronegative 9-substituents would cause a negative interaction. The full occupation of the lipophilic area would lead to a full agonist, whereas a partial occupation or a negative interaction with the lipophilic area would result in a partial agonist. Thus, 2-(4-chlorophenyl)-5,6-dihydrothieno[2',3':2,3]-thiepino[4,5-*c*]pyridazin-3(2*H*)-ones have a wide spectrum of pharmacological activities, compared with 2-aryl-5,6-dihydro[1]benzothiepino[5,4-*c*]pyridazin-3(2*H*)-ones [9] which exhibited partial agonistic properties. 2-(4-Chlorophenyl)-5,6-dihydrothieno-[2',3':2,3]thiepino[4,5-*c*]pyridazin-3(2*H*)-ones serve as a tool for exploring the effects of structural modifications on compounds which bind to BZRs, and they should lead to agents useful in the treatment of anxiety and sleep disorders. Studies to evaluate further structural modifications of 2-(4-chlorophenyl)-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-*c*]pyridazin-3(2*H*)-one nucleus are currently underway and the results will be published in due course.

Experimental protocols

Chemistry

All melting points were determined on Büchi 530 melting point apparatus, and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded with Jeol JNM-EX 270 spectrometer (Me_4Si as an internal standard). Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), q (quartet), brs (broad singlet), and m (multiplet). Chemical shifts are expressed in ppm and coupling constants in hertz (Hz). The IR spectra were recorded with a Jeol JIR-6500W spectrophotometer. The mass spectra were taken on Jeol JMS-DX 300 system. The elemental analyses were performed for C, H, N, and results were within ±0.4% of the theoretical values. Silica-gel plates (Merck F254) and silica gel 60 (Merck, 70-230 mesh) were used for analytical and column chromatography, respectively.

Table II. Biological activity of example compounds.

Compound	*Antibicuculline ED_{50} (mg/kg, mice, po)*	*Anticonflict activity MED (mg/kg, rats, po)*	*Rotarod ED_{50} (mg/kg, rats, po)*
14c	0.2	2.5	1.7
17c	1.4	25	> 300
1	0.4	10	8.8

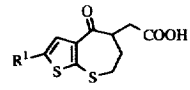
*4-Oxo-4,5,6,7-tetrahydrothieno[2,3-b]thiepin-5-acetic acids **9i–iv***
A typical example is given to represent the general procedure.

*4-(2-Thienylthio)butyric acid **4i** [27].* Under nitrogen, *n*-butyllithium (1.6 M in hexane, 250 ml, 0.4 mol) was added dropwise to a solution of thiophene (32 g, 0.38 mol) in tetrahydrofuran (500 ml) at –20°C. The solution was stirred at –20°C for an additional 0.5 h, and powdered sulfur (12.8 g, 0.4 mol) was added portionwise below –20°C. The solution was stirred for 0.5 h, and ethyl 4-bromobutyrate (77.9 g, 0.4 mol) was added to the stirred mixture. The solution was kept at 0°C for 1 h and then was brought to room temperature and stirred overnight. Water was added; the layers were separated. The aqueous layer was extracted with ethyl acetate, and the combined ethyl acetate layer and extracts were washed with brine, dried over $MgSO_4$, and evaporated. The residue was dissolved in EtOH (250 ml). A solution of KOH (30 g, 0.54 mol) in water (250 ml) was added, the mixture stirred for 2 h at room temperature. The solution was made acid and extracted with chloroform, and washed with brine, and concentrated *in vacuo*. The residue was chromatographed on silica-gel column to give **4i** (51 g, 66%); ^{1}H-NMR ($CDCl_3$) δ: 1.92 (2H, tt, *J* = 7.3, 7.3 Hz, $SCH_2CH_2CH_2$), 2.52 (2H, t, *J* = 7.3 Hz), 2.84 (2H, t, *J* = 7.3 Hz), 6.97 (1H, dd, *J* = 4.0, 5.3 Hz, ArH), 7.12 (1H, d, *J* = 4.0 Hz, ArH), 7.34 (1H, d, *J* = 5.3 Hz, ArH); IR (KBr) cm^{-1}: 1705 (C=O); MS *m/z* 202 (M^+); anal $C_8H_{10}O_2S_2$ (C, H, N).

*6,7-Dihydro-5H-thieno[2,3-b]thiepin-4-one **5i**.* To a solution of **4i** (130 g, 0.64 mol) in toluene (2.0 l) was added with stirring celite (250 g) and phosphorus pentoxide (182 g, 1.28 mol). The mixture was refluxed for 2 h and then filtered. The filtrate was washed with 2% $NaHCO_3$, dried over $MgSO_4$, and concentrated *in vacuo* to give **5i** (70 g, 59%). Recrystallization from hexane/isopropylether gave colorless crystals, mp 55–57°C (reference 12, mp 53–54°C); ^{1}H-NMR ($CDCl_3$) δ: 2.26 (2H, tt, *J* = 6.6, 6.6 Hz, $SCH_2CH_2CH_2$), 3.02 (2H, t, *J* = 6.6 Hz), 3.06 (2H, t, *J* = 6.6 Hz), 7.08 (1H, d, *J* = 5.9 Hz, ArH), 7.42 (1H, d, *J* = 5.9 Hz, ArH); IR (KBr) cm^{-1}: 1655 (C=O); MS *m/z* 184 (M^+); anal $C_8H_8OS_2$ (C, H, N).

*5-Trimethylammoniomethyl-6,7-dihydro-5H-thieno[2,3-b]thiepin-4-one iodide **7i**.* A solution of dimethylamine hydrochloride (69.7 g, 0.85 mol) in 37% HCHO (69.3 g, 0.85 mol)

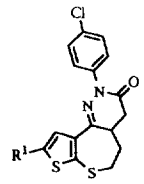
Table III. Physicochemical data for 4-oxo-4,5,6,7-tetrahydrothieno[2,3-*b*]thiepin-5-acetic acids.



Compound	*R*[1][a]	*Yield*[b] *(%)*	*Mp (°C)*	*Recrystallization solvent*	*Formula*
9i	H	18	151–152	EtOH/H_2O	$C_{10}H_{10}O_3S_2$
9ii	Me	25	183–186	EtOH/H_2O	$C_{11}H_{12}O_3S_2$
9iii	Et	28	180–182	EtOH/H_2O	$C_{12}H_{14}O_3S_2$
9iv	Pr	31	177–179	$CHCl_3$	$C_{13}H_{16}O_3S_2$

[a]Pr, *n*-propyl; [b]yield from corresponding thiophenes.

Table IV. Physicochemical data for 2-(4-chlorophenyl)-4,4a,5,6-tetrahydrothieno[2',3':2,3]thiepino[4,5-*c*]pyridazin-3(2*H*)-ones.



Compound	*R*[1][a]	*Yield*[b] *(%)*	*Mp (°C)*	*Recrystallization solvent*	*Formula*
11i	H	83	134–135	EtOH/$CHCl_3$	$C_{16}H_{13}ClN_2OS_2$
11ii	Me	81	153–154	MeOH/$CHCl_3$	$C_{17}H_{15}ClN_2OS_2$
11iii	Et	75	101–104	EtOH/$CHCl_3$	$C_{18}H_{17}ClN_2OS_2$
11iv	Pr	80	Oil	–	$C_{19}H_{19}ClN_2OS_2$

[a]Pr, *n*-propyl; [b]isolated yield.

was stirred at room temperature for 0.5 h. Acetic anhydride (270 ml) was added dropwise at 70–90°C. After being stirred for 0.5 h, **5i** (105 g, 0.57 mol) was added to the mixture at 70°C. The mixture was stirred at 70–75 °C for 3 h. After cooling, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in chilled water, neutralized with 28% NH_4OH and extracted by $CHCl_3$. The extract was washed with water, dried over $MgSO_4$, and evaporated below 40°C. The residue was dissolved in acetone (500 ml), and iodomethane (133 g, 0.93 mol) was added to the resulting solution below 5°C in an ice bath. The mixture was allowed to stand at room temperature for 3 h, and then the crystals formed were collected by filtration and washed with acetone to give **7i** (145 g, 66%). Recrystallization from EtOH/H_2O gave colorless crystals, mp 190–192°C; ^{1}H-NMR ($CDCl_3$) δ: 1.94–2.06 (1H, m), 2.41–2.54 (1H, m), 2.68–2.82 (1H, m), 3.06 (9H, s), 3.42–3.67 (2H, m), 3.80–3.90 (1H, m), 4.28–4.38 (1H, m), 7.36 (1H, d, *J* = 5.3 Hz, ArH), 7.45 (1H, d, *J* = 5.3 Hz, ArH); IR (KBr) cm^{-1}: 1670 (C=O); anal $C_{12}H_{18}INOS_2$ (C, H, N).

4-Oxo-4,5,6,7-tetrahydrothieno[2,3-b]thiepin-5-acetonitrile ***8i***. To a solution of **7i** (145 g, 0.38 mol) in methanol (500 ml) was added a solution of KCN (60 g, 0.92 mol) in water (150 ml) dropwise at room temperature. The solution was stirred at room temperature for 1 h and poured into ice-water. The resulting mixture was extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. After the addition of isopropyl ether to the residue, the crystals formed were collected by filtration (75 g, 89%). Recrystallization from EtOH gave **8i** as colorless crystals, mp 68–70°C; ^{1}H-NMR ($CDCl_3$) δ: 1.99–2.11 (1H, m), 2.53–2.90 (4H, m), 3.31–3.40 (1H, m), 3.71–3.76 (1H, m), 7.12 (1H, d, *J* = 5.3 Hz, ArH), 7.46 (1H, d, *J* = 5.3 Hz, ArH); IR (KBr) cm^{-1}: 2250 (CN), 1655 (C=O); MS *m/z* 223 (M^+); anal $C_{10}H_9NOS_2$ (C, H, N).

4-Oxo-4,5,6,7-tetrahydrothieno[2,3-b]thiepin-5-acetic acid ***9i***. To a solution of conc HCl (300 ml) and acetic acid (300 ml) was added **8i** (75 g, 0.34 mol). The solution was refluxed for 4 h, and poured into ice-water. The precipitate was collected by filtration, washed with water, and recrystallized from EtOH/H_2O to give **9i** (63.8 g, 78%) as colorless needles, mp 151–152°C; ^{1}H-NMR ($CDCl_3$) δ: 1.92–2.05 (1H, m), 2.27–2.42 (1H, m), 2.53 (1H, dd, *J* = 17.2, 4.6 Hz), 2.73–2.85 (1H, m), 3.08 (1H, dd, *J* = 17.2, 8.6 Hz), 3.24–3.33 (1H, m), 3.75–3.86 (1H, m), 7.08 (1H, d, *J* = 5.3 Hz, ArH), 7.41 (1H, d, *J* = 5.3 Hz, ArH), 8.70 (1H, brs, COOH); IR (KBr) cm^{-1}: 1705

(COOH), 1660 (C=O); MS *m/z* 242 (M^+); anal $C_{10}H_{10}O_3S_2$ (C, H, N).

The other compounds (**9ii–iv**) in table III were prepared in a similar manner.

*2-(4-Chlorophenyl)-4,4a,5,6-tetrahydrothieno[2',3':2,3]thiepino[4,5-c]pyridazin-3(2H)-ones **11i–iv***

A typical example is given to represent the general procedure.

*2-(4-Chlorophenyl)-4,4a,5,6-tetrahydrothieno[2',3':2,3]thiepino[4,5-c]pyridazin-3(2H)-one **11i**.* A mixture of **9i** (58.7 g, 0.24 mol), sodium acetate (24 g, 0.29 mol), and 4-chlorophenylhydrazine halfsulfate (55.8 g, 0.29 mol) in EtOH (800 ml) was refluxed for 15 h. After evaporation of the solvent, the residue was dissolved in acetic acid (800 ml). The mixture was refluxed for 3 h, poured into ice-water, and extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residual solid was recrystallized with EtOH/$CHCl_3$ give **11i** (69.5 g, 83%) as a pale yellow powder, mp 134–135°C; ^{1}H-NMR ($CDCl_3$) δ: 2.10–2.19 (1H, m), 2.25–2.32 (1H, m), 2.64 (1H, dd, *J* = 16.5, 2.0 Hz), 2.92–3.06 (2H, m), 3.12–3.21 (1H, m), 3.76–3.83 (1H, m), 7.13 (1H, d, *J*= 5.3 Hz, H-10), 7.33 (1H, d, *J* = 5.3 Hz, H-9), 7.36 (2H, d, *J* = 8.6 Hz, ArH), 7.55 (2H, d, *J* = 8.6 Hz, ArH); IR (KBr) cm^{-1}: 1695 (C=O); MS *m/z*: 348 (M^+); anal $C_{16}H_{13}ClN_2OS_2$ (C, H, N).

The other compounds (**11ii–iv**) in table IV were prepared in a similar manner.

*2-(4-Chlorophenyl)-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-c]pyridazin-3(2H)-ones **12a–15a***

A typical example is given to represent the general procedure.

*2-(4-Chlorophenyl)-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-c]pyridazin-3(2H)-one **12a**.* To a solution of **11i** (69 g, 0.20 mol) in acetic acid containing 15% HBr (400 ml) was added dropwise dimethylsulfoxide (14.8 ml, 0.21 mol) at room temperature. The reaction mixture was stirred for 0.5 h, and poured into ice-water, and extracted with $CHCl_3$. The extract was washed with water and 2% $NaHCO_3$, dried over $MgSO_4$, and concentrated *in vacuo*. The residual solid was recrystallized with ethyl acetate/isopropyl ether to give **12a** (56 g, 82%) as a pale yellow powder, mp 140–142°C; ^{1}H-NMR ($CDCl_3$) δ: 2.85 (2H, t, *J* = 6.6 Hz, SCH_2CH_2), 3.34 (2H, t, *J* = 6.6 Hz, SCH_2CH_2), 6.95 (1H, s, C=CHCO), 7.30 (1H, d, *J* = 5.3 Hz, H-10), 7.33 (1H, d, *J* = 5.3 Hz, H-9), 7.44 (2H, d, *J* = 8.6 Hz, ArH), 7.67 (2H, d, *J* = 8.6 Hz, ArH); IR (KBr) cm^{-1}: 1680 (C=O); MS *m/z*: 346 (M^+); anal $C_{16}H_{11}ClN_2OS_2$ (C, H, N).

The other compounds (**13a–15a**) in table I were prepared in a similar manner.

*2-(4-Chlorophenyl)-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-c]pyridazin-3(2H)-one 7-oxides **12b–20b***

A typical example is given to represent the general procedure.

*2-(4-Chlorophenyl)-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-c]pyridazin-3(2H)-one 7-oxide **12b**.* To a stirred solution of **12a** (0.55 g, 1.6 mmol) in acetic acid (20 ml) was added dropwise 30% H_2O_2 (0.5 g, 4.4 mmol) below 10°C. The mixture was stirred at room temperature for 6 h, poured into ice-water, and extracted with $CHCl_3$. The extract was washed with 2% $NaHSO_3$ and water, dried over $MgSO_4$, and concentrated *in vacuo*. The residual solid was recrystallized with EtOH/$CHCl_3$ to give **12b** (0.4 g, 70%) as a colorless powder, mp 184–186°C (dec); ^{1}H-NMR ($CDCl_3$) δ: 2.98 (1H, ddd, *J* = 14.5, 7.9, 4.6 Hz, SCH_2CH_2), 3.36 (1H, ddd, *J* = 14.5, 7.9, 4.0 Hz, SCH_2CH_2), 3.50 (1H, ddd, *J* = 13.9, 7.9, 4.0 Hz, SCH_2CH_2), 3.67 (1H, ddd, *J* = 13.9, 7.9, 4.6 Hz, SCH_2CH_2), 6.98 (1H, s, C=CHCO), 7.44 (2H, d, *J* = 8.6 Hz, ArH), 7.56 (1H, d, *J* = 5.3 Hz, H-10), 7.64 (2H, d, *J* = 8.6 Hz, ArH), 7.68 (1H, d, *J* = 5.3 Hz, H-9); IR (KBr) cm^{-1}: 1675 (C=O); MS *m/z*: 362 (M^+); anal $C_{16}H_{11}ClN_2O_2S_2$ (C, H, N).

The other compounds (**13b–20b**) in table I were prepared in a similar manner.

*2-(4-Chlorophenyl)-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-c]pyridazin-3(2H)-one 7,7-dioxides **12c–20c***

A typical example is given to represent the general procedure.

*2-(4-Chlorophenyl)-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-c]pyridazin-3(2H)-one 7,7-dioxide **12c**.* To a stirred solution of **12a** (1.0 g, 2.9 mmol) in CH_2Cl_2 (20 ml) was added 80% *m*-chloroperbenzoic acid (1.3 g, 6.0 mmol) below 10°C. The mixture was stirred at room temperature for 5 h. The resulting solution was washed with 2% $NaHCO_3$, dried over $MgSO_4$, and concentrated *in vacuo*. The residual solid was recrystallized with EtOH/$CHCl_3$ to give **12c** (0.9 g, 82%) as colorless needles, mp 277–279°C; ^{1}H-NMR ($CDCl_3$) δ: 3.04 (2H, t, *J* = 6.6 Hz, SCH_2CH_2), 4.02 (2H, t, *J* = 6.6 Hz, SCH_2CH_2), 7.24 (1H, s, C=CHCO), 7.55 (1H, d, *J* = 5.3 Hz, H-10), 7.58 (2H, d, *J* = 8.6 Hz, ArH), 7.72 (2H, d, *J* = 8.6 Hz, ArH), 8.15 (1H, d, *J* = 5.3 Hz, H-9); IR (KBr) cm^{-1}: 1675 (C=O); MS *m/z*: 378 (M^+); anal $C_{16}H_{11}ClN_2O_3S_2$ (C, H, N).

The other compounds (**13c–20c**) in table I were prepared in a similar manner.

*9-Bromo-2-(4-chlorophenyl)-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-c]pyridazin-3(2H)-one **17a***

To a stirred solution of **12a** (2.0 g, 5.8 mmol) in acetic acid (80 ml) was added bromine (1.0 g, 6.3 mmol) at 10°C. The reaction mixture was kept at room temperature for 2 h, and was then poured into ice-water. The precipitate was collected by filtration, washed with H_2O, recrystallized from EtOH/$CHCl_3$ to give **17a** (1.9 g, 77%) as colorless needles, mp 148–151°C; ^{1}H-NMR ($CDCl_3$) δ: 2.87 (2H, t, *J* = 6.6 Hz, SCH_2CH_2), 3.35 (2H, t, *J* = 6.6 Hz, SCH_2CH_2), 6.94 (1H, s, C=CHCO), 7.27 (1H, s, H-10), 7.44 (2H, d, *J* = 9.2 Hz, ArH), 7.65 (2H, d, *J* = 9.2 Hz, ArH); IR (KBr) cm^{-1}: 1680 (C=O); MS *m/z*: 426 (M^+); anal $C_{16}H_{10}BrClN_2OS_2$ (C, H, N).

*9-Acyl-2-(4-chlorophenyl)-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-c]pyridazin-3(2H)-ones **18a**, **22a**, **23a***

A typical example is given to represent the general procedure.

*9-Butyryl-2-(4-chlorophenyl)-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-c]pyridazin-3(2H)-one **23a**.* To an ice-cooled suspension of $AlCl_3$ (3.8 g, 29 mmol) in CH_2Cl_2 (50 ml) was added butyrylchloride (0.9 g, 8.4 mmol) and the mixture was stirred at 0–10°C for 0.5 h. After addition of **12a** (2.0 g, 5.8 mmol), the mixture was refluxed for 1 h and then poured into ice-water. The resulting mixture was extracted with CH_2Cl_2. The extract was washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residual solid was recrystallized with EtOH/$CHCl_3$ to give **23a** (1.5 g, 62%) as colorless needles, mp 152–153°C; ^{1}H-NMR ($CDCl_3$) δ: 0.99 (3H, t, *J* = 7.3 Hz, $CH_3CH_2CH_2CO$), 1.77 (2H, tq, *J* = 7.3, 7.3 Hz, $CH_3CH_2CH_2CO$), 2.84 (2H, tt, *J* = 7.3, 7.3 Hz, $CH_3CH_2CH_2CO$), 2.93 (2H, t, *J* = 6.6 Hz, SCH_2CH_2), 3.42 (2H, t, *J* = 6.6 Hz, SCH_2CH_2), 6.96 (1H, s, C=CHCO), 7.46 (2H, d, *J* = 8.6 Hz, ArH), 7.66 (2H, d, *J* = 8.6 Hz, ArH), 7.83 (1H, s, H-10); IR (KBr) cm^{-1}: 1650 (C=O), 1670 (C=O); MS *m/z*: 388 (M^+); anal $C_{20}H_{17}ClN_2O_2S_2$ (C, H, N).

The other compounds (**18a** and **22a**) were prepared in a similar manner.

9-Butyl-2-(4-chlorophenyl)-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-c]pyridazin-3(2H)-one ***16a***
To a stirred solution of **23a** (1.0 g, 2.4 mmol) in trifluoroacetic acid (15 ml) was added triethylsilane (0.6 g, 5.2 mmol) at room temperature. The reaction mixture was stirred at room temperature for 15 h, poured into ice-water, and extracted with $CHCl_3$. The extract was washed with 2% $NaHCO_3$, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on a silica gel using $CHCl_3$ as an eluent to give **16a** (0.6 g, 62%), which was recrystallized from EtOH to afford a colorless powder, mp 125–130°C; ^{1}H-NMR ($CDCl_3$) δ: 0.94 (3H, t, J = 7.3 Hz, $CH_3CH_2CH_2CH_2$), 1.40 (2H, tq, J = 7.3, 7.3 Hz, $CH_3CH_2CH_2CH_2$), 1.67 (2H, tt, J = 7.3, 7.3 Hz, $CH_3CH_2CH_2CH_2$), 2.78 (2H, t, J = 7.3, $CH_3CH_2CH_2CH_2$), 2.84 (2H, t, J = 6.6 Hz, SCH_2CH_2), 3.31 (2H, t, J = 6.6 Hz, SCH_2CH_2), 6.93 (1H, s, C=CHCO), 6.98 (1H, s, H-10), 7.44 (2H, d, J = 8.6 Hz, ArH), 7.66 (2H, d, J = 8.6 Hz, ArH); IR (KBr) cm^{-1}: 1680 (C=O); MS *m/z*: 402 (M^+); anal $C_{20}H_{19}ClN_2OS_2$ (C, H, N).

2-(4-Chlorophenyl)-9-nitro-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-c]pyridazin-3(2H)-one ***19a***
To a stirred solution of **12a** (2.5 g, 7.2 mmol) in acetic acid (100 ml) and acetic anhydride (1 ml) was added fuming HNO_3 (0.6 ml) at 10°C. The reaction mixture was stirred for 0.5 h, followed by poured into ice-water. The resulting mixture was extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on a silica gel using $CHCl_3$ as an eluent to give **19a** (1.3 g, 40%), which was recrystallized from EtOH/$CHCl_3$ to afford a yellow powder, mp 181–182°C; ^{1}H-NMR ($CDCl_3$) δ: 2.98 (2H, t, J = 6.6 Hz, SCH_2CH_2), 3.48 (2H, t, J = 6.6 Hz, SCH_2CH_2), 6.97 (1H, s, C=CHCO), 7.45 (2H, d, J = 8.6 Hz, ArH), 7.64 (2H, d, J = 8.6 Hz, ArH). 8.09 (1H, s, H-10); IR (KBr) cm^{-1}: 1670 (C=O); MS *m/z*: 391 (M^+); anal $C_{16}H_{10}ClN_3O_3S_2$ (C, H, N).

2-(4-Chlorophenyl)-9-formyl-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-c]pyridazin-3(2H)-one ***21a***
A solution of phosphorus oxychloride (3.5 g, 22.8 mmol) and *N*-methylformanilide (3.1 g, 22.8 mmol) was stirred for 0.5 h at room temperature. To the solution was added **12a** (4.0 g, 11.5 mmol), the mixture was stirred for 8 h at room temperature and was then poured into water. The resulting mixture was extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel using $CHCl_3$ as an eluent to give **21a** (3.5 g, 81%), which was recrystallized from EtOH/$CHCl_3$ to afford a colorless powder, mp 182–183°C; ^{1}H-NMR ($CDCl_3$) δ: 2.97 (2H, t, J = 6.6 Hz, SCH_2CH_2), 3.45 (2H, t, J = 6.6 Hz, SCH_2CH_2), 6.96 (1H, s, C=CHCO), 7.45 (2H, d, J = 8.6 Hz, ArH), 7.65 (2H, d, J = 8.6 Hz, ArH). 7.95 (1H, s, H-10), 9.82 (1H, s, CHO). IR (KBr) cm^{-1}: 1665 (C=O); MS *m/z*: 374 (M^+); anal $C_{17}H_{11}ClN_2O_2S_2$ (C, H, N).

2-(4-Chlorophenyl)-9-cyano-5,6-dihydrothieno[2',3':2,3]thiepino[4,5-c]pyridazin-3(2H)-one ***20a***
To a stirred solution of **21a** (1.0 g, 2.7 mmol) in EtOH (50 ml) was added hydroxylamine hydrochloride (0.3 g, 4.3 mmol) and $NaHCO_3$ (0.36 g, 4.3 mmol) at room temperature. The reaction mixture was refluxed for 5 h and was then poured into ice-water. The resulting mixture was extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was dissolved in tetrahydrofuran (10 ml), and triethylamine (0.8 g, 7.9 mmol) and trifluoroacetic anhydride (0.56 g, 2.7 mmol) were added to the resulting solution below 5°C in an ice bath. The reaction mixture was stirred for 1 h, and was then poured into ice-water. The resulting mixture was extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residual solid was recrystallized with EtOH/$CHCl_3$ to give **20a** (0.6 g, 60%) as colorless needles, mp 128–129°C (dec); ^{1}H-NMR ($CDCl_3$) δ: 2.92 (2H, t, J = 6.6 Hz, SCH_2CH_2), 3.44 (2H, t, J = 6.6 Hz, SCH_2CH_2), 6.98 (1H, s, C=CHCO), 7.45 (2H, d, J = 8.6 Hz, ArH), 7.63 (2H, d, J = 8.6 Hz, ArH). 7.79 (1H, s, H-10); IR (KBr) cm^{-1}: 2225 (CN), 1680 (C=O); MS *m/z*: 371 (M^+); anal $C_{17}H_{10}ClN_3OS_2$ (C, H, N).

2-(4-Chlorophenyl)-10-propionyl-5,6-dihydro[1]benzothiepino[5,4-c]pyridazin-3(2H)-one ***24a***
To an ice-cooled suspension of $AlCl_3$ (5.9 g, 44 mmol) in 1,2-dichloroethane (100 ml) was added propionylchloride (1.2 g, 13 mmol) and the mixture was stirred at 0–10°C for 0.5 h. After addition of **2a** (3.0 g, 8.8 mmol), the mixture was stirred for 2 h at 60°C and then poured into ice-water. The resulting mixture was extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on a silica gel using $CHCl_3$ as an eluent to give **24a** (1.8 g, 52%), which was recrystallized from EtOH/$CHCl_3$ to afford colorless needles, mp 197–198°C; ^{1}H-NMR ($CDCl_3$) δ: 1.23 (3H, t, J = 7.3 Hz, CH_3CH_2CO), 2.75 (2H, t, J = 6.6 Hz, SCH_2CH_2), 3.04 (2H, q, J = 7.3 Hz, CH_3CH_2CO), 3.30 (2H, t, J = 6.6 Hz, SCH_2CH_2), 6.92 (1H, s, C=CHCO), 7.45 (2H, d, J = 9.2 Hz, ArH), 7.68 (2H, d, J = 9.2 Hz, ArH), 7.73 (1H, d, J = 7.9 Hz, ArH), 7.98 (1H, dd, J = 2.0, 7.9 Hz, ArH), 8.13 (1H, d, J = 2.0 Hz, ArH); IR (KBr) cm^{-1}: 1680 (C=O); MS *m/z*: 396 (M^+); anal $C_{21}H_{17}ClN_2O_2S$ (C, H, N).

2-(4-Chlorophenyl)-10-propyl-5,6-dihydro[1]benzothiepino-[5,4-c]pyridazin-3(2H)-one ***25a***
This compound was synthesized from **24a** in the same way as described for the preparation of **16a**, mp 178–179°C; ^{1}H-NMR ($CDCl_3$) δ: 0.96 (3H, t, J = 7.3 Hz, $CH_3CH_2CH_2$), 1.66 (2H, tq, J = 7.3, 7.3 Hz, $CH_3CH_2CH_2$), 2.63 (2H, t, J = 7.3 Hz, $CH_3CH_2CH_2$), 2.72 (2H, t, J = 6.6 Hz, SCH_2CH_2), 3.22 (2H, t, J = 6.6 Hz, SCH_2CH_2), 6.89 (1H, s, C=CHCO), 7.23 (1H, dd, J = 2.0, 7.9 Hz, ArH), 7.40 (1H, d, J = 2.0 Hz, ArH), 7.44 (2H, d, J = 8.6 Hz, ArH), 7.53 (1H, d, J = 7.9 Hz, ArH), 7.68 (2H, d, J = 8.6 Hz, ArH); IR (KBr) cm^{-1}: 1670 (C=O); MS *m/z*: 382 (M^+); anal $C_{21}H_{19}ClN_2OS$ (C, H, N).

2-(4-Chlorophenyl)-10-propyl-5,6-dihydro[1]benzothiepino-[5,4-c]pyridazin-3(2H)-one 7-oxide ***25b***
This compound was synthesized from **25a** in the same way as described for the preparation of **12b**, mp 201–202°C; ^{1}H-NMR ($CDCl_3$) δ: 0.98 (3H, t, J = 7.3 Hz, $CH_3CH_2CH_2$), 1.70 (2H, tq, J = 7.3, 7.3 Hz, $CH_3CH_2CH_2$), 2.68–2.90 (4H, m), 3.07–3.17 (1H, m), 3.85–3.97 (1H, m), 6.96 (1H, s, C=CHCO), 7.44 (2H, d, J = 8.6 Hz, ArH), 7.46 (1H, d, J = 2.0 Hz, ArH), 7.53 (1H, dd, J = 2.0, 7.9 Hz, ArH), 7.67 (2H, d, J = 8.6 Hz, ArH), 7.82 (1H, d, J = 7.9 Hz, ArH); IR (KBr) cm^{-1}: 1675 (C=O); MS *mz*: 398 (M^+); anal $C_{21}H_{19}ClN_2O_2S$ (C, H, N).

Pharmacology

Benzodiazepine receptor binding assay
Preparation of a synaptosome fraction and [^{3}H]diazepam binding studies were carried out according to the method of

Möhler and Okada [2]. Crude synaptosomal membranes were suspended in a 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl and 5 mM KCl. The reaction was started by the addition of a 900 µl aliquot of crude synaptosomal membranes to 100 µl solution containing [^{3}H]diazepam (final concentration was 2 nM) and a known concentration of the test compounds. After the mixture was incubated for 20 min at 0°C, the binding was stopped by addition of 3 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl and 5 mM KCl. The samples were then filtered under vacuum through Whatman GF/B filters and immediately washed 4 times with 3 ml of ice-cold buffer. The radioactivity on the filters was measured by a liquid scintillation counter Binding in the presence of 1 µM unlabelled diazepam was defined as non-specific binding. Specific binding was defined as the difference between the total binding and the non-specific binding. The K_i values were determined by the relationship $K_i = IC_{50}/(1 + c/K_d)$, where IC_{50} was the concentration of the test compounds which caused a 50% reduction of the specific binding *vs* the control, c was the concentration of [^{3}H]diazepam (2 nM), and K_d was the dissociation constant determined by Scatchard's plot. The K_i values are means ± SE of at least three determinations.

GABA-induced chloride current in frog sensory neuron
The experiment was carried out according to the published method [16]. Bull frog dorsal root ganglion neurons were isolated. Isolated neuronal cell bodies were perfused internally and externally by a suction pipette technique with respective test solutions for recording the chloride current. The external solution contained Tris-HCl 89, CsCl 2, $MgCl_2$ 5, TEA-Cl 25, glucose 5, and HEPES 10 (each unit, mM) and was adjusted at pH 7.4 with appropriate Tris-base. The internal solution contained CsCl 95, Cs-aspartate 10, TEA-Cl 25, HEPES 10 and EGTA 2.5 (each unit, mM) and was adjusted to pH 7.2. Neurons were voltage-clamped at a holding membrane potential of –50 mV with a single electrode. Test compounds were applied by using a concentration–clamp technique. Augmentative action of test compound on the GABA response was examined on I_{Cl} induced by 3×10^{-6} M GABA. The results were presented as relative values of peak I_{Cl} elicited by 3×10^{-6} M GABA alone. The relative I_{Cl} values represented the mean of at least three determinations and the SE for these values were generally ≤ 10% of the mean.

Anticonvulsant test (antibicuculline test)
The experiment was a modification of the method of Lippa and Regan [25]. Groups of 7–14 ddY male mice were challenged with bicuculline (0.6 mg/kg iv) 1 h after the oral administration of the test compounds. The ED_{50} values were calculated by the probit method as the dose which prevented tonic extension in half of the animals.

Anticonflict test (water-lick test)
The experiment was carried out by a modification of the method of Vogel *et al* [26]. Groups of 10–14 male Wister rats were deprived of water for 72 h, and were placed in the test chamber and allowed to drink from the water spout. Licking was automatically accompanied by a 100 V, 0.2–0.3 mA, 300 ms electrical stimulus across the grid floor and spout every 20 licks. After the rat received the first electrical stimulus, the number of stimuli was recorded automatically during the subsequent 3 min test. The test compounds were administered orally 1 h before the test. The MED was defined as the lowest dose to produce a statistically significant increase in the punished responses compared with control.

Rotarod test
Groups of 10–14 male Wister rats were used. The rats were gently placed on the rod (5 cm in diameter rotating at 5 rpm) 1h after oral administration of the test compounds. The ED_{50} value was calculated by the probit method as the dose which caused half of the animals to drop from the rotarod within 1 min.

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