Eur J Med Chem (1997) 32, 607–615

Synthesis and evaluation of novel 2-aryl-2,5,6,7-tetrahydro-3*H*-thieno[2',3':6,7]cyclohepta[1,2-*c*]pyridazin-3-ones and 2-aryl-5,6-dihydrothieno[2,3-*h*]cinnolin-3(2*H*)-ones as anxiolytics

H Tanaka*, S Kirihara, H Yasumatsu, T Yakushiji, T Nakao

Research Laboratories, Yoshitomi Pharmaceutical Industries Ltd, 7–25, Koyata 3-chome, Iruma-shi, Saitama 358, Japan

(Received 8 November 1996; accepted 6 February 1997)

Summary — A series of 2-aryl-2,5,6,7-tetrahydro-3*H*-thieno[2',3':6,7]cyclohepta[1,2-*c*]pyridazin-3-ones and 2-aryl-5,6-dihydrothieno[2,3-*h*]cinnolin-3(2*H*)-ones were synthesized and evaluated for their affinity to benzodiazepine receptors (BZRs) in the excised brain of rats and also for their intrinsic efficacy in augmentation of the γ-aminobutyric acid-induced chloride currents in the dissociated sensory neurons of frogs. The synthesized compounds showed a high affinity to BZRs. In these compounds, the substituents at the 2-position and at either the 8- or the 9-position and the ring size of the condensed ring affected the biological activity of the compounds. Thus, an introduction of 4-methyl- or 4-chloro-substituted phenyl ring into the 2-position, an introduction of methyl or ethyl into either the 8- or the 9-position, and an expansion of the 6-membered condensed ring to a 7-membered ring brought about a continuous shift of compounds from inverse to full agonists. Among the synthesized compounds, 8-(1-hydroxyethyl)-2-(4-methylphenyl)-5,6-dihydrothieno[2,3-*h*]cinnolin-3(2*H*)-one, which can be classified as a BZR partial agonist, was found to exhibit an anxioselective feature.

benzodiazepine receptor / γ-aminobutyric acid / chloride current / anticonvulsive action / partial agonist

Introduction

Since the discovery of specific binding sites for benzodiazepines in mammalian brain [1, 2], the mechanism of action of benzodiazepine has been rapidly clarified. The pharmacological effects of the benzodiazepines result from their affinity for a specific binding site on the $GABA_A$ receptor, known as the benzodiazepine receptor (BZR). The heterogeneity of the $GABA_A$ receptor population has been confirmed by molecular biology studies which have identified several different receptor subtypes composed of multiple subtypes (α, β, γ, δ, and ρ) [3]. The $GABA_A$ receptor is associated with a transmembrane chloride ion channel, and this macromolecular complex has several major binding domains including benzodiazepine sites [4]. The BZR ligands can be classified as ranging from inverse to full agonists according to their modulatory effect on GABAergic transmission. Because BZR full agonists exert a maximal response at the $GABA_A$ receptor they frequently exhibit muscle relaxation, ethanol potentiation and physical dependency as side effects [5]. The partial agonists are of interest due to their lower but positive intrinsic efficacy, which should be intermediary between that of full agonists (represented by diazepam) and zero intrinsic efficacy (antagonists such as flumazenil). The partial agonists would be sufficient to maintain the anxiolytic and anticonvulsant responses but insufficient to induce unwanted side effects seen with conventional full agonists [6]. In a previous paper [7], we described the synthesis and analyzed the structure–activity relationship of 5,6-dihydrothieno[2',3':2,3]thiepino[4,5-*c*]pyridazin-3(2*H*)-ones (TTP; fig 1), some of which are BZR partial agonists and others full agonists. Among these compounds, R^1-substituents at the 9-position are known to play a role not only in their affinity but also in their intrinsic efficacy.

To systematically evaluate the influence of substituents at the thiophene ring on both the receptor affinity and activity profile, in this paper we have described a series of 2,5,6,7-tetrahydro-3*H*-thieno[2',3':6,7]-cyclohepta[1,2-*c*]pyridazin-3-ones (TCP), which has a methylene unit at the 7-position instead of a thioether moiety in TTP. Moreover, we synthesized a series of 5,6-dihydrothieno[2,3-*h*]cinnolin-3(2*H*)-ones (TCN) in which the thioether moiety was removed from TTP.

*Correspondence and reprints

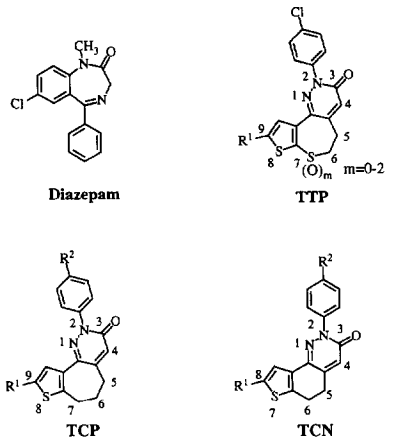


Fig 1. Chemical structures of diazepam, TTP, TCP and TCN.

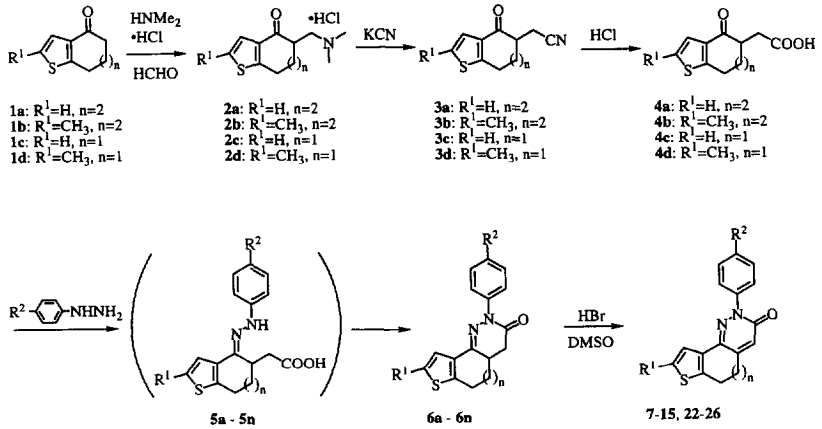
In the compounds synthesized here, we evaluated both their affinity to BZRs in the excised brain of rats, and their intrinsic efficacy regarding the GABA-induced chloride currents in the dissociated sensory neurons of frogs. These compounds were then classified into their continuous shift from inverse to full agonists.

Chemistry

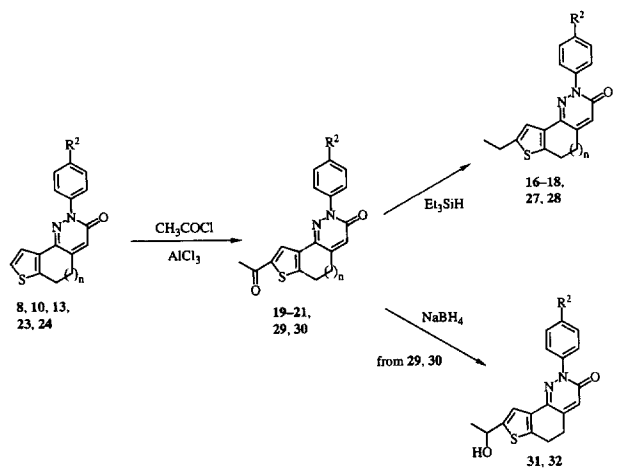
Compounds belonging to the TCP and TCN series (**7–32**) were derived from known 5,6,7,8-tetrahydrocyclohepta[*b*]thiophen-4-ones (**1a** and **1b**) [8, 9] and 6,7-dihydro-5*H*-benzo[*b*]thiophen-4-ones (**1c** and **1d**) [10, 11] in a similar manner to that described in our previous paper [7, 12]. As shown in scheme 1, the ketones **1a–1d** were converted into the nitriles **3a–3d** by way of the Mannich bases **2a–2d**, the hydrolysis of which gave the carboxylic acids **4a–4d**, respectively. The carboxylic acids **4a–4d** were cyclocondensed with arylhydrazines to give 2-aryl-2,4,4*a*,5,6,7-hexahydro-3*H*-thieno[2',3':6,7]cyclohepta[1,2-*c*]pyridazin-3-ones (**6a–6i**) and 2-aryl-4,4*a*,5,6-tetrahydrothieno-[2,3-*h*]cinnolin-3(2*H*)-ones (**6j–6n**) via intermediate hydrazones (**5a–5n**). An introduction of a double bond to the site between the 4- and 4a-position of **6a–6n** by the previously reported method (DMSO/HBr) [13] led to **7–15**, **22–26**. Acetyl compounds (**19–21**, **29** and **30**) and ethyl compounds (**16–18**, **27** and **28**) were prepared according to the synthesis outlined in scheme 2. Thus Friedel–Crafts reaction of **8**, **10**, **13**, **23** and **24** afforded the acetyl compounds **19–21**, **29** and **30** respectively. Reduction of **19–21**, **29** and **30** by Et_3SiH gave the ethyl compounds **16–18**, **27** and **28** respectively. On the other hand, reduction of **29** and **30** by $NaBH_4$ yielded the alcohol compounds **31** and **32** respectively.

Pharmacological results and discussion

Compounds belonging to the TCP and TCN series were evaluated for their affinity to BZRs via an assay on their ability to displace [^{3}H]diazepam binding to the cerebral cortex of rats [1]. Here the BZR affinity of these compounds is indicated as the K_i value (nM) for competition with [^{3}H]diazepam at the binding site. Then the efficacy of the compounds towards BZRs was evaluated via a concentration-clamp technique under single-electrode voltage-clamp conditions [14]. In such an experiment, all the full agonists increase the peak amplitude of the chloride currents (I_{Cl}) induced by GABA, and the partial agonists dose-



Scheme 1. Synthetic route to TCP (n = 2) and TCN (n = 1).



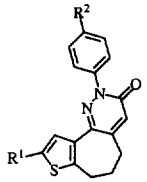
Scheme 2. Introduction of substituents at the 8- (or 9-) position (n = 1, 2).

dependently augment this GABA response with an amplitude that is significantly smaller than that of diazepam [15]. The relative I_{Cl} (r-I_{Cl}) value, which is a ratio of the I_{Cl} induced by GABA in the presence of an appropriate test compound to that induced by GABA itself, was used as a predictor of the type of activity observed in whole animal models for the BZR ligands. The structure and pharmacological data for TCP and TCN are shown in tables I and II respectively.

TCP

In 9-unsubstituted TCP (**7–13**), the R^2-substituent on the phenyl ring at the 2-position altered not only the BZR-binding affinity but also the r-I_{Cl} value. Although compound **13** in which R^2 is methoxy was the most potent in affinity to the BZRs among the 9-unsubstituted TCP, replacement of the methoxy with hydrogen, methyl or fluoro slightly reduced the affinity (**13** vs **7**, **8**, **9**). Regarding the halogen substituents at the 4-position of the phenyl ring, the rank order of BZR-binding potency was as follows: Cl > Br, F > I (**9** vs **10** vs **11** vs **12**). These results show that the substituents on the phenyl ring contribute to the binding ability of TCP to the BZRs. In contrast to the case of the TTP series [7], an introduction of methyl

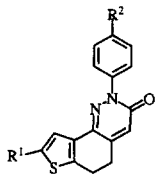
Table I. Physicochemical and pharmacological data of TCP.



Compound	*R¹*	*R²*	*Yield*[a] *(%)*	*Mp (°C)*	*Recryst solv*[b]	*Formula*	*[³H]Diazepam*[c] *K_i (nM)*	*Relative*[c] *I_{Cl}*
7	H	H	65	114–115	IPA–hexane	$C_{17}H_{14}N_2OS$	14 ± 1	0.43
8	H	CH_3	76	128–130	AcOEt–IPE	$C_{18}H_{16}N_2OS$	11 ± 3	1.19
9	H	F	96	125–127	EtOH	$C_{17}H_{13}FN_2OS$	19 ± 1	NT[d]
10	H	Cl	72	128–130	EtOH	$C_{17}H_{13}ClN_2OS$	8.8 ± 0.5	1.44
11	H	Br	65	148–150	$CHCl_3$–EtOH	$C_{17}H_{13}BrN_2OS$	16 ± 1	NT[d]
12	H	I	78	163–164	$CHCl_3$–EtOH	$C_{17}H_{13}IN_2OS$	27 ± 2	NT[d]
13	H	CH_3O	80	138–140	EtOH	$C_{18}H_{16}N_2O_2S$	3.5 ± 0.9	0.94
14	CH_3	Cl	74	150–151	EtOH	$C_{18}H_{15}ClN_2OS$	14 ± 4	2.70
15	CH_3	CH_3O	73	162–164	EtOH	$C_{19}H_{18}N_2O_2S$	1.8 ± 0.2	1.57
16	CH_3CH_2	CH_3	77	109–111	IPE–hexane	$C_{20}H_{20}N_2OS$	4.6 ± 0.4	2.74
17	CH_3CH_2	Cl	59	116–119	EtOH–hexane	$C_{19}H_{17}ClN_2OS$	4.8 ± 0.5	3.43
18	CH_3CH_2	CH_3O	52	155–157	AcOEt	$C_{20}H_{20}N_2O_2S$	1.3 ± 0.2	2.34
19	CH_3CO	CH_3	80	186–188	$CHCl_3$–EtOH	$C_{20}H_{18}N_2O_2S$	30 ± 4	NT[d]
20	CH_3CO	Cl	59	164–166	EtOH	$C_{19}H_{15}ClN_2O_2S$	12 ± 2	1.65
21	CH_3CO	CH_3O	83	197–198	$CHCl_3$–EtOH	$C_{20}H_{18}N_2O_3S$	7.3 ± 0.2	1.15
Diazepam							5.3 ± 1.0	2.54

[a]Isolated yield; [b]IPA, isopropyl alcohol; AcOEt, ethyl acetate; IPE, isopropyl ether; [c]values represent the average of three or more experiments. See *Experimental protocols* for details; [d]NT, not tested.

Table II. Physicochemical and pharmacological data of TCN.



Compound	*R¹*	*R²*	*Yield*[a] *(%)*	*Mp (°C)*	*Recryst solv*[b]	*Formula*	*[³H]Diazepam*[c] *K_i (nM)*	*Relative*[c] *I_{Cl}*
22	H	H	65	163–165	EtOH–IPE	$C_{16}H_{12}N_2OS$	76 ± 6	0.35
23	H	CH_3	62	146–148	AcOEt–EtOH	$C_{17}H_{14}N_2OS$	22 ± 2	0.98
24	H	Cl	56	158–161	$CHCl_3$–EtOH	$C_{16}H_{11}ClN_2OS$	17 ± 2	1.15
25	H	CH_3O	52	165–168	$CHCl_3$–EtOH	$C_{17}H_{14}N_2O_2S$	13 ± 1	0.51
26	CH_3	Cl	70	192–194	EtOH	$C_{17}H_{13}ClN_2OS$	12 ± 2	1.59
27	CH_3CH_2	CH_3	67	133–135	AcOEt–hexane	$C_{19}H_{18}N_2OS$	10 ± 2	1.57
28	CH_3CH_2	Cl	62	162–164	AcOEt–hexane	$C_{18}H_{15}ClN_2OS$	9.6 ± 1.2	1.47
29	CH_3CO	CH_3	69	240–242	$CHCl_3$–MeOH	$C_{19}H_{16}N_2O_2S$	9.5 ± 2.4	1.24
30	CH_3CO	Cl	84	229–231	$CHCl_3$–EtOH	$C_{18}H_{13}ClN_2O_2S$	4.9 ± 0.5	1.38
31	$CH_3CH(OH)$	CH_3	85	158–160	EtOH–IPE	$C_{19}H_{18}N_2O_2S$	6.4 ± 0.5	1.65
32	$CH_3CH(OH)$	Cl	81	146–148	$CHCl_3$–EtOH	$C_{18}H_{15}ClN_2O_2S$	3.5 ± 0.7	1.86
Diazepam							5.3 ± 1.0	2.54

[a]Isolated yield; [b]IPA, isopropyl ether; AcOEt, ethyl acetate; [c]values represent the average of three or more experiments. See *Experimental protocols* for details.

or ethyl at the 9-position slightly affected the affinity of the ligands to the BZRs (**10** vs **14** vs **17**; **13** vs **15** vs **18**). The affinity of the 9-acetyl-compounds **19–21** towards the BZRs was slightly decreased in comparison to the 9-ethyl compounds (**16–18**). On the basis of the r-I_{Cl} values, the 9-unsubstituted compounds could be classified into two groups: BZR inverse agonists (**7** and **13**) and BZR agonists (**8** and **10**). The R^2-substituents affect the r-I_{Cl} values. Furthermore, the introduction of an alkyl substituent into the 9-position of TCP increased the r-I_{Cl} value. In particular, the introduction of an ethyl resulted in a remarkable increase of the value (**8** vs **16**; **10** vs **14** vs **17**; **13** vs **15** vs **18**). The r-I_{Cl} values of **14**, **16** and **17** were higher than that of diazepam. Therefore compounds **14**, **16** and **17** should be classified as BZR full agonists. The r-I_{Cl} values of the 9-acetyl compounds (**20** and **21**) were lower than those of the ethyl compounds (**17** vs **20**; **18** vs **21**). This tendency has also been recognized in the TTP series [7].

TCN

8-Unsubstituted TCN (**22–25**) exhibited a lower affinity towards the BZRs than the corresponding 9-unsubstituted TCP (**7**, **8**, **10**, and **13**). The introduction of an alkyl, acetyl or 1-hydroxyethyl group at the 8-position slightly increased the BZR affinity of TCN. The r-I_{Cl} values of **22–25** ranged from 0.35 to 1.15, indicating the presence of a BZR inverse agonist (**22**, **25**), antagonist (**23**) and agonist (**24**). The introduction of a methyl or ethyl at the 8-position resulted in an increase of the r-I_{Cl} value (**23** vs **27**; **24** vs **26**, **28**). However, by the introduction of an alkyl this increase was smaller than in the case of TCP (**16** vs **27**; **17** vs **28**). Therefore, not only the introduction of an alkyl at the 8-position but also the expansion of a condensed ring from a 6-membered ring (TCN) to a 7-membered ring (TCP) seems necessary to bring about a high intrinsic efficacy of the BZRs. In contrast to TCP, the 8-acetyl compounds (**29** and **30**) exhibited slightly higher r-I_{Cl} values than the corresponding 8-unsubstituted compounds (**23** and **24**). Interestingly, a conversion of the acetyl group in **29** and **30** into a 1-hydroxyethyl group resulted in an increase of the r-I_{Cl} value.

An expansion of the condensed ring, an introduction of alkyl substituents on the thiophene ring (as an R^1-substituent), and the introduction of a 4-methyl or 4-chloro substituent on the 2-phenyl ring (as an R^2-substituent) resulted in an increase of the r-I_{Cl} value. In other words, these transformations tended to change the pharmacological properties of the compounds from the inverse agonistic to the agonistic side. For instance, compounds **17** and **28** are distinguishable only at ring size. In spite of such a minor

difference, compound **17** shows a higher r-I_{Cl} value than diazepam and can be classified as a full agonist, but **28** with a smaller r-I_{Cl} value should be classified as a partial agonist.

Cook et al [16–18] have reported that BZR agonists interact not only with two hydrogen-bond donating groups, termed H_1 and H_2, of the BZRs but also with three lipophilic regions, L_1, L_2 and L_3. We have already reported that compound **33** exhibits a full agonistic property and that full occupation of the lipophilic region by the fused thiophene ring and the 9-propyl of **33** should result in a full agonist [7] (fig 2). This lipophilic region could correspond to the region L_3, whose occupation may enhance agonist activity [19, 20]. According to this BZR binding mode, the interaction of each compound **17** and **28** with the BZRs would be mediated by the imino nitrogen atom at the 1-position and the carbonyl oxygen atom at the 3-position, forming hydrogen bonds with two donor sites (H_1 and H_2) of the BZRs, and the fused thiophene ring and 9-ethyl-substituent would interact with the lipophilic region L_3. In the case of **17** therefore, full occupation of the lipophilic region L_3 of the BZRs with the fused thiophene ring and the 9-ethyl-substituent should result in a full agonist like compound **33** (fig 2b). On the other hand, in the case of **28** partial occupation of the same region should result in a partial agonist (fig 2c). Because the pyridazinone ring of each compound should be fixed by the hydrogen bond with H_1 and H_2 and by the interaction between the R^2-substituent and the BZRs, the fused thiophene ring and the 9-ethyl-substituent in **28** cannot completely reach the lipophilic region L_3.

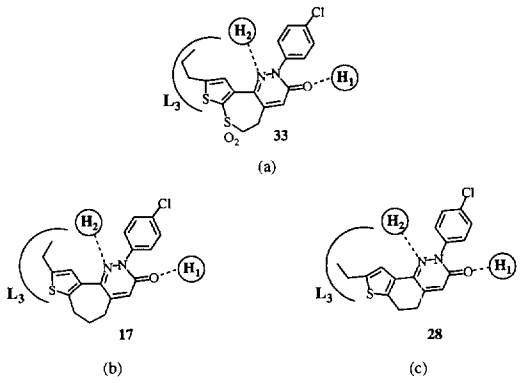


Fig 2. Binding interactions of **33** (**a**), **17** (**b**), **28** (**c**) with the pharmacophore model [18] for the BZR agonist site. H_1, H_2 are the hydrogen bond donor sites on the receptor protein.

In vivo study

The compounds shown in table III were chosen for in vivo tests as BZR partial agonists. Here, all these compounds show a r-I_{Cl} value between 1.00 (the value of antagonists) and 2.54 (the value of diazepam as a typical full agonist). The compounds were tested in mice for their anticonvulsive property by using the convulsant bicuculline [21]. Some compounds, which are listed in table III, were inactive at a dose up to 100 mg/kg. The lack of activity in such compounds may be due to a rapid metabolism into inactive compounds in vivo or to their insufficient efficacy against convulsion. Among the tested compounds **31** showed the most potent activity, with an r-I_{Cl} value in partial agonist range. Thus, compound **31** is a promising anxiolytic agent with fewer unfavorable side effects. We therefore evaluated the pharmacological profile of **31** in comparison with that of diazepam by determining both the anxiolytic activity via the water-lick conflict paradigm in rats (anticonflict test) [22] and the effect on motor coordination in rats (rotarod test) (table IV). The potency of **31** was 4-fold that of diazepam in the anticonflict test but less than that of diazepam in the rotarod test. Thus **31** shows a remarkable improvement in the ratio of the minimum effective dose (MED) value in the model of anti-anxiety (anticonflict test) to the ED_{50} value of muscle relaxant effects (rotarod test) in comparison with diazepam.

Table III. Anticonvulsive potency of BZR partial agonists against bicuculline-induced seizure.

Compound	*Anticonvulsive action ED_{50} (mg/kg, po)*
8	> 100
10	> 100
15	> 100
18	100
20	18
21	> 100
24	> 100
26	> 100
27	20
28	8.0
29	> 100
30	> 100
31	4.3
32	> 100
Diazepam	0.4

Table IV. Pharmacological activity of **31** and diazepam.

Compound	*Anticonflict activity MED (mg/kg, rats iv)*	*Rotarod ED50 (mg/kg, rats iv)*	*Ratio (Rotarod/ anticonflict)*
31	0.25	3.8	15
Diazepam	1.0	0.9	0.9

MED: minimum effective dose.

Conclusion

In conclusion, both TCP and TCN possess a high affinity to BZRs and comprise a continuous spectrum of agents with a graduated range of pharmacological efficacy towards BZRs. Furthermore, on the basis of anticonflict potency and r-I_{Cl} value indicative of partial agonism, **31** shows promise as an anxiolytic agent with diminished side effects. These preliminary results provide a firm platform from which drug candidates for the treatment of anxiety can be developed.

Experimental protocols

Chemistry

All melting points were determined on a Büchi 530 melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded with a JEOL JNM-FX 100 spectrometer (Me_4Si as internal standard). Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), brs (broad singlet), and m (multiplet). Chemical shifts are expressed in ppm. The IR spectra were recorded with a JASCO IR-810 spectrophotometer. The mass spectra were recorded on JEOL JMS-01SG-2 system. The elemental analyses were performed for C, H, N; results were within ± 0.4% of theoretical values. Silica gel plates (Merck F254) and silica gel 60 (Merck, 70–230 mesh) were used for analytical and column chromatography respectively. The physicochemical data of newly synthesized compounds are listed in tables I, II, V and VI.

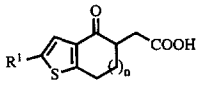
4-Oxo-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophen-5-acetic acids and 4-oxo-4,5,6,7-tetrahydrobenzo[b]thiophen-5-acetic acids ***4a–4d***

A typical example is given to represent the general procedure.

4,5,6,7-Tetrahydro-5-dimethylaminomethylbenzo[b]thiophen-4-one hydrochloride ***2c***. Acetic anhydride (60 mL, 0.64 mol) was added dropwise to the mixture of dimethylamine hydrochloride (14 g, 0.17 mol) and 37% HCHO (14 g, 0.17 mol) at 70–90 °C. After stirring for 0.5 h, 4,5,6,7-tetrahydrobenzo[*b*]-thiophen-4-one [10] (20 g, 0.13 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 crystallized from acetone to produce **2c** (29 g, 90%). Recrystallization from EtOH gave colorless crystals, mp = 193–195 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.68–2.20 (1H, m), 2.40–2.70 (1H, m), 2.80 (6H, s, $N(CH_3)_2$), 2.96–3.32 (4H, m), 3.40–3.84 (1H, m), 7.18 (1H, d, J = 6 Hz, ArH), 7.44 (1H, d, J = 6 Hz, ArH); IR (KBr) cm^{-1}: 1665 (C=O); MS *m/z*: 209 (M^+); anal $C_{11}H_{15}NOS$·HCl (C, H, N).

4,5,6,7-Tetrahydro-4-oxo-benzo[b]thiophen-5-acetonitrile ***3c***. A solution of KCN in H_2O (50 mL) was added to a stirred solution of **2c** (24.5 g, 0.1 mol) in MeOH (200 mL) at room temperature. The reaction mixture was stirred for 5 h at 45–50 °C, then poured into H_2O and extracted with $CHCl_3$. The extract was washed with brine, dried over $MgSO_4$ and concentrated in

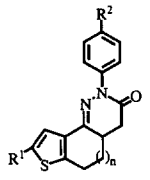
Table V. Physicochemical data for 4-oxo-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophen-5-acetic acids (n = 2) and 4-oxo-4,5,6,7-tetrahydrobenzo[*b*]thiophen-5-acetic acids (n = 1).



Compound	*R^1*	*n*	*Yield*[a] *(%)*	*Mp (°C) Recryst solv*[b]	*Formula*	*Analysis Calcd (found)*	
						C	*H*
4a	H	2	51	130–131 EtOH–H_2O	$C_{11}H_{12}O_3S$	58.91 (58.93)	5.39 (5.42)
4b	CH_3	2	57	156–158 EtOH–H_2O	$C_{12}H_{14}O_3S$	60.48 (60.49)	5.92 (5.98)
4c	H	1	60	199–121 IPE	$C_{10}H_{10}O_3S$	57.13 (57.36)	4.79 (4.64)
4d	CH_3	1	63	126–128 EtOH–H_2O	$C_{11}H_{12}O_3S$	58.91 (58.90)	5.39 (5.47)

[a]Yield from **1**; [b]IPE, isopropyl ether.

Table VI. Physicochemical data for 4,4*a*-dihydro-TCP (n = 2) and 4,4*a*-dihydro-TCN (n = 1).



Compound	*R¹*	*R²*	*n*	*Yield*[a] *(%)*	*Mp (°C)*	*Recryst solv*[b]	*Formula*
6a	H	H	2	57	68–70	Hexane–IPE	$C_{17}H_{16}N_2OS$
6b	H	CH_3	2	69	105–107	$CHCl_3$–EtOH	$C_{18}H_{18}N_2OS$
6c	H	F	2	57	106–108	$CHCl_3$–EtOH	$C_{17}H_{15}FN_2OS$
6d	H	Cl	2	70	156–158	$CHCl_3$–EtOH	$C_{17}H_{15}ClN_2OS$
6e	H	Br	2	69	161–162	IPA–IPE	$C_{17}H_{15}BrN_2OS$
6f	H	I	2	91	143–145	$CHCl_3$–EtOH	$C_{17}H_{15}IN_2OS$
6g	H	CH_3O	2	91	139–141	$CHCl_3$–EtOH	$C_{18}H_{18}N_2O_2S$
6h	CH_3	Cl	2	84	119–121	EtOH	$C_{18}H_{17}ClN_2OS$
6i	CH_3	CH_3O	2	81	136–138	IPA	$C_{19}H_{20}N_2O_2S$
6j	H	H	1	57	117–119	EtOH	$C_{16}H_{14}N_2OS$
6k	H	CH_3	1	69	157–160	EtOH	$C_{17}H_{16}N_2OS$
6l	H	Cl	1	92	169–171	AcOEt	$C_{16}H_{13}ClN_2OS$
6m	H	CH_3O	1	91	190–192	AcOEt	$C_{17}H_{16}N_2O_2S$
6n	CH_3	Cl	1	84	137–139	IPA	$C_{17}H_{15}ClN_2OS$

[a]Isolated yield; [b]IPA, isopropyl alcohol; AcOEt, ethyl acetate; IPE, isopropyl ether.

vacuo. The residue was chromatographed on a silica gel column with $CHCl_3$ to give **3c** (16.5 g, 87%), which was recrystallized from hexane/ethyl acetate to afford a colorless powder, mp = 64–65 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.88–2.36 (1H, m), 2.40–3.40 (6H, m), 7.10 (1H, d, J = 6 Hz, ArH), 7.36 (1H, d, J = 6 Hz, ArH); IR (KBr) cm^{-1}: 2250 (CN), 1675 (C=O); MS *m/z*: 191 (M^+); anal $C_{10}H_9NOS$ (C, H, N).

4,5,6,7-Tetrahydro-4-oxo-benzo[b]thiophen-5-acetic acid ***4c***. Conc HCl (50 mL) was added to a stirred solution of **3c** (16.0 g, 84 mmol) in acetic acid (70 mL) at room temperature. The reaction mixture was heated to reflux for 3 h, followed by pouring into ice–water and extracted with $CHCl_3$. The extract was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The remaining solid was recrystallized from isopropyl-ether to give **4c** (13.4 g, 76%) as a colorless prisms, mp = 119–121 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.80–2.64 (3H, m), 2.84–3.28 (4H, m), 7.07 (1H, d, J = 6 Hz, ArH), 7.38 (1H, d, J = 6 Hz, ArH), 10.95 (1H, brs, COOH); IR (KBr) cm^{-1}: 1685 (C=O), 1705 (COOH); MS *m/z*: 210 (M^+); anal $C_{10}H_{10}O_3S$ (C, H, N). The other compounds (**4a**, **b**, **d**) in table V were similarly prepared from **1a**, **b**, **d** respectively.

2-Aryl-2,4,4a,5,6,7-hexahydro-3H-thieno[2',3':6,7]cyclohepta[1,2-c]pyridazin-3-ones and 2-aryl-4,4a,5,6-tetrahydrothieno[2,3-h]cinnolin-3(2H)-ones ***6a–6n***
A typical example is given to represent the general procedure.

2-(4-Chlorophenyl)-4,4a,5,6-tetrahydrothieno[2,3-h]cinnolin-3(2H)-one ***6l***. A mixture of **4c** (5 g, 23.8 mmol) and 4-chlorophenylhydrazine (4.3 g, 30 mmol) in EtOH (100 mL) was refluxed for 5 h. After evaporation of the solvent, the residue was dissolved in acetic acid (100 mL). The mixture was refluxed for 2 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 ethyl acetate to give **6l** (6.95 g, 92%) as a pale yellow powder, mp = 169–171 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.42–1.98 (1H, m), 2.10–3.22 (6H, m), 7.11 (1H, d, J = 6 Hz, ArH), 7.33 (2H, d, J = 9 Hz, ArH), 7.42 (1H, d, J = 6 Hz, ArH), 7.55 (2H, d, J = 9 Hz, ArH); IR (KBr) cm^{-1}: 1690 (C=O); MS *m/z*: 316 (M^+); anal $C_{16}H_{13}ClN_2OS$ (C, H, N). The other compounds in table VI were similarly prepared from the corresponding **4a–d** and arylhydrazines.

2-Aryl-2,5,6,7-tetrahydro-3H-thieno[2',3':6,7]cyclohepta[1,2-c]pyridazin-3-ones and 2-aryl-5,6-dihydrothieno[2,3-h]cinnolin-3(2H)-ones ***7–15, 22–26***
A typical example is given to represent the general procedure.

2-(4-Chlorophenyl)-5,6-dihydrothieno[2,3-h]cinnolin-3(2H)-one ***24***. To a solution of **6l** (1.2 g, 3.8 mmol) in acetic acid containing 15% HBr (20 mL) was added dropwise dimethylsulfoxide (0.27 mL, 3.8 mmol) at room temperature. The reaction mixture was stirred for 1.5 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 residue was chromatographed on a silica gel column using $CHCl_3$ as eluent to give **24** (0.67 g, 56%) as a colorless powder, ^{1}H-NMR ($CDCl_3$) δ: 3.05 (4H, s, CH_2CH_2), 6.83 (1H, s, C=CHCO), 7.13 (1H, d, J = 6 Hz, ArH), 7.38 (2H, d, J = 9 Hz, ArH), 7.40 (1H, d, J = 6 Hz, ArH), 7.62 (2H, d, J = 9 Hz, ArH). IR (KBr) cm^{-1}: 1680 (C=O). MS *m/z*: 314 (M^+); anal $C_{16}H_{11}ClN_2OS$ (C, H. N). The other compounds (**7–15**, **22**, **23**, **25**, **26**) in tables I, II were similarly prepared from **6a–n**.

*9-Acetyl-2-aryl-2,5,6,7-tetrahydro-3H-thieno[2',3':6,7]cyclohepta[1,2-c]pyridazin-3-ones (**19–21**) and 8-acetyl-2-aryl-5,6-dihydrothieno[2,3-h]cinnolin-3(2H)-ones **29**, **30***

A typical example is given to represent the general procedure.

*8-Acetyl-2-(4-chlorophenyl)-5,6-dihydrothieno[2,3-h]cinnolin-3(2H)-one **30**.* To an ice-cooled suspension of $AlCl_3$ (8.5 g, 63.7 mmol) in CH_2Cl_2 (150 mL) was added acetylchloride (2.0 g, 25.5 mmol) and the mixture was stirred at 0–10 °C for 0.5 h. After addition of **24** (4.0 g, 12.7 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 **30** (3.8 g, 84%) as colorless needles, ^{1}H-NMR ($CDCl_3$) δ: 2.58 (3H, s, CH_3CO), 3.09 (4H, brs, CH_2CH_2), 6.88 (1H, s, C=CHCO), 7.44 (2H, d, J = 9 Hz, ArH), 7.64 (2H, d, J = 9 Hz, ArH), 7.96 (1H, s, H9). IR (KBr) cm^{-1}: 1680 (C=O), 1665 (C=O). MS *m/z*: 356 (M^+); anal $C_{18}H_{13}ClN_2O_2S$ (C, H, N). The other compounds (**19–21**, **29**) in tables I, II were similarly prepared from **8**, **10**, **13** and **23** respectively.

*2-Aryl-9-ethyl-2,5,6,7-tetrahydro-3H-thieno[2',3':6,7]cyclohepta[1,2-c]pyridazin-3-ones and 2-aryl-8-ethyl-5,6-dihydrothieno[2,3-h]cinnolin-3(2H)-ones **16–18**, **27**, **28***

A typical example is given to represent the general procedure.

*2-(4-Chlorophenyl)-8-ethyl-5,6-dihydrothieno[2,3-h]cinnolin-3(2H)-one **28**.* To a stirred solution of **30** (2.0 g, 5.6 mmol) in trifluoroacetic acid (20 mL) was added triethylsilane (1.4 g, 12 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 water, 2% $NaHCO_3$, dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on a silica gel using $CHCl_3$ as eluent to give **28** (1.2 g, 62%), which was recrystallized from ethyl acetate/hexane to afford a colorless powder, ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, t, J = 7 Hz, CH_3CH_2), 2.82 (2H, q, J = 7 Hz, CH_3CH_2), 3.02 (4H, brs, CH_2CH_2), 6.83 (1H, s, C=CHCO), 7.11 (1H, s, H9), 7.42 (2H, d, J = 9 Hz, ArH), 7.64 (2H, d, J = 9 Hz, ArH). IR (KBr) cm^{-1}: 1670 (C=O). MS *m/z*: 342 (M^+); anal $C_{18}H_{15}ClN_2OS$ (C, H, N). The other compounds (**16–18**, **27**) in tables I, II were similarly prepared from **19–21** and **29** respectively.

*2-Aryl-8-(1-hydroxyethyl)-5,6-dihydrothieno[2,3-h]cinnolin-3(2H)-ones **31**, **32***

A typical example is given to represent the general procedure.

*2-(4-Chlorophenyl)-8-(1-hydroxyethyl)-5,6-dihydrothieno[2,3-h]cinnolin-3(2H)-ones **32**.* To a stirred solution of **30** (3.8 g, 10.6 mmol) in MeOH/$CHCl_3$ (1:1) (80 mL) was added $NaBH_4$ (0.8 g, 21 mmol) at 0 °C. The reaction mixture was stirred for 1 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 ethyl acetate/hexane to give **32** (3.1 g, 81%) as colorless powder, ^{1}H-NMR ($CDCl_3$) δ: 1.59 (3H, d, J = 7 Hz, $CH_3CH(OH)$), 2.20 (1H, brs, $CH_3CH(OH)$), 3.03 (4H, brs, CH_2CH_2), 5.05 (1H, q, J = 7 Hz, $CH_3CH(OH)$), 6.82 (1H, s, C=CHCO), 7.25 (1H, s, H9), 7.40 (2H, d, J = 9 Hz, ArH), 7.62 (2H, d, J = 9 Hz, ArH). IR (KBr) cm^{-1}: 1655 (C=O). MS *m/z*: 358 (M^+); anal $C_{18}H_{15}ClN_2O_2S$ (C, H, N). Compound **31** in table II was similarly prepared from **29**.

Pharmacology

Benzodiazepine receptor binding assay

Preparation of a synaptosome fraction and [^{3}H]diazepam binding studies were carried out according to the method Möhler and Okada [1]. Crude synaptosomal membranes, prepared from cerebral cortex of male Wister rats, 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 mL aliquot of crude synaptosomal membranes to 100 mL solution containing [^{3}H]diazepam (final concentration 2 nM) and a known concentration of test compounds. After the mixture had been incubated for 20 min at 0 °C, the binding was stopped by addition of 3 mL 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 four times with 3 mL ice-cold buffer. The radioactivity on the filters was measured by a liquid scintillation counter. Binding in the presence of 1 mM unlabelled diazepam was defined as nonspecific binding. Specific binding was defined as the difference between total binding and nonspecific binding. The experiments were carried out in triplicate. 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 the concentration of [^{3}H]diazepam (2 nM), and K_d the dissociation constant determined by Scatchard plot. The K_i values were means ± SE of at least three determinations.

GABA-induced chloride current in frog sensory neuron

The experiment was carried out according to the method of Akaike et al [14]. Bullfrog dorsal root ganglion neurons were isolated. Isolated neuronal cell bodies were perfused internally and externally by suction pipette technique with respective test solutions to record the chloride current. The external solution contained Tris–Cl 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 an 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 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 via a concentration-clamp technique. Increasing action of test compound on the GABA response was examined on I_{Cl} induced by 3 μM GABA. The results were presented as relative values of peak I_{Cl} elicited by 3 μM 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 carried out using a modification of the method of Lippa and Regan [21]. Groups of 7–14 ddY male mice were challenged with bicuculline (0.6 mg/kg, iv) 1 h after oral administration of the test compounds. The ED_{50} values were calculated by the probit method as the dose which prevented tonic extension in 50% of the animals.

Anticonflict test (water-lick test)
The test was carried out using a modification of the method of Vogel et al [22]. Groups of eight to 20 female Wistar rats weighing 150–200 g were used. They were deprived of water for 48 h before the test. The experimental apparatus was composed of a light and a dark compartment equipped with a nozzle for water supply where the rats were allowed movement between the two compartments. Fifteen min after iv administration of the test compound, the rat was placed into the test apparatus where an electric shock was given once every 20th lick through the nozzle and grid floor. After the rat received the first electric shock, the number of shocks were recorded during the subsequent 3-min test period. The criterion was set as eight shocks during the test period. The MED was defined as the lowest dose producing a statistically significant difference in the incidence of rats attaining the criteria for the polyethyleneglycol 400-treated group assessed via Fisher's exact probability test.

Rotarod test
Groups of seven female Wistar rats were used. The rats were gently placed on a rod (diameter 5 cm rotating at 7.5 rpm) 15 min after iv administration of the test compound. The ED_{50} value was calculated by the probit method as the dose which caused 50% of the animals to fall from the rotarod within 1 min.

References

1 Möhler H, Okada T (1977) *Life Sci* 20, 2101–2110
2 Squires RF, Braestrup C (1977) *Nature* 266, 732–734
3 Sieghart W (1995) *Pharm Rev* 47, 181–234
4 Smith GB, Olsen RW (1995) *Trends Pharmacol Sci* 16, 162–168
5 Bellantuono C, Reggi V, Tognoni G, Garattini S (1980) *Drugs* 19, 195–219
6 Haefely WE, Martin JR, Schoch P (1990) *Trends Pharmacol Sci* 11, 452–456
7 Tanaka H, Kirihara S, Yasumatsu H, Yakushiji T, Nakao T (1995) *Eur J Med Chem* 30, 859–868
8 Nishimura S, Nakamura M, Suzuki M, Imoto E (1962) *Nippon Kagaku Zasshi* 83, 343–347
9 Lechartier JP, Demerseman P, Buisson JP, Cheutin A, Desvoye ML, Royer R (1969) *Bull Soc Chim Fr* 797–803
10 Jones G, Robinson MJ (1977) *J Chem Soc Perkin Trans I* 505–510
11 Cagniant P, Cagniant D (1955) *Bull Soc Chim Fr* 680–686
12 Nakao T, Obata M, Kawakami M et al (1991) *Chem Pharm Bull* 39, 2556–2563
13 Nakao T, Obata M, Yamaguchi Y, Tahara T (1991) *Chem Pharm Bull* 39, 524–526
14 Akaike N, Inoue M, Krishtal OA (1986) *J Physiol* 379, 171–185
15 Yakushiji T, Fukuda T, Oyama Y, Akaike N (1989) *Br J Pharmacol* 98, 735–740
16 Hollinshead SP, Trudell ML, Skolnick P, Cook JM (1990) *J Med Chem* 33, 1062–1069
17 Zhang W, Koehler KF, Harris B, Skolnick P, Cook JM (1994) *J Med Chem* 37, 745–757
18 Zhang W, Koehler KF, Zhang P, Cook JM (1995) *Drug Des Discov* 12, 193–248
19 Diaz-Arauzo H, Evoniuk GE, Skolnick P, Cook JM (1991) *J Med Chem* 34, 1754–1756
20 Diaz-Arauzo H, Koehler KF, Hagen TJ, Cook JM (1991) *Life Sci* 49, 207–216
21 Lippa AS, Regan B (1977) *Life Sci* 21, 1779–1784
22 Vogel JR, Beer B, Clody DC (1971) *Psychopharmacologia* 21, 1–12