methyl-2-(methylthio)phenyl]imidazolidine-2,4-dione, 120121-47-3; 5-[5-chloro-2-(methylthio)-3-(trifluoromethyl)phenyl]-imidazolidine-2,4-dione, 120121-48-4; 5-[3,5-dichloro-2-(methylthio)phenyl]imidazolidine-2,4-dione, 120121-49-5.

Supplementary Material Available: Coordinates, anistropic temperature factors, distances, and angles for compound **2b** and 4 (8 pages). Ordering information is given on any current masthead page.

Thienylpyrazoloquinolines with High Affinity to Benzodiazepine Receptors: Continuous Shift from Inverse Agonist to Agonist Properties Depending on the Size of the Alkyl Substituent

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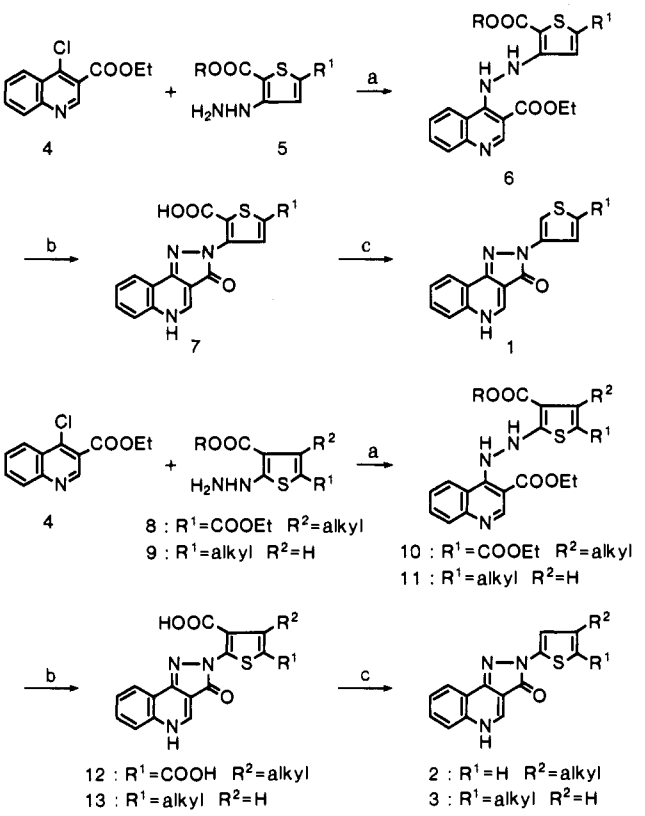
2-(5-Alkylthien-3-yl)- (1), 2-(4-alkylthien-2-yl)- (2), and 2-(5-alkylthien-2-yl)-2,5-dihydro-3*H*-pyrazolo[4,3-*c*]quinolines (3) were prepared in four steps starting from ethyl 4-chloroquinoline-3-carboxylate (4) and hydrazinothiophenecarboxylates 5, 8, and 9. All the assayed compounds possessed high affinities for benzodiazepine receptors (K_i = 0.3–2.6 nM). The activities of agonists and inverse agonists were assessed on the basis of inhibition or facilitation of pentylenetetrazole-induced convulsions, respectively. Introduction of alkyl groups of different sizes into the unsubstituted inverse agonistic compounds results in a corresponding shift in the activity from an inverse agonist to an antagonist to an agonist. The susceptibility of such a shift increases in the order of 1 < 2 < 3. This tendency may be explained by slight differences in the geometry of the alkyl substituents among the three series.

A recent study[1] using the gene-cloning technique has found that the GABA/benzodiazepine receptor[2] has a membrane-spanning ion channel similar to that of the nicotinic acetylcholine receptor, and the binding sites for GABA and benzodiazepines (BZ) have been proposed to be located in the N-terminal extracellular domains. Although the exact structural features of the binding sites remain obscure, it is more likely that the BZ receptor ligand allosterically influences the GABA binding site which is presumably located close to the BZ binding site.

The BZ receptor ligands are thought to comprise a continuous spectrum of agents with a graduated range of pharmacological efficacies at the receptor:[3] (1) full inverse agonists (negative efficacy; anxiogenic/convulsant), (2) partial inverse agonists (intermediate negative efficacy; proconvulsant), (3) pure antagonists (nil efficacy; antagonism toward the other classes), (4) partial agonists, and (5) full agonists (positive efficacy; anxiolytic/anticonvulsant). The inverse agonist β-CCM enhances the performance in several animal models of learning and memory,[4] whereas the agonist diazepam impairs such performance in humans,[5] suggesting that partial inverse agonists lacking anxiogenic or convulsant effects may provide a new type of nootropic drugs. Some series of the BZ receptor ligands have been described[3,6] in which slight structural modifications can produce a change in the activity from an inverse agonist to an antagonist or to an agonist. Also, several papers[7] have reported the structural requirements for the agonists, antagonists, and inverse agonists from comparisons of various ligands belonging to chemically different classes, but no definite conclusion has been reached.

We have previously described[8] the structure–activity relationships of thienylpyrazoloquinolines, in which **1b** (S-135[9]) with a 5-methylthien-3-yl group possessed potent inverse agonistic activity, while its isomer **3b** with a 5-methylthien-2-yl group had agonist activity. The 4-methylthien-2-yl isomer **2b** exhibited weak inverse agonist properties. Moreover, the coplanarity of the thiophene ring with the pyrazoloquinoline skeleton was found to be necessary for high-affinity binding to the BZ receptors. Assuming that the thiophene ring is a regular pentagon, the

Scheme I[a]



[a] (a) EtOH/room temperature. (b) 1 N aqueous NaOH/EtOH/room temperature, then reflux. (c) Cu/quinoline/190–200 °C.

† Division of Organic Chemistry.
‡ Division of Pharmacology.

(1) Schofield, P. R.; Darlison, M. G.; Fujita, N.; Burt, D. R.; Stephenson, F. A.; Rodriguez, H.; Rhee, L. M.; Ramachandran, J.; Reale, V.; Glencorse, T. A.; Seeburg, P. H.; Barnard, E. A. *Nature (London)* **1987**, *328*, 221.
(2) A recent review: Haefely, W.; Kyburz, E.; Gerecke, M.; Möhler, H. *Advances in Drug Research*; Testa, B., Ed.; Academic: Orlando, 1985; Vol. 14, pp 165–322.

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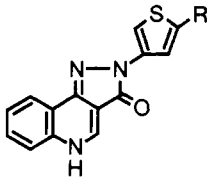
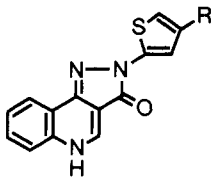
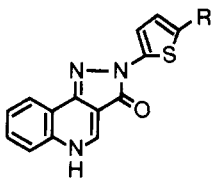
Table I. Pharmacological Activities of Thienylpyrazoloquinolines 1-3



1



2



3

compd	R	K_i,[a] nM (mean ± SD)	inverse agonist act.[b] PTZ = 75 mg/kg sc ED_{50},[d] mg/kg iv	inverse agonist act.[b] PTZ = 90 mg/kg sc ED_{50},[d] mg/kg iv	agonist act.:[c] PTZ = 125 mg/kg sc ED_{50},[d] mg/kg iv
1a	H	0.73 ± 0.44	0.43 (0.28-0.73)	0.14 (0.04-0.29)	
1b	Me	0.32 ± 0.02	0.47 (0.33-0.66)	0.13 (0.09-0.18)	
1c	Et	0.41 ± 0.14	inactive to 10	50% at 10[e]	
1d	*n*-Pr	1.04 ± 0.18		inactive to 10	inactive to 10
1e	*i*-Pr	0.95 ± 0.21		inactive to 10	inactive to 10
1f	*n*-Bu	1.86 ± 0.30			50% at 5[e,f]
1g	*i*-Bu	2.27 ± 0.34		inactive to 10	7.86 (5.32-13.14)
2a	H	0.41 ± 0.18	62.5% at 2.5[e,f]	0.57 (0.25-1.15)	
2b	Me	0.48 ± 0.16	inactive to 10	5.15 (3.54-7.18)	
2c	Et	0.60 ± 0.17			5.15 (3.54-7.18)
2d	*n*-Pr	1.01 ± 0.17			3.54 (2.59-4.83)
3a = 2a	H	0.41 ± 0.18	62.5% at 2.5[e,f]	0.57 (0.25-1.15)	
3b	Me	0.32 ± 0.02			0.16 (0.11-0.23)
3c	Et	1.02 ± 0.43			0.32 (0.11-0.48)
3d	*n*-Pr	0.86 ± 0.21			0.74 (0.43-1.12)
3e	*i*-Pr	1.02 ± 0.18			0.89 (0.58-1.32)
3f	*n*-Bu	1.48 ± 0.48			0.22 (0.14-0.37)
3g	*i*-Bu	2.04 ± 0.31			0.61 (0.43-0.84)
β-CCE		1.14 ± 0.08	1.00 (0.62-1.62)	0.46 (0.27-0.71)	
CGS 8216		0.22 ± 0.01	inactive to 10	0.20 (0.09-0.50)	
CGS 9896		0.83 ± 0.15			0.07 (0.04-0.12)
diazepam		5.02 ± 0.37			0.15 (0.10-0.18)

[a] Displacing potential to [^{3}H]diazepam binding in rat cerebral cortex. [b] Mouse proconvulsant activity. See text for schedule details. [c] Mouse pentylenetetrazole anticonvulsant test. See text for schedule details. [d] ED_{50} values and their 95% confidence limits were calculated by the probit method. [e] Percentage of the animals affected at that dose. [f] ED_{50} could not be obtained because of poor dose dependency.

methyl groups of **1b**, **2b**, and **3b** can be completely superposed on one another. Nevertheless, when the models are constructed by using the bond lengths and angles of the thiophene obtained from single-crystal X-ray analyses,[10] the methyl groups of **1b**, **2b**, and **3b** possess somewhat distinct geometries as shown in Figure 1.[11] In order to know how the geometrical differences of these methyl groups are responsible for the intrinsic activities on a receptor level, analogues bearing the higher alkyl group on the thiophene were prepared and their pharmacological properties were examined.

Chemistry

2-(5-Alkylthien-3-yl)- (**1**), 2-(4-alkylthien-2-yl)- (**2**), and 2-(5-alkylthien-2-yl)-2,5-dihydro-3*H*-pyrazolo[4,3-*c*]-quinolin-3-ones (**3**) were prepared according to our previously reported method[8] as shown in Scheme I. Treatment of hydrazinothiophenecarboxylate **5** with ethyl 4-chloroquinoline-3-carboxylate (**4**) in ethanol at room temperature gave an adduct **6**, which was cyclized and hydrolyzed by sodium hydroxide in aqueous ethanol to afford an acid **7**. Decarboxylation of **7** with copper in quinoline at ca. 190 °C provided the desired pyrazoloquinoline **1**.

Similar sequential treatment with hydrazinothiophenes **8** and **9** produced analogues **2** and **3**, respectively.

Pharmacological Methods

Binding test to the BZ receptor was carried out as described[12] with minor modifications, using [^{3}H]diazepam and the receptor preparation obtained from the cerebral

(3) Braestrup, C.; Honoré, T.; Nielsen, M.; Petersen, E. N.; Jensen, H. *Biochem. Pharmacol.* **1984**, *33*, 859.
(4) Venault, P.; Chapouthier, G.; Prado de Carvalho, L.; Simiand, J.; Morre, M.; Dodd, R. H.; Rossier, J. *Nature(London)* **1986**, *321*, 864.
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(8) (a) Takada, S.; Shindo, H.; Sasatani, T.; Matsushita, A.; Eigyo, M.; Kawasaki, K.; Murata, S. *J. Med. Chem.* **1987**, *30*, 454. (b) Takada, S.; Shindo, H.; Sasatani, T.; Chomei, N.; Matsushita, A.; Eigyo, M.; Kawasaki, K.; Murata, S.; Takahara, Y.; Shintaku, H. *J. Med. Chem.* **1988**, *31*, 1738.
(9) For the detailed pharmacological properties of S-135, see: Matsushita, A.; Kawasaki, K.; Matsubara, K.; Eigyo, M.; Shindo, H.; Takada, S. *Prog. Neuro-Psychopharmacol. Biol. Psychiat.* **1988**, *12*, 951.
(10) The single-crystal X-ray analyses of **1b**, **2b**, and **3b** were performed by Dr. M. Shiro in these laboratories.
(11) Our previous paper[8b] has described the planarity of the ligand as being one of the structural requirements for high-affinity binding to BZ receptors. Thus, two planar conformers can be depicted in each molecule of **1b**, **2b**, and **3b** through rotation of the thiophene ring by using crystal-structure coordinates and computer graphics. This figure shows one of the two possible superpositions in which all the methyl groups are placed on the same side.
(12) Möhler, H.; Okada, T. *Science (Washington, D.C.)* **1977**, *198*, 849.

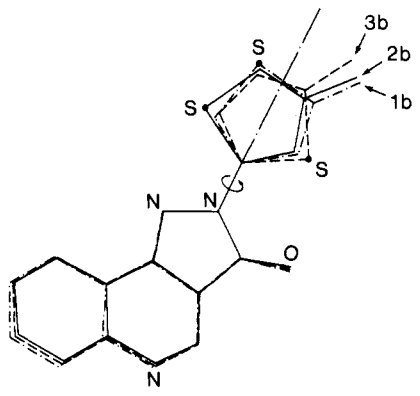

Figure 1. Superposition of **1b**, **2b**, and **3b**.

cortex of Wistar rats. The detailed procedure has been described in our previous paper.[8b]

Inverse agonist activity was evaluated by potentiation of the pentylenetetrazole (PTZ) induced convulsions. Groups of 8–16 male mice were challenged with a subconvulsive dose (75 or 90 mg/kg, sc) of PTZ immediately after intravenous injection of the test compounds. The dose requied to produce tonic convulsions and death in 50% of the animals during a 2-h observation period was calculated by the probit method.

Agonist activity was evaluated by inhibition of the PTZ-induced convulsions. Groups of 8–16 male mice were challenged with a convulsive dose (125 mg/kg, sc) of PTZ immediately after intravenous injection of the test compounds. The dose required to prevent tonic convulsions and death in 50% of the animals during a 2-h observation period was calculated by the probit method.

Both the inverse agonist and agonist activities are apparently mediated via BZ receptors because these effects were completely antagonized by the BZ antagonist Ro 15-1788.[13]

Antagonist activity was assessed by disruption of the anticonvulsant actions of diazepam against PTZ. Groups of 8–16 male mice were challenged with an effective dose (1 mg/kg, sc) of diazepam. This dose offers 100% protection against the convulsions induced by PTZ (125 mg/kg, sc). Thirty minutes later, the animals were administered a convulsive dose (125 mg/kg) of PTZ immediately after intravenous injection of the test compounds. The dose required to produce tonic convulsions and death in 50% of the animals during a 2-h observation period was calculated by the probit method.

Results and Discussion

The pharmacological activities of compounds **1a–g**, **2a–d**, and **3a–g** are shown in Table I, where the inverse agonist activities were assessed by potentiattion of the convulsions induced by two subconvulsive doses (75 and 90 mg/kg, sc) of PTZ. Since the higher intrinsic activities of inverse agonists corresponds to the ability to potentiate the convulsant action of the lower dose of PTZ, the assay using 75 mg/kg of PTZ indicates higher negative efficacy (closer to the full inverse agonist properties) than the one using 90 mg/kg of PTZ. For example, β-CCE[14] exhibited greater intrinsic activities than CGS 8216 in the inverse agonist assays. Although **2a** and **3a** are identical, their data were duplicated for the sake of convenience. Our previous reports[8] have described the oral activities of some of the present compounds, which are mostly compatible with the intravenous activities shown herein. Using the intravenous activities to elucidate the structure–activity relationships is preferable because oral administration may more readily affect the absorption and metabolism.

All of the listed analogues possessed high affinity (K_i = 0.3–2.6 nM) to the BZ receptors although the higher alkyl chain tended to slightly reduce the affinity. In the in vivo activities, the parent unsubstituted compounds (R = H) **1a** and **2a** (**3a**) were ranked as the most potent inverse agonists in each series. When R was substituted by a methyl group (R = Me), **1b** possessed the same order of inverse agonist activities as **1a**, while a lowering of the inverse agonist potential of **2b** was observed, with **3b** exhibiting agonist activity. Ethyl substitution in this series further reduced the inverse agonist potency of **1c** and transformed **2c** into a weak agonist. Compound **3c** was less affected by this substitution, retaining agonist activity comparable to that of **3b**. Introduction of the higher alkyl groups did not significantly alter the agonistic potency of **2** and **3** (compare **2d** with **2c** and **3d–g** with **3c**), while with **1**, introduction of *n*-propyl and isopropyl groups led to a loss of inverse agonist activity in **1d** and **1e**. Further substitution with *n*-butyl and isobutyl groups at this position in **1** led to weak agonist activity in **1f** and **1g**. In addition, the evaluation of **1d** and **1e** in the antagonism of the anticonvulsant actions of diazepam against PTZ showed these compounds to possess antagonist activity (**1d**, ED_{50} = 6.04 mg/kg, 95% confidence limit 3.74–10.77 mg/kg; **1e**, 50% inhibition at 10 and 20 mg/kg). Therefore, introduction of alkyl substituents of increasing size into **1a**, **2a**, and **3a**, the parent compounds of each series, led to a continuous shift in the activity from an inverse agonist (through an antagonist in the case of **1**) to an agonist. Among the three series of the derivatives, the susceptibility of such a shift increases in the order of **1** < **2** < **3**. Interestingly, this order appears to be related to the geometrical differences of the alkyl substituents among the three series. As shown in the Figure 1,[11] the distance from each methyl group of **1b**, **2b**, and **3b** to the rotation axis of the thiophene ring decreases in the order of **1b** > **2b** > **3b**. These findings indicate that an alkyl group closer to the rotation axis of the thiophene ring may cause a greater change in the activity from an inverse agonist to an agonist. Several structural models[7] for the BZ receptor have been published on the basis of crystallographic data and computer graphics studies of the various BZ receptor ligands. These models have suggested the presence of the particular region conferring agonist propertis in the BZ receptor with respect to CGS 9896,[6a] β-CCP,[15] and some other BZ ligands. We consider that the alkyl portion of the present agonistic compounds may be able to occupy this region, while the inverse agonistic analogues may not. Therefore, we concluded that the gradual occupation of this region by introducing alkyl groups of different sizes into the parent inverse agonistic compounds results in a change in the activity from an inverse agonist to an antagonist to an agonist depending on the size of the introduced alkyl group.

Experimental Section

Unless otherwise noted, all reactions were carried out under an atmosphere of nitrogen. Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian EM-390 spectrometer. Chemical shifts are given in parts per million relative to tetramethylsilane as the internal standard. Elemental analyses were

(13) Hunkeler, W.; Möhler, H.; Pieri, L.; Polc, P.; Bonetti, E. P.; Cumin, R.; Schaffner, R.; Haefely, W. *Nature (London)* **1981**, *290*, 514.

(14) (a) Nielsen, M.; Braestrup, C. *Nature (London)* **1980**, *286*, 606. (b) Oakley, N. R.; Jones, B. J. *Eur. J. Pharmacol.* **1980**, *68*, 381.

(15) Braestrup, C.; Nielsen, M. *Nature (London)* **1981**, *294*, 472.

Table II. Physical Properties of 6 and 11 Prepared by Method A

compd	R^1	R^2	R	yield, %	mp, °C	formula[a]
6e	*i*-Pr		Me	98	145–146	$C_{21}H_{23}N_3O_4S$
6f	*n*-Bu		Me	98	118–119	$C_{22}H_{25}N_3O_4S$
6g	*i*-Bu		Et	98	145–147	$C_{23}H_{27}N_3O_4S$
11d	*n*-Pr	H	Me	80	173–174	$C_{21}H_{23}N_3O_4S$
11e	*i*-Pr	H	Me	85	173–174	$C_{21}H_{23}N_3O_4S$
11g	*i*-Bu	H	Me	83	161–163	$C_{22}H_{25}N_3O_4S$

[a] Analyses for C, H, N were within ±0.4% of the theoretical values unless otherwise noted.

Table III. Physical Properties of 7 and 13 Prepared by Method B

compd	R^1	R^2	yield, %	mp, °C	formula[a]
7e	*i*-Pr		88	273–274 dec	$C_{18}H_{15}N_3O_3S$[b]
7f	*n*-Bu		98	245–246 dec	$C_{19}H_{17}N_3O_3S$
7g	*i*-Bu		98	250–251 dec	$C_{19}H_{17}N_3O_3S$
13d	*n*-Pr	H	81	282–284 dec	$C_{18}H_{15}N_3O_3S \cdot {}^1/_4H_2O$
13e	*i*-Pr	H	82	294–296 dec	$C_{18}H_{15}N_3O_3S$
13g	*i*-Bu	H	79	305–307 dec	$C_{19}H_{17}N_3O_3S \cdot {}^1/_5H_2O$

[a] Analyses for C, H, N were within ±0.4% of the theoretical values unless otherwise noted. [b] C: calcd, 61.18; found, 60.76.

performed by the analytical department of Shionogi Research Laboratories and are within 0.4% of the theoretical values unless otherwise noted.

The starting materials **5**, **8**, and **9** were prepared by the method of Hentschel and Gewald.[16] The synthetic methods and physical properties of compounds **1a–c**, **2a**, **2b**, **3a–c**, and **3f** have been described in a previous paper.[8b] The other compounds were prepared essentially in the same manner.

Ethyl 4-[2-[2-(Methoxycarbonyl)-5-*n*-propylthien-3-yl]-hydrazino]quinoline-3-carboxylate (6d) (Method A). To a solution of ethyl 4-chloroquinoline-3-carboxylate (**4**: 377 mg, 1.60 mmol) in ethanol (10 mL) was added methyl 3-hydrazino-5-*n*-propylthiophene-2-carboxylate (**5d**: 343 mg, 1.60 mmol). After being stirred at room temperature for 2 h, the mixture was concentrated in vacuo. The residue was dissolved in chloroform and washed with cold aqueous sodium carbonate and water. The solution was dried ($MgSO_4$) and concentrated in vacuo. Purification of the residue by silica gel column chromatography (toluene–methanol 3:1) gave **6d** (616 mg, 93% yield) as orange crystals: mp 152–153 °C; NMR ($CDCl_3$) δ 0.92 (3 H, t, J = 7 Hz), 1.43 (3 H, t, J = 7 Hz), 1.62 (2 H, m), 2.65 (2 H, t, J = 7 Hz), 3.73 (3 H, s), 4.40 (2 H, q, J = 7 Hz), 6.57 (1 H, br), 7.20–8.02 (3 H, m), 8.50 (1 H, br s), 8.97 (1 H, d, J = 9 Hz), 9.19 (1 H, s), 10.8 (1 H, br). Anal. ($C_{21}H_{23}N_3O_4S$) C, H, N.

Compounds **6** and **11** in Table II were prepared in a similar manner.

2-(2-Carboxy-5-*n*-propylthien-3-yl)-2,5-dihydro-3*H*-pyrazolo[4,3-*c*]quinolin-3-one (7d) (Method B). A suspension of **6d** (870 mg, 2.10 mmol) and 1 N aqueous sodium hydroxide (2.32 mL, 2.32 mmol) was stirred at room temperature for 2 h. After additional 1 N aqueous sodium hydroxide (4 mL, 4 mmol) was added, the mixture was refluxed for 2 h. The resulting solution was treated with active charcoal and filtered. To the filtrate were added acetic acid (2 mL) and water (100 mL) to give a solid which was collected by filtration and washed with water and ethanol, affording **7d** (627 mg, 84% yield) as pale yellow crystals: mp 245–246 °C; NMR (DMSO-d_6) δ 0.96 (3 H, t, J = 7 Hz), 1.68 (2 H, m), 2.81 (2 H, t, J = 7 Hz), 7.57 (1 H, br s), 7.53–7.90 (3 H, m), 8.05–8.17 (1 H, m), 8.90 (1 H, s). Anal. ($C_{18}H_{15}N_3O_3S$) C, H, N.

Compounds **7** and **13** in Table III were prepared in a similar manner.

2-(5-*n*-Propylthien-3-yl)-2,5-dihydro-3*H*-pyrazolo[4,3-*c*]-quinolin-3-one (1d) (Method C). The mixture of **7d** (613 mg, 1.73 mmol), copper powder (61 mg), and quinoline (7 mL) was stirred at 190 °C for 2 h. The copper was removed by filtration and washed with ethanol and then the filtrate was concentrated and mixed with 1 N aqueous sodium hydroxide (6 mL) and water (30 mL), followed by extraction with ether to remove the quinoline. The separated aqueous layer was treated with active charcoal and filtered. The filtrate was acidified with acetic acid and the resulting precipitate was collected by filtration, washed with water, and dried. The solid was recrystallized from ethanol to afford **1d** (320 mg, 60% yield) as yellow crystals: mp 263–265 °C; NMR (DMSO-d_6) δ 0.97 (3 H, t, J = 7 Hz), 1.69 (2 H, m), 2.81 (2 H, t, J = 7 Hz), 7.45–7.78 (5 H, m), 8.15–8.28 (1 H, m), 8.72 (1 H, s). Anal. ($C_{17}H_{15}N_3OS \cdot {}^1/_4H_2O$) C, H, N.

Compounds **1** and **3** in Table IV were prepared in a similar manner.

Table IV. Physical Properties of 1 and 3 Prepared by Method C

compd	R^1	R^2	yield, %	mp, °C	formula[a]
1e	*i*-Pr		62	281–282 dec	$C_{17}H_{15}N_3OS$
1f	*n*-Bu		77	246–247 dec	$C_{18}H_{17}N_3OS$
1g	*i*-Bu		77	265–269 dec	$C_{18}H_{17}N_3OS$
3d	*n*-Pr	H	63	267–270 dec	$C_{17}H_{15}N_3OS$
3e	*i*-Pr	H	58	312–313 dec	$C_{17}H_{15}N_3OS$
3g	*i*-Bu	H	72	285–287 dec	$C_{18}H_{17}N_3OS \cdot {}^1/_6H_2O$

[a] Analyses for C, H, N were within ±0.4% of the theoretical values unless otherwise noted.

Ethyl 4-[2-[4-Alkyl-3,5-bis(ethoxycarbonyl)thien-2-yl]-hydrazino]quinoline-3-carboxylate (10). To a solution of ethyl 4-chloroquinoline-3-carboxylate (**4**: 1 mmol) in ethanol (15 mL) was added diethyl 4-alkyl-2-hydrazinothiophene-3,5-dicarboxylate (**8**: 1.05 equiv). After being stirred at room temperature for 4 h, the mixture was concentrated in vacuo. The residue was dissolved in chloroform–methanol (10:1, 5 mL) and washed with cold aqueous sodium bicarbonate and water. The solution was dried ($MgSO_4$) and concentrated in vacuo to give a solid, which was crystallized from ethanol, affording **10** as pale yellow crystals.

10c (R^1 = COOEt, R^2 = Et, R^3 = Et): mp 235–237 °C, 95% yield; NMR (DMSO-d_6) δ 1.10 (3 H, t, J = 7 Hz), 1.26, 1.31, and 1.34 (3 H each, t, J = 7 Hz), 3.22 (2 H, m), 4.20 (2 H, q, J = 7 Hz), 4.32 (4 H, m), 7.25–7.58 (3 H, m), 8.10–8.25 (1 H, m), 8.22 (1 H, s), 11.55 (1 H, br). Anal. ($C_{24}H_{27}N_3O_6S$) H, N, C: calcd, 59.37; found, 58.95.

10d (R^1 = COOEt, R^2 = *n*-Pr, R^3 = Et): mp 201–203 °C, 66% yield; NMR (DMSO-d_6) δ 0.90 (3 H, t, J = 7 Hz), 1.23, 1.27, and 1.30 (3 H each, t, J = 7 Hz), 4.19 (2 H, q, J = 7 Hz), 4.31 (4 H, q, J = 7 Hz), 7.22–7.73 (3 H, m), 8.10–8.25 (1 H, m), 8.21 (1 H, s). Anal. ($C_{25}H_{29}N_3O_6S$) C, H, N.

2-(4-Alkylthien-2-yl)-2,5-dihydro-3*H*-pyrazolo[4,3-*c*]-quinolin-3-one (2). A suspension of **10** (1 mmol) and 1 N aqueous sodium hydroxide (1.1 mL) in ethanol (40 mL) was stirred at room temperature for 3 h. The resulting solution was treated with active charcoal and filtered. The filtrate was acidified with 10% aqueous acetic acid (3 mL). After removal of the ethanol in vacuo, the resulting solid was filtered and washed with water and ethanol. The mixture of the solid in 1 N aqueous sodium hydroxide (5 mL) and ethanol (20 mL) was refluxed for 2 h. The mixture was treated with active charcol and filtered. The filtrate was concentrated and acidified with acetic acid, and the resulting solid (crude **12**) was collected by filtration and washed with ethanol. This solid and copper powder (0.1 part by weight) were suspended in quinoline (4 mL) and heated at 190 °C for 1.5 h. After being allowed to cool, the mixture was diluted with ether (80 mL) and extracted with 0.3 N aqueous sodium hydroxide (3 × 15 mL). The aqueous extract was washed with ether, treated with active charcoal, and filtered. The filtrate was acidified with acetic acid (1 mL) and the resulting solid was collected by filtration and washed with water. The solid was purified by silica gel column chromatography (chloroform–methanol 20:1) to afford **2** as yellow crystals.

2c (R^1 = H, R^2 = Et): mp 263–264 °C dec, 70% yield; NMR (DMSO-d_6) δ 1.21 (3 H, t, J = 7 Hz), 2.76 (2 H, q, J = 7 Hz), 6.71 (1 H, m), 7.27 (1 H, m), 7.50–7.77 (3 H, m), 8.07–8.18 (1 H, m), 8.77 (1 H, s), 12.80 (1 H, br). Anal. ($C_{16}H_{13}N_3OS \cdot {}^1/_3H_2O$) C, H, N.

2d (R^1 = H, R^2 = *n*-Pr): mp 268–270 °C dec, 52% yield; NMR (DMSO-d_6) δ 0.90 (3 H, t, J = 7 Hz), 1.62 (2 H, m), 2.55 (2 H, m), 6.70 (1 H, m), 7.23 (1 H, m), 8.45–8.80 (3 H, m), 8.15–8.28 (1 H, m), 8.77 (1 H, s), 12.90 (1 H, br). Anal. ($C_{17}H_{15}N_3OS \cdot {}^1/_3H_2O$) C, H, N.

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Registry No. **1a**, 104679-66-5; **1b**, 104679-67-6; **1c**, 104679-75-6; **1d**, 119945-92-5; **1e**, 119945-93-6; **1f**, 119945-94-7; **1g**, 119945-95-8; **2a**, 104679-79-0; **2b**, 104635-74-7; **2c**, 119945-96-9; **2d**, 119945-97-0; **3b**, 104635-65-6; **3c**, 104635-66-7; **3d**, 119945-98-1; **3e**, 119945-99-2; **3f**, 104635-69-0; **3g**, 119946-00-8; **4**, 13720-94-0; **5d**, 119946-01-9; **5e**, 119946-02-0; **5f**, 119946-03-1; **5g**, 119946-04-2; **6d**, 119946-09-7; **6e**, 119946-10-0; **6f**, 119946-11-1; **6g**, 119946-12-2; **7d**, 119946-18-8; **7e**, 119946-19-9; **7f**, 119946-20-2; **7g**, 119946-21-3; **8c**, 119946-05-3; **8d**, 119946-06-4; **9d**, 119970-50-2; **9e**, 119946-07-5; **9g**, 119946-08-6; **10c**, 119946-13-3; **10d**, 119946-14-4; **11d**, 119946-15-5; **11e**, 119946-16-6; **11g**, 119946-17-7; **12c**, 119946-22-4; **12d**, 119946-23-5; **13d**, 119946-24-6; **13e**, 119946-25-7; **13g**, 119946-26-8.

Synthesis, in Vitro Acetylcholine-Storage-Blocking Activities, and Biological Properties of Derivatives and Analogues of *trans*-2-(4-Phenylpiperidino)cyclohexanol (Vesamicol)

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Eighty-four analogues and derivatives of the acetylcholine-storage-blocking drug *trans*-2-(4-phenylpiperidino)-cyclohexanol (vesamicol) were synthesized, and their potencies were evaluated with the acetylcholine active-transport assay utilizing purified synaptic vesicles from *Torpedo* electric organ. The parent drug exhibits enantioselectivity, with (−)-vesamicol being 25-fold more potent than (+)-vesamicol. The atomic structure and absolute configuration of (+)-vesamicol were determined by X-ray crystallography. The absolute configuration of (−)-vesamicol is 1*R*,2*R*. Structure–activity evidence indicates that (−)-vesamicol does not act as an acetylcholine analogue. Alterations to all three rings can have large effects on potency. Unexpectedly, analogues locking the alcohol and ammonium groups trans-diequatorial or trans-diaxial both exhibit good potency. A potent benzovesamicol family has been discovered that is suitable for facile elaboration of the sort useful in affinity labeling and affinity chromatography applications. A good correlation was found between potencies as assessed by the acetylcholine transport assay and LD_{50} values in mouse.

The biochemical and physiological mechanisms of acetylcholine (ACh) storage by nerve terminal synaptic vesicles are being studied by many groups.[1] In addition to a proton-pumping ATPase and ACh transporter, a receptor for the compound *trans*-2-(4-phenylpiperidino)cyclohexanol is present.[2] When the receptor is occupied by drug, noncompetitive inhibition of ACh storage occurs.[3] The drug (formerly called AH5183 but now called vesamicol[1,4]) has been of particular value to the study of ACh metabolism in intact nerve terminal preparations (reviewed in ref 1). While it is entirely satisfactory for biochemical studies utilizing highly purified *Torpedo* electric organ synaptic vesicles, vesamicol exhibits some nonspecificity in intact preparations. For example, before neuromuscular block sets in as a result of the ACh storage block, the amplitude of muscle contraction in response to indirect stimulation actually increases.[5] This has been shown in α-bungarotoxin-blocked preparations to be nonneural in origin,[6] and thus to be unrelated to the primary site of action.

Because vesamicol is proving to be an important tool in both biochemical and physiological studies of the cholinergic nerve terminal, we sought to understand its mode of action and to develop its potential further through a structure–activity study. No structure–activity work has been published previously on this drug. We sought to determine whether the pharmacological potency of the drug exhibits enantioselectivity and whether the drug is mimicking ACh to inhibit uptake. To exploit vesamicol for receptor identification and purification in the future, we need to know which parts of the drug are critical to its potency and where we can attach steric bulk without compromising the potency. Also, we would like to increase the drug potency and specificity in order to facilitate physiological studies on and anatomical mapping[7] of cholinergic nerve terminals in mammalian preparations. Thus, we report here the synthesis of a number of new vesamicol derivatives and analogues. Their potencies as inhibitors of ACh active transport were assessed in the purified *Torpedo* electric organ synaptic vesicle assay and, for some of the compounds, in vivo toxicities also were determined. The X-ray crystallographic structure of (+)-vesamicol also was determined.

Chemistry

Most of the new analogues were synthesized by addition of secondary amines (usually substituted piperidines or piperazines) to epoxides under S_N2 conditions followed in some cases by further derivatization. Thus, except where noted, the amino alcohol substituents in the products are in the trans relationship. A number of required specialized epoxides[8] were generous gifts from Dr. Bruce F. Rickborn (Department of Chemistry, University of California, Santa Barbara, CA 93106) and were used to make compounds

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