Thieno[2,3-*d*]pyrimidine-3-acetic Acids A New Class of Nonpeptide Endothelin Receptor Antagonists

Nobuo Cho,*[,a] Yoshi Nara,[a] Mioko Harada,[a] Tsukasa Sugo,[a] Yasushi Masuda,[a] Akemi Abe,[b] Keiji Kusumoto,[b] Yasuaki Itoh,[a] Tetsuya Ohtaki,[a] Toshifumi Watanabe,[b] and Shuichi Furuya[a]

Discovery Research Division, Takeda Chemical Industries, Ltd.,[a] Wadai 10, Tsukuba-shi, Ibaraki 300–4293, Japan and Pharmaceutical Research Division, Takeda Chemical Industries, Ltd.,[b] 17–85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532–8686, Japan. Received July 13, 1998; accepted July 31, 1998

On the basis of structural information for the cyclic hexapeptide endothelin (ET) receptor antagonist, TAK-044, a series of thieno[2,3-*d*]pyrimidine-2,4-dione derivatives bearing a carboxyl group and aromatic rings that were important for receptor binding were designed, synthesized, and evaluated for ET receptor binding affinities and inhibitory activities against ET-induced vasoconstriction.

Optimization of each substituent in the thieno[2,3-*d*]pyrimidine ring led to the discovery of a novel and potent nonpeptide ET receptor antagonist, 6-(4-methoxymethoxyphenyl)-5-methylsulfonylaminomethyl-1-(2-methylthiobenzyl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetic acid (32g), which binded to human ET_A and ET_B receptor subtypes with affinities (IC_{50}) of 7.6 and 100 nM, respectively.

Compound 32g effectively antagonized ET-induced vasoconstriction and the inhibitory effect mediated by the ET_B receptor was more potent than that of bosentan, while the inhibitory effect mediated by the ET_A receptor was slightly less potent than that of bosentan.

Key words thieno[2,3-*d*]pyrimidine-3-acetic acid; endothelin receptor antagonist; endothelin receptor binding affinity; ET-induced vasoconstriction; bosentan

The discovery of endothelin-1 (ET-1), an extremely potent and long-acting vasoconstrictor peptide comprised of 21 amino acid residues, led to considerable pathophysiologic interest.[1] ET-1 is one member of a family of isopeptides, including ET-2 and ET-3,[2] that are structurally and functionally related to the cardiotoxic sarafotoxins.[3] In addition, further studies have revealed the existence of two receptor subtypes designated ET_A and ET_B,[4] which are both G protein-coupled receptors (GPCR). The ET_A receptor binds ET-1 and ET-2 with greater affinity than ET-3, and mediates vasoconstriction[5,6] and smooth muscle proliferation.[7] In contrast, the ET_B receptor does not discriminate between ET-1, ET-2, and ET-3, and mediates both vasoconstriction[8] and vasodilation.[9]

Due to their peculiar pharmacological effects, ETs are thought to play a major role in chronic diseases such as myocardial infarction, heart failure, renal failure, pulmonary hypertension, and subarachnoid hemorrhage.[10] Therefore, blockade of the ET receptors might be therapeutically effective in the treatment of the above conditions.

A large number of peptide ET receptor antagonists, represented by FR-139317[11] and BQ-123,[12] have been reported. Moreover, nonpeptide ET receptor antagonists have been reported during the course of our work described in this paper. These include Ro 46-2005,[13] Ro 47-0203 (**1**, bosentan),[14] BMS 182874,[15] SB 209670,[16] A-127722,[17] L-749329,[18] and PD 156707.[19]

In our search for a novel nonpeptide ET antagonist, we first focused on the rigidity of the cyclic hexapeptide ET antagonist TAK-044 (**2**),[20] which results from the presence of β-turn and γ-turn structures. Introduction of crucial functional moieties for ET receptor binding, *i.e.* a carboxyl group and aromatic rings,[21] onto a bicyclic heterocycle 'scaffold' which mimics the fixed main chain of TAK-044, provides nonpeptide ET receptor ligands. Using the same strategy, we have also discovered a novel, potent, and orally active nonpeptide LHRH receptor antagonist.[22] In addition, several heterocyclic compounds bearing important functional groups for receptor binding have been described in the literature as synthetic nonpeptide antagonists of GPCRs, such as angiotensin II,[23] cholecystokinin,[24] substance P,[25] and vasopressin.[26]

The thieno[2,3-*d*]pyrimidine-2,4-dione nucleus, bearing a phenyl ring attached to the thiophene ring was selected as a bicyclic heterocycle scaffold. Among the early compounds synthesized based on this structure, the thieno[2,3-*d*]pyrimidine-3-acetic acid derivative (**7d**), bearing a *p*-methoxyphenyl group at the 6-position, was found to be a lead compound possessing micromolar binding affinities for cloned human ET receptors (Fig. 1).

In this paper, we describe our synthetic studies starting from the lead compound (**7d**) which culminated in the discovery of a new class of potent nonpeptide ET antagonists, represented by, 6-(4-methoxymethoxyphenyl)-5-methylsulfonylaminomethyl-1-(2-methylthiobenzyl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetic acid (**32g**), 5-ethylsulfonylaminomethyl-6-(4-methoxymethoxyphenyl)-1-(2-methylthiobenzyl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetic acid (**32h**), and 6-(4-methoxymethoxyphenyl)-1-(2-methylthiobenzyl)-2,4-dioxo-5-propylsulfonylaminomethyl-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetic acid (**32i**).

Chemistry The thieno[2,3-*d*]pyrimidine-3-acetic acids bearing various alkyl and aralkyl groups at the 1-position (**7a—m**) were generally synthesized by the route shown in Chart 1. The 2-aminothiophene derivatives **4a—c** were prepared from the corresponding phenylacetone **3a—c** using the previously reported procedure.[27] In addition, compound **4d** was obtained from **4c** by catalytic hydrogenation over 10% palladium–charcoal. Compounds **4a**, **4b**, and **4d** were con-

* To whom correspondence should be addressed.

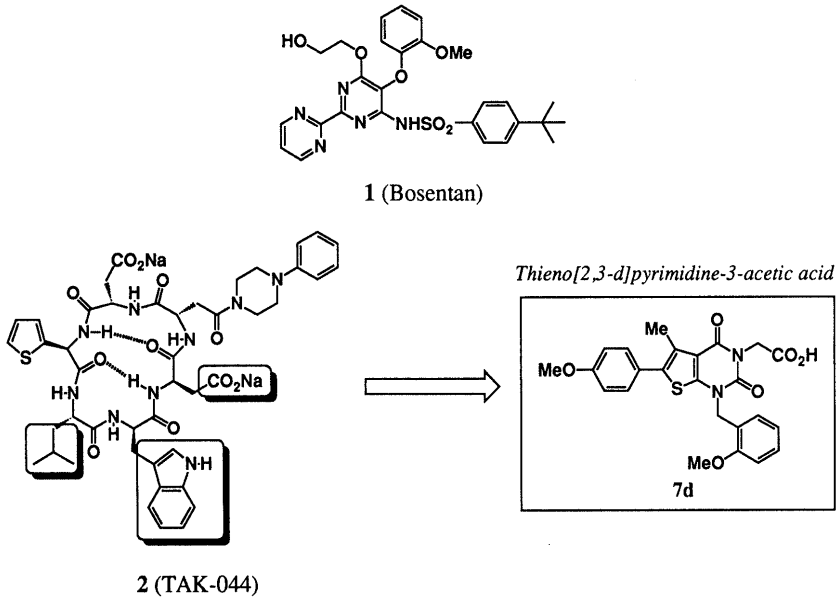
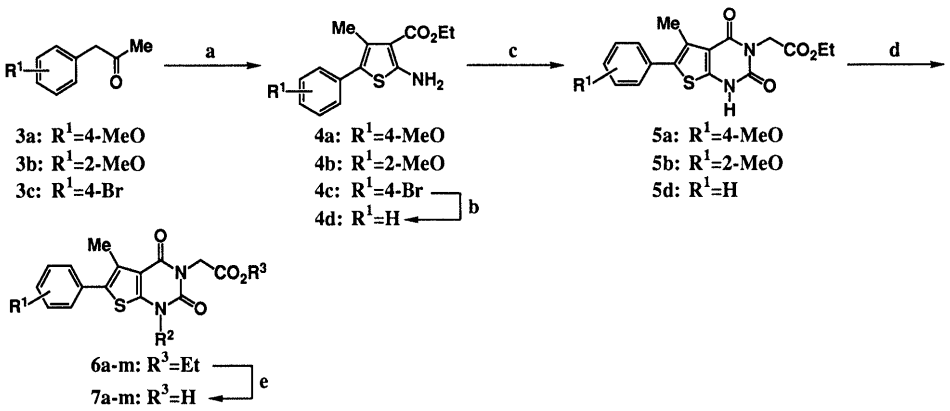


Fig. 1



a: R^1=4-MeO, R^2=*n*-Bu
b: R^1=4-MeO, R^2=$(CH_2)_2$Ph(2-OMe)
c: R^1=4-MeO, R^2=CH_2Ph
d: R^1=4-MeO, R^2=CH_2Ph(2-OMe)
e: R^1=4-MeO, R^2=CH_2Ph(3-OMe)
f: R^1=4-MeO, R^2=CH_2Ph(4-OMe)
g: R^1=4-MeO, R^2=CH_2Ph(2-Me)
h: R^1=4-MeO, R^2=CH_2Ph(2-F)
i: R^1=4-MeO, R^2=CH_2Ph(2-CN)
j: R^1=4-MeO, R^2=CH_2Ph(2-NO_2)
k: R^1=4-MeO, R^2=CH_2Ph(2-SMe)
l: R^1=2-MeO, R^2=CH_2Ph(2-OMe)
m: R^1=H, R^2=CH_2Ph(2-OMe)

(a) 1) $NCCH_2CO_2Et$, AcOH, NH_4OAc, benzene, reflux, 2) S, Et_2NH, EtOH; (b) H_2, Pd-C, AcONa, MeOH; (c) 1) $OCNCH_2CO_2Et$, Py, 2) NaOEt, EtOH; (d) R^2-X, K_2CO_3, DMF; (e) 1N NaOH, EtOH-THF.

Chart 1

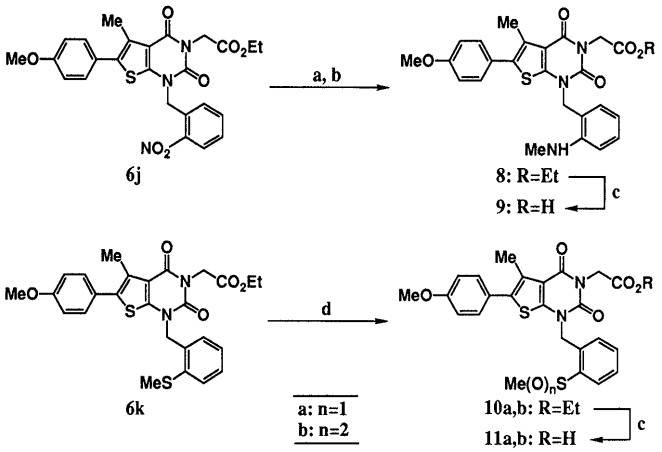
verted to the thieno[2,3-*d*]pyrimidine-2,4-dione derivatives **5a**, **5b**, and **5d** by reaction with ethyl isocyanatoacetate, followed by cyclization with sodium ethoxide. After alkylation of **5a**, **5b**, and **5d** at the 1-position in the presence of potassium carbonate, saponification of the ethyl ester group produced the carboxylic acids **7a**—**m**. The nitro compound **6j** was reduced with iron powder–acetic acid, formylated with acetic formic anhydride,[28] and then reacted with borane–methyl sulfide complex[29] to afford **8**, which was hydrolyzed to yield the methylamino derivative **9**. Oxidation of the methylthio group of **6k** with *m*-chloroperbenzoic acid (1 or 2 equivalents) and subsequent alkaline hydrolysis furnished **11a**, **b** (Chart 2).

Using the general procedure shown in Chart 1, it would be cumbersome to synthesize the substituted phenyl derivatives at the 6-position starting from the corresponding phenylacetones. Thus, a Suzuki aryl-coupling reaction[30] with an arylboronic acid and a palladium catalyst was examined as an alternative method. The key intermediates **14a**, **b** were prepared by reaction of **13** with 1 eq of *N*-bromosuccinimide (NBS) and subsequent alkylation. Compound **13** was readily obtained from the 2-aminothiophene **12**. The 6-bromo derivatives **14a**, **b** reacted with arylboronic acids in the presence of tetrakis(triphenylphosphine)palladium (0) and 2 M aqueous sodium carbonate to produce **15a**, **b**, which were converted to the carboxylic acids **16a**, **b** by alkaline hydrolysis (Chart 3). The arylboronic acids were prepared from the corresponding bromobenzene derivatives by treatment with *n*-

butyllithium, followed by reaction with triisopropyl borate and subsequent acidic hydrolysis.[31]

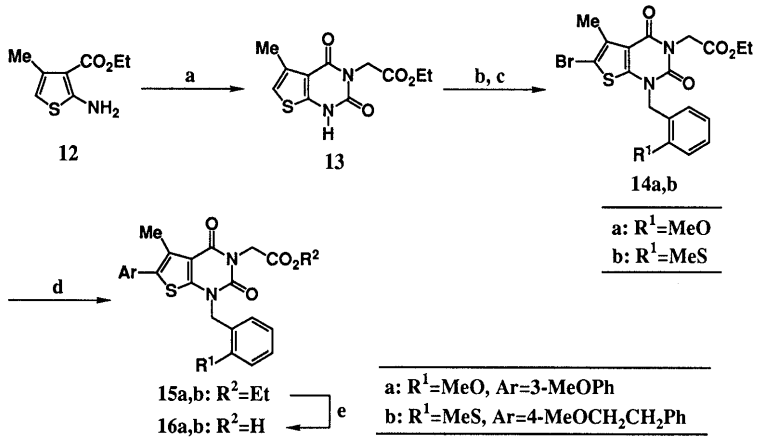
The synthesis of *para*-substituted phenyl derivatives at the 6-position was achieved by the procedures shown in Charts 4 and 5. After demethylation of **5a** with aluminum chloride, acetylation of **17** and subsequent benzylation yielded **18**. Compound **18** was then treated with aqueous potassium carbonate, followed by alkylation to afford **19a—f** (Chart 4). Compound **22** was obtained by the Friedel–Crafts acylation of **5d**, followed by benzylation. In addition, nitration of **5d** with sodium nitrate and concentrated sulfuric acid produced **24**, which was converted to the amide **26** by benzylation, reduction, and subsequent acylation (Chart 5). Saponification of these esters **19a—f**, **22**, **25**, and **26** furnished the corresponding carboxylic acids **20a—f**, **23**, **27**, and **28**.

Chart 6 shows the route used for the preparation of 5-acyl- and 5-sulfonylaminomethyl derivatives. After bromination of **19d** with NBS in the presence of 2,2′-azobis(isobutyronitrile), the aminomethyl derivative **29** was obtained by reaction with potassium phthalimide and subsequent hydrazinolysis (Gabriel synthesis). Compound **29** was reacted with acid chlorides, sulfonyl chlorides, and isocyanate to give **30a—j**. Compound **33** was prepared from **30g** by reaction with iodomethane in the presence of sodium hydride. The esters **29**, **30a—j**, and **33** were converted to the carboxylic acids **31**,



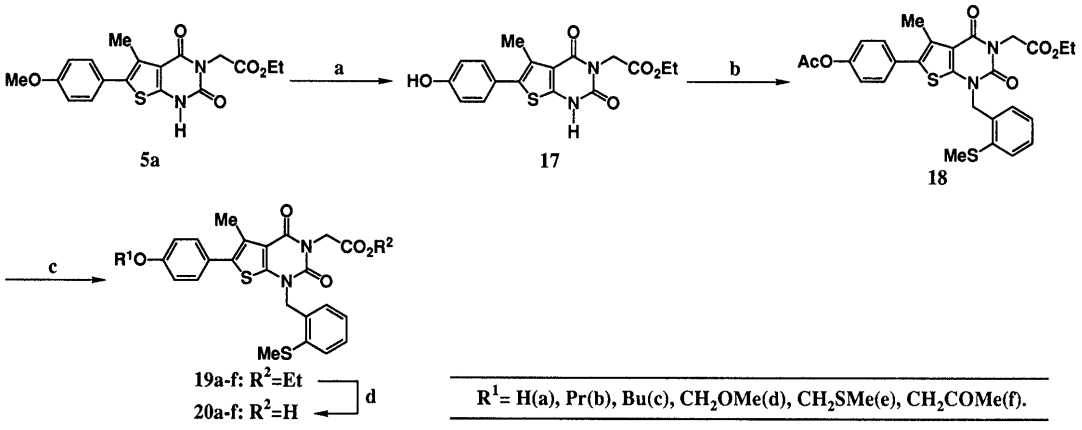
(a) Fe, AcOH, MeOH; (b) 1) HCO_2H, Ac_2O, THF, 2) $Me_2S{:}BH_3$, CH_2Cl_2; (c) 1N NaOH, EtOH-THF; (d) *m*-CPBA(1eq. or 2eq.), CH_2Cl_2.

Chart 2



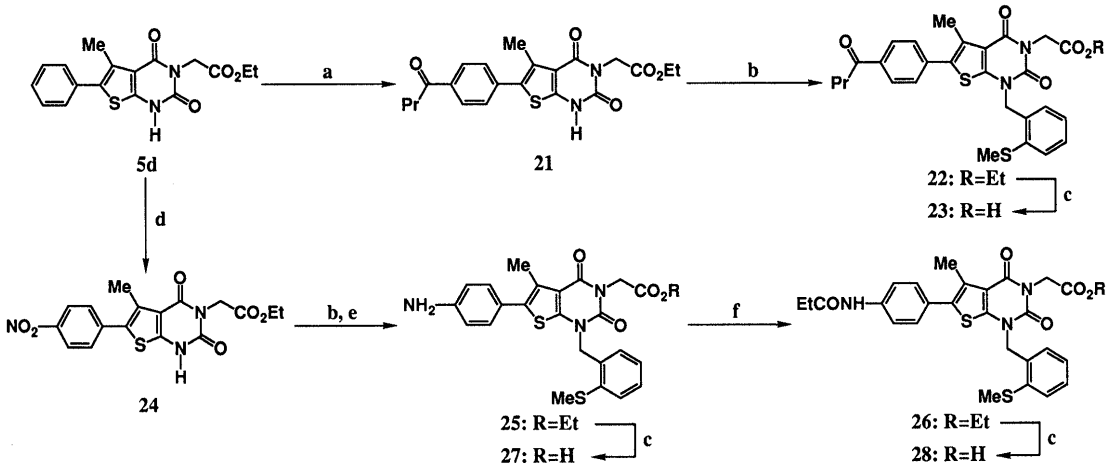
(a) 1) $OCNCH_2CO_2Et$, Py, 2) NaOEt, EtOH; (b) NBS, $CHCl_3$; (c) 2-methoxy- or 2-methylthiobenzyl chloride, K_2CO_3, DMF; (d) $ArB(OH)_2$, $Pd(PPh_3)_4$, 2M Na_2CO_3, DME, under Ar, reflux; (e) 1N NaOH, EtOH-THF.

Chart 3



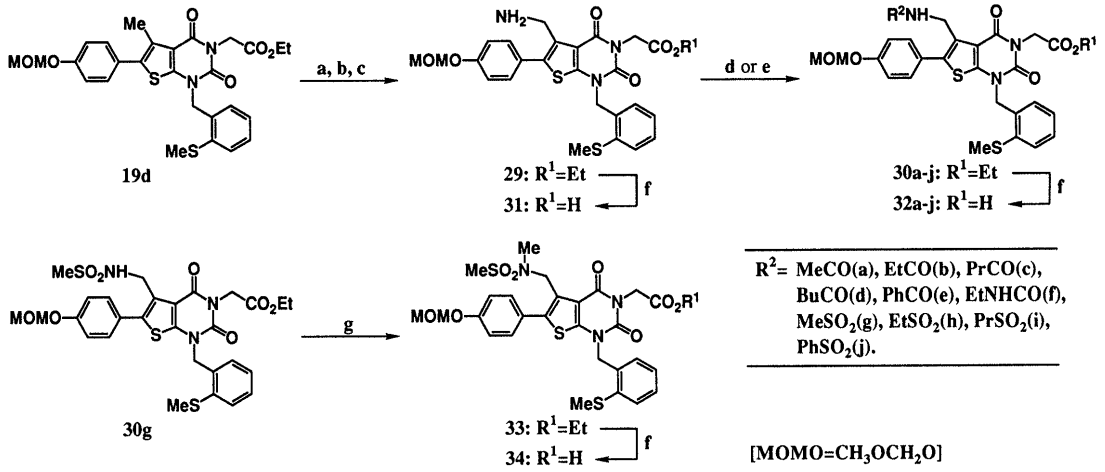
(a) $AlCl_3$, MeSSMe, CH_2Cl_2; (b) 1) Ac_2O, Py, 2) 2-methylthiobenzyl chloride, K_2CO_3, DMF; (c) [R^1=H] aq.K_2CO_3, MeOH-THF, [$R^1 \neq$H] 1) aq.K_2CO_3, MeOH-THF, 2) R^1-X, K_2CO_3 or NaH, DMF; (d) 1N NaOH, EtOH-THF.

Chart 4



(a) PrCOCl, $AlCl_3$, $PhNO_2$, 50°C; (b) 2-methylthiobenzyl chloride, K_2CO_3, DMF; (c) 1N NaOH, EtOH-THF; (d) $NaNO_3$, conc.H_2SO_4; (e) Fe, conc.HCl, EtOH; (f) EtCOCl, Et_3N, CH_2Cl_2.

Chart 5



(a) NBS, AIBN, CCl_4, reflux; (b) potassium phthalimide, DMF; (c) $H_2NNH_2 \cdot H_2O$, EtOH, reflux; (d) R^2Cl, Et_3N or Py, CH_2Cl_2; (e) EtNCO, Py; (f) 1N NaOH, EtOH-THF; (g) MeI, NaH, DMF.

Chart 6

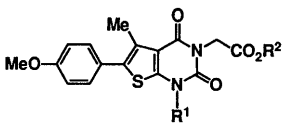
32a—j, and **34** by alkaline hydrolysis.

Biology All the compounds prepared were evaluated for inhibition of [^{125}I]ET-1 binding to cloned human ET_A and ET_B receptors. The *in vitro* antagonistic activities of compounds exhibiting high ET receptor binding affinities were examined for the ability to inhibit the constriction of ring preparations of porcine coronary artery induced by ET-1 at 3 nM and that of vein preparations induced by sarafotoxin S6c, an ET_B selective agonist, at 1 nM.

Results and Discussion

First, the ethyl ester (**6d**) was prepared in order to confirm the role of the carboxylic function in the initial lead **7d** as the recognition site to interact with ET receptors. Subsequently, we then carried out modification of the substituent at the 1-position of **7d** (**7a—k**, **9**, **11a**, **b**). The results are summarized in Table 1. Ester (**6d**) had insignificant binding to both ET_A and ET_B receptors at the concentration examined, whereas the corresponding acid (**7d**) exhibited significant binding affinities with IC_{50} values of 1.9 μM for the ET_A receptor and 12 μM for the ET_B receptor. These results demonstrate that the carboxylic acid moiety at the 3-position was important for ET receptor binding. With respect to the substituents at the 1-position, butyl (**7a**) and *o*-methoxyphenethyl (**7b**) derivatives were less potent than the *o*-methoxybenzyl derivative (**7d**). Therefore, substituted benzyl derivatives were next examined. Deletion of the *o*-methoxy moiety on the phenyl ring of **7d** (**7c**) caused *ca.* 5-fold decrease in the affinities for both ET_A and ET_B receptors. Transposition of the methoxy group of **7d** into the *meta* position (**7e**) maintained comparable affinities to **7d**, but transposition to the *para* position (**7f**) decreased binding affinities. In the series of *ortho*-substituted benzyl derivatives, electron-donating substituents tended to improve binding affinities. Methyl (**7g**) and methylamino (**9**) derivatives were almost equipotent to **7d**. Moreover, the methylthio derivative (**7k**) had 3-fold higher affinity compared with **7d**. On the other hand, bulky and electron-withdrawing substituents, such as methylsulfinyl (**11a**) and methylsulfonyl (**11b**) derivatives, reduced affinities.

Table 1. SAR of Thieno[2,3-*d*]pyrimidine-3-acetic Acids at the 1- and 3-Positions



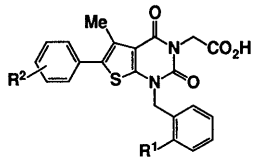
Compd.	R^1	R^2	IC_{50} (μM)[a]	
			ET_A (Sf9)	ET_B (Sf9)
7a	Bu	H	160	230
7b		H	31	76
7c	R = H	H	11	40
7d	R = 2-OMe	H	1.9	12
6d	R = 2-OMe	Et	>20	>20
7e	R = 3-OMe	H	5.1	16
7f	R = 4-OMe	H	110	120
7g	R = 2-Me	H	1.8	14
7h	R = 2-F	H	5.1	20
7i	R = 2-CN	H	13	63
7j	R = 2-NO_2	H	18	67
9	R = 2-NHMe	H	1.2	6
7k	R = 2-SMe	H	0.71	6.8
11a	R = 2-SOMe	H	170	440
11b	R = 2-SO_2Me	H	100	190

a) All data are expressed as means of three or more determinations.

Table 2 shows the effects of substituents on the phenyl group at the 6-position on receptor affinities. In the series of 1-position *o*-methoxybenzyl derivatives, 6-(*p*-methoxyphenyl) derivative (**7d**) was the most potent compound, in comparison with 6-phenyl (**7m**), 6-(*o*-methoxyphenyl) (**7l**), and 6-(*m*-methoxyphenyl) (**16a**) derivatives. This result led us to examine 6-(*p*-substituted)phenyl derivatives. In the series of 1-position *o*-methylthiobenzyl derivatives, introduction of a *p*-alkoxy group into the 6-phenyl ring enhanced binding affinities. The affinities increased in the order of hydroxy (**20a**)<methoxy (**7k**)<butoxy (**20c**)<propoxy (**20b**). Replacement of the 2-methylene moiety in the 4-propoxy group of **20b** with an oxygen (**20d**, IC_{50} values of 0.085 μM for the ET_A receptor and 0.92 μM for the ET_B receptor) or a sulfur atom (**20e**, IC_{50} values of 0.066 μM for the ET_A and 0.66 μM for the ET_B) markedly enhanced binding affinities. However, replacement of the phenolic oxygen of **20d** with methylene (**16b**), or the methoxyl oxygen of **20d** with a carbonyl moiety (**20f**) resulted in loss of this effect. Moreover, ketone (**23**) and amide (**28**) were 10- and 480-fold less potent than **20b** in the affinity for the ET_A receptor, respectively. These results revealed that both the phenolic oxygen and the other oxygen or sulfur atom of **20d** and **20e** were important for high binding affinities, presumably due to the hydrogen bond accepting characteristic of the methoxymethoxy and methylthiomethoxy groups. In addition, the *p*-methoxymethoxy and *p*-methylthiomethoxy groups on the 6-phenyl ring were stable and no degradation of **20d** and **20e** was observed in this binding experiment [Tris–HCl, pH=7.2] (data not shown).

The structure–activity relationship (SAR) of the substituents at the 5-position was investigated, whereby the *p*-methoxymethoxyphenyl moiety was used as the substituent at the 6-position, which exhibited apparent *in vitro* antagonistic activities as discussed later (Table 3). Although introduction of an amino moiety to the 5-methyl group of **20d** (**31**) attenuated binding affinities, acylation of the aminomethyl group of **31** (**32a**—**c**) tended to enhance activities. Introduction of a larger acyl group, as in the case of **32d** and **32e**, tended to decrease activities. In addition, the ethylureido derivative (**32f**) was 2-fold less potent than the butyrylamino

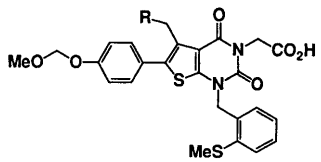
Table 2. SAR of Thieno[2,3-*d*]pyrimidine-3-acetic Acids at the 1- and 6-Positions



Compd.	R^1	R^2	IC_{50} (μM)[a]	
			ET_A (Sf9)	ET_B (Sf9)
7m	MeO	H	12	39
7d	MeO	4-OMe	1.9	12
16a	MeO	3-OMe	130	94
7l	MeO	2-OMe	90	130
7k	MeS	4-OMe	0.71	6.8
20a	MeS	4-OH	1.6	11
20b	MeS	4-OPr	0.25	2.6
20c	MeS	4-OBu	0.49	4.5
20d	MeS	4-OCH_2OMe	0.085	0.92
20e	MeS	4-OCH_2SMe	0.066	0.66
20f	MeS	4-OCH_2COMe	0.4	7.1
23	MeS	4-COPr	2.4	13
16b	MeS	4-CH_2CH_2OMe	1.9	11
27	MeS	4-NH_2	4.0	12
28	MeS	4-NHCOEt	120	77

a) All data are expressed as means of three or more determinations.

Table 3. SAR of Thieno[2,3-*d*]pyrimidine-3-acetic Acids at the 5-Position



Compd.	R	IC_{50} (μM)[a]	
		ET_A (Sf9)	ET_B (Sf9)
20d	H	0.085	0.92
31	NH_2	0.62	3.8
32a	NHCOMe	0.018	0.57
32b	NHCOEt	0.047	1.0
32c	NHCOPr	0.036	0.7
32d	NHCOBu	0.18	1.6
32e	NHCOPh	1.5	0.54
32f	NHCONHEt	0.069	2.0
32g	$NHSO_2Me$	0.0076	0.10
32h	$NHSO_2Et$	0.0061	0.054
32i	$NHSO_2Pr$	0.022	0.047
32j	$NHSO_2Ph$	2.1	3.7
34	$N(Me)SO_2Me$	0.42	0.13

a) All data are expressed as means of three or more determinations.

Table 4. *In Vitro* Antagonistic Activities and Binding Affinities of Thieno[2,3-*d*]pyrimidine-3-acetic Acids for the ET Receptor Subtypes

Compd.	Relaxation (%)								Binding affinity	
	Artery (ET-1 3 nM)[a]				Vein (S6c 1 nM)[b]					
	Concentration (μM)[c]				Concentration (μM)[c]				IC_{50} (nM)[d]	
	0.01	0.1	1	10	0.01	0.1	1	10	ET_A	ET_B
TAK-044	67	87			54	98			0.82	118
Bosentan		0	62	93		28	48	88	7	240
20d			3[e]	49[e]			58[e]	99[e]	85	920
20e				0[e]			66[e]		66	660
32g			36[e]			66[e]	100[e]		7.6	100
32h			33[e]			53[e]	98[e]		6.1	54
32i			0[e]			66[e]	99[e]		22	47

All data are expressed as means of three or more determinations. *a*) Percentage values in relaxation of isolated porcine coronary artery constricted by ET-1 at 3 nM. *b*) Percentage values in relaxation of isolated porcine coronary vein constricted by sarafotoxin S6c at 1 nM. *c*) Concentrations of the compound used in these experiments. *d*) IC_{50} values of [^{125}I]ET-1 binding to the cloned human ET_A and ET_B receptors (Sf9). *e*) These data were obtained using the corresponding potassium salts of the test compounds.

derivative (**32c**). In the case of sulfonamide derivatives, a tendency similar to the results described for the acylamino derivatives was observed. Small alkyl sulfonamides (**32g—i**) were more potent than **20d**, whereas the phenylsulfonylamino derivative (**32j**) was less potent than **20d**. Furthermore, *N*-methylation of the methylsulfonylamino moiety (**34**) decreased affinity for the ET_A receptor compared with **32g**. The methylsulfonylamino (**32g**, IC_{50} values of 0.0076 μM for the ET_A and 0.10 μM for the ET_B) and the ethylsulfonylamino (**32h**, IC_{50} values of 0.0061 μM for the ET_A and 0.054 μM for the ET_B) derivatives had the optimum ET receptor binding affinities in this series. The propylsulfonylamino derivative (**32i**) had the most potent activity for the ET_B receptor (IC_{50} value of 0.047 μM) in this assay.

Next, the *in vitro* antagonistic activities of the compounds (**20d**, **e**, **32g**, **h**, **i**) were examined. As already mentioned, the ET_B receptor mediates both vasoconstriction and vasodilation. Taking into account the relationship between ETs and chronic diseases, the inhibitory effects of these compounds on vasoconstriction mediated by ET_A and ET_B receptors were evaluated. Artery constriction is predominantly mediated by the ET_A receptor, and vein constriction is mediated predominantly by the ET_B receptor.[5] Inhibition of constriction of ring preparations of porcine coronary artery induced by ET-1 and that of vein preparations induced by sarafotoxin S6c was investigated.

Table 4 shows the *in vitro* antagonistic activities and binding affinities of TAK-044, bosentan, and thieno[2,3-*d*]pyrimidine-3-acetic acids (**20d**, **e**, **32g**, **h**, **i**). Bosentan, **20d**, **32g**, and **32h** at 1 μM caused 62, 3, 36, and 33% relaxation of pre-constricted artery, respectively. Moreover, bosentan, **20d**, **20e**, **32g**, **32h**, and **32i** at 1 μM caused 48, 58, 66, 100, 98, and 99% relaxation of pre-constricted vein, respectively. Inhibition of the effects mediated by the ET_B receptor by compounds **32g**, **32h**, and **32i** was more potent than with bosentan, whereas for the effects mediated by the ET_A receptor, **32g** and **32h** were slightly less potent than bosentan. With compounds **20d**, **20e**, **32g**, **32h**, and **32i**, the order of the potencies of relaxation of the artery and vein almost corresponded to those of the binding affinities for the cloned ET_A and ET_B receptor subtypes, respectively. The ability of all compounds to relax vein was more potent than the ability to relax artery. On the other hand, the affinities of all compounds for the ET_A receptor are higher than those for the ET_B receptor.

These results suggest that compounds **32g**, **32h**, and **32i** are selective ET_B antagonists in the functional assay, but non-selective ET antagonists in the receptor binding assay. Recently, intensive studies have suggested that there are pharmacologically discernible ET_B receptor subtypes and/or major species differences.[6,32] The cloned human ET_B receptor was used for the binding assay, but a ring preparation of porcine coronary vein was used for the functional assay. This might explain the difference between binding affinity and antagonistic activity for the ET_B receptor, but the details remain unclear.

In summary, we selected a thieno[2,3-*d*]pyrimidine-2,4-dione nucleus bearing a phenyl ring as a scaffold, and introduced a carboxyl group and aromatic rings, which were important for receptor binding, to this bicyclic heterocycle. SAR studies of the lead compound (**7d**) led to the discovery of novel and potent nonpeptide ET antagonists, **32g**, **32h**, and **32i**. These compounds represent a new class of nonpeptide ET antagonists possessing potent ET_B antagonistic activities.

Experimental

Chemistry All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. The proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on either a Varian Gemini-200 (200 MHz) or a JEOL JNM-LA300 (300 MHz) spectrometer. Chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, quint=quintet, sext=sextet, m=multiplet, dd=doublets of doublet, br s=broad singlet. The infrared (IR) spectra were recorded on a JACSO FT/IR-7000 spectrometer. FAB mass spectra were recorded on a JEOL JMS-HX110. Elemental analyses were within ±0.3% of theoretical values and were determined in Takeda Research Analytical Laboratories, Osaka. Flash chromatography was performed with Merck silica gel 60 (Art. 9385, 230—400 mesh). The yields were not optimized.

Ethyl 2-Amino-5-(4-methoxyphenyl)-4-methylthiophene-3-carboxylate (4a) A mixture of 4-methoxyphenylacetone **3a** (16.5 g, 0.10 mol), ethyl cyanoacetate (12.2 g, 0.10 mol), ammonium acetate (1.55 g, 20 mmol), acetic acid (4.6 ml, 80 mmol), and benzene (20 ml) was refluxed for 24 h while water was azeotropically removed using Dean–Stark apparatus. After removal of the solvent *in vacuo*, the residue was diluted with CH_2Cl_2 and washed with aqueous $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give a dark brown oil, which was dissolved in EtOH (30 ml). To the solution were added sulfur powder (3.21 g, 0.10 mol) and diethylamine (10.4 ml, 0.10 mol). The reaction mixture was

Table 5. Physicochemical Data of Ethyl 2,4-Dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetates

Compd.	Yield (%)	mp (°C) Recryst. solvent[a]	Formula	Analysis (%) Calcd (Found) C	H	N	^{1}H-NMR (in $CDCl_3$) δ
6a	81	134.5—136 EA–H	$C_{22}H_{26}N_2O_5S$	61.38 (61.36)	6.09 6.24	6.51 6.36)	0.98 (3H, t, J=7.6 Hz), 1.30 (3H, t, J=7.1 Hz), 1.43 (2H, sext, J=7.6 Hz), 1.80 (2H, quint, J=7.6 Hz), 2.50 (3H, s), 3.86 (3H, s), 3.96 (2H, t, J=7.6 Hz), 4.24 (2H, q, J=7.1 Hz), 4.78 (2H, s), 6.97 (2H, d, J=8.8 Hz), 7.36 (2H, d, J=8.8 Hz)
6b	81	113—115 EA–H	$C_{27}H_{28}N_2O_6S$	63.76 (63.72	5.55 5.40	5.51 5.31)	1.31 (3H, t, J=7.2 Hz), 2.48 (3H, s), 3.11 (2H, t, J=7.7 Hz), 3.85 (3H, s), 3.86 (3H, s), 4.14 (2H, t, J=7.7 Hz), 4.25 (2H, q, J=7.2 Hz), 4.79 (2H, s), 6.82—7.33 (8H, m)
6c	81	126—128 EA–H	$C_{25}H_{24}N_2O_5S$	64.64 (64.65	5.21 5.44	6.03 5.77)	1.31 (3H, t, J=7.2 Hz), 2.48 (3H, s), 3.84 (3H, s), 4.26 (2H, q, J=7.2 Hz), 4.83 (2H, s), 5.19 (2H, s), 6.94 (2H, d, J=8.8 Hz), 7.30—7.38 (7H, m)
6d	78	141—142 EE–H	$C_{26}H_{26}N_2O_6S$	63.14 (63.01	5.30 5.48	5.66 5.48)	1.30 (3H, t, J=7.2 Hz), 2.49 (3H, s), 3.83 (3H, s), 3.87 (3H, s), 4.25 (2H, q, J=7.2 Hz), 4.83 (2H, s), 5.24 (2H, s), 6.86—6.96 (4H, m), 7.09—7.14 (1H, m), 7.22—7.31 (3H, m)
6e	91	144—145 EA–EE	$C_{26}H_{26}N_2O_6S$	63.14 (62.93	5.30 5.38	5.66 5.59)	1.30 (3H, t, J=7.1 Hz), 2.48 (3H, s), 3.79 (3H, s), 3.84 (3H, s), 4.25 (2H, q, J=7.1 Hz), 4.83 (2H, s), 5.16 (2H, s), 6.80—6.97 (5H, m), 7.22—7.32 (3H, m)
6f	87	167—168 EA–EE	$C_{26}H_{26}N_2O_6S$	63.14 (62.88	5.30 5.28	5.66 5.40)	1.30 (3H, t, J=7.1 Hz), 2.47 (3H, s), 3.78 (3H, s), 3.84 (3H, s), 4.25 (2H, q, J=7.1 Hz), 4.82 (2H, s), 5.12 (2H, s), 6.86 (2H, d, J=8.8 Hz), 6.94 (2H, d, J=8.8 Hz), 7.29 (2H, d, J=8.8 Hz), 7.35 (2H, d, J=8.8 Hz)
6g	78	127— 128.5 EA–H	$C_{26}H_{26}N_2O_5S$	65.25 (65.21	5.48 5.27	5.85 5.66)	1.31 (3H, t, J=7.2 Hz), 2.40 (3H, s), 2.50 (3H, s), 3.83 (3H, s), 4.25 (2H, q, J=7.2 Hz), 4.85 (2H, s), 5.22 (2H, s), 6.90—7.29 (8H, m)
6h	77	125—127 EA–H	$C_{25}H_{23}FN_2O_5S$	62.23 (62.42	4.80 4.89	5.81 5.93)	1.31 (3H, t, J=7.2 Hz), 2.49 (3H, s), 3.84 (3H, s), 4.26 (2H, q, J=7.2 Hz), 4.84 (2H, s), 5.28 (2H, s), 6.93 (2H, d, J=8.9 Hz), 7.05—7.15 (2H, m), 7.25—7.31 (4H, m)
6i	80	176—177 EA–H	$C_{26}H_{23}N_3O_5S$	63.79 (63.84	4.74 4.84	8.58 8.41)	1.32 (3H, t, J=7.2 Hz), 2.50 (3H, s), 3.84 (3H, s), 4.26 (2H, q, J=7.2 Hz), 4.85 (2H, s), 5.46 (2H, s), 6.93 (2H, d, J=9.0 Hz), 7.28 (2H, d, J=9.0 Hz), 7.30—7.74 (4H, m)
6j	62	154—156 EA–H	$C_{25}H_{23}N_3O_7S$	58.93 (58.78	4.55 4.46	8.25 8.26)	1.31 (3H, t, J=7.2 Hz), 2.53 (3H, s), 3.83 (3H, s), 4.25 (2H, q, J=7.2 Hz), 4.83 (2H, s), 5.65 (2H, s), 6.92 (2H, d, J=8.8 Hz), 7.27 (2H, d, J=8.8 Hz), 7.22—8.21 (4H, m)
6k	88	144—145 E	$C_{26}H_{26}N_2O_5S_2$	61.16 (60.98	5.13 5.34	5.49 5.34)	1.30 (3H, t, J=7.1 Hz), 2.53 (3H, s), 3.87 (3H, s), 4.25 (2H, q, J=7.1 Hz), 4.83 (2H, s), 5.25 (2H, s), 6.87—6.94 (2H, m), 7.10—7.15 (1H, m), 7.23—7.44 (6H, m)
6l	90	147—148 EA	$C_{26}H_{26}N_2O_6S \cdot 0.1H_2O$	62.91 (62.73	5.32 5.29	5.64 5.48)	1.30 (3H, t, J= 7.1 Hz), 2.36 (3H, s), 3.80 (3H, s), 3.86 (3H, s), 4.25 (2H, q, J=7.1 Hz), 4.83 (2H, s), 5.24 (2H, s), 6.86—7.02 (4H, m), 7.12—7.39 (4H, m)
6m	89	151—152 EA	$C_{25}H_{24}N_2O_5S$	64.64 (64.72	5.21 5.21	6.03 6.23)	1.30 (3H, t, J=7.1 Hz), 2.53 (3H, s), 3.87 (3H, s), 4.25 (2H, q, J=7.1 Hz), 4.83 (2H, s), 5.25 (2H, s), 6.87—6.94 (2H, m), 7.10—7.15 (1H, m), 7.23—7.44 (6H, m)
22	91	124—125 EA–EE	$C_{29}H_{30}N_2O_5S_2 \cdot 0.3EtOAc$	62.85 (62.56	5.65 5.46	4.85 4.54)	1.01 (3H, t, J=7.3 Hz), 1.31 (3H, t, J=7.1 Hz), 1.78 (2H, q, J=7.3 Hz), 2.54 (3H, s), 2.58 (3H, s) 2.94 (2H, t, J=7.3 Hz), 4.26 (2H, q, J=7.1 Hz), 4.80 (2H, s), 5.36 (2H, s), 7.01—7.36 (4H, m), 7.44 (2H, d, J=8.4 Hz), 7.98 (2H, d, J=8.4 Hz)

a) Recrystallization solvent: EA=ethyl acetate, EE=ethyl ether, E=ethanol, H=hexane.

stirred at 60 °C for 2 h. After evaporation of the solvent *in vacuo*, the residue was diluted with water and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). The solution was concentrated *in vacuo* and the residue purified by flash column chromatography (CH_2Cl_2). The resulting oily product was crystallized from ether–hexane to give **4a** (11.5 g, 40%) as pale yellow plates, mp 79—80 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.37 (3H, t, J=7.1 Hz), 2.28 (3H, s), 3.83 (3H, s), 4.31 (2H, q, J=7.1 Hz), 6.05 (2H, br s), 6.91 (2H, d, J=8.8 Hz), 7.27 (2H, d, J=8.8 Hz). IR (KBr): 3426, 3328, 1651, 1586, 1550, 1505, 1485 cm^{-1}. FAB-MS *m/e*: 291 (M^+). *Anal.* Calcd for $C_{15}H_{17}NO_3S$: C, 61.83; H, 5.88; N, 4.81; S, 11.01. Found: C, 61.81; H, 5.75; N, 4.74; S, 10.82.

Compounds **4b** and **4c** were prepared from **3b** and **3c** by a similar procedure to that used for the preparation of **4a**, in 12 and 47% yields, respectively. **4b**: mp 70—71 °C (from ether–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, J=7.1 Hz), 2.16 (3H, s), 3.82 (3H, s), 4.30 (2H, q, J=7.1 Hz), 6.05 (2H, br s), 6.93—7.01 (2H, m), 7.22—7.36 (2H, m). *Anal.* Calcd for $C_{15}H_{17}NO_3S$: C, 61.83; H, 5.88; N, 4.81; S, 11.01. Found: C, 61.97; H, 5.88; N, 4.83; S, 11.06. **4c**: mp 105—106 °C (from ether–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.37 (3H, t, J=7.1 Hz), 2.30 (3H, s), 4.32 (2H, q, J=7.1 Hz), 6.12 (2H, s), 7.21 (2H, d, J=8.4 Hz), 7.49 (2H, d, J=8.4 Hz). FAB-MS *m/e*: 339 (M^+, ^{79}Br), 341 (M^+, ^{81}Br). *Anal.* Calcd for $C_{14}H_{14}BrNO_2S$: C, 49.42; H, 4.15; N, 4.12; S, 9.42. Found: C, 49.43; H, 4.06; N, 4.13; S, 9.46.

Ethyl 2-Amino-4-methyl-5-phenylthiophene-3-carboxylate (4d) A mixture of **4c** (10 g, 29.4 mmol) and sodium acetate (4.90 g, 59.7 mmol) in MeOH (300 ml) was hydrogenated over 10% palladium–charcoal (containing 50% H_2O, 1.0 g) with vigorous stirring at room temperature for 5 h. The reaction mixture was filtered through Celite and the filtrate was concentrated *in vacuo*. The residue was diluted with water and extracted with $CHCl_3$. The extract was washed with brine and dried ($MgSO_4$). The solution was concentrated *in vacuo* to give the product (7.40 g, 96%) as a pale yellow oil. Crystallization from ether-hexane afforded colorless needles, mp 64—65 °C (lit.[27] mp 95 °C). ^{1}H-NMR ($CDCl_3$) δ: 1.37 (3H, t, J=7.1 Hz), 2.33 (3H, s), 4.32 (2H, q, J=7.1 Hz), 6.09 (2H, br), 7.24—7.42 (5H, m). IR (KBr): 3388, 3278, 1665, 1584, 1549, 1481 cm^{-1}. *Anal.* Calcd for $C_{14}H_{15}NO_2S$: C, 64.34; H, 5.79; N, 5.36. Found: C, 64.51; H, 5.77; N, 5.29.

Ethyl 6-(4-Methoxyphenyl)-5-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetate (5a) A mixture of **4a** (5.0 g, 17.2 mmol) and ethyl isocyanatoacetate (2.9 ml, 25.8 mmol) in pyridine (20 ml) was stirred at 45 °C for 3 h. After evaporation of the solvent *in vacuo*, the residue was dissolved in absolute EtOH (30 ml). To this solution was added ethanolic sodium ethoxide [prepared from sodium (0.79 g, 34.3 mmol) and absolute EtOH (30 ml)]. After being stirred at room temperature for 1.5 h, the mixture

Table 6. Physicochemical Data of 2,4-Dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetic Acids

Compd.	Yield (%)	mp (°C) Recryst. solvent[a)]	Formula	Analysis (%) Calcd (Found) C	H	N	^{1}H-NMR δ
7a	96	195—197 E	$C_{20}H_{22}N_2O_5S$	59.69 (59.57	5.51 5.38	6.96 6.80)	0.94 (3H, t, *J*=7.2 Hz), 1.37 (2H, sext, *J*=7.2 Hz), 1.68 (2H, quint, *J*=7.2 Hz), 2.44 (3H, s), 3.83 (3H, s), 3.95 (2H, t, *J*=7.2 Hz), 4.58 (2H, s), 7.07 (2H, d, *J*=8.8 Hz), 7.43 (2H, d, *J*=8.8 Hz)[b)]
7b	82	201.5—203 E	$C_{25}H_{24}N_2O_6S$	62.49 (62.38	5.03 5.07	5.83 5.71)	2.46 (3H, s), 3.09 (2H, t, *J*=7.1 Hz), 3.82 (3H, s), 3.84 (3H, s), 4.13 (2H, t, *J*=7.1 Hz), 4.83 (2H, s), 6.79—7.31 (8H, m)[c)]
7c	78	194 E	$C_{23}H_{20}N_2O_5S$	63.29 (63.06	4.62 4.57	6.42 6.51)	2.49 (3H, s), 3.85 (3H, s), 4.91 (2H, s), 5.20 (2H, s), 6.94 (2H, d, *J*=9.0 Hz), 7.31—7.38 (7H, m)[c)]
7d	93	216—218 EE–H	$C_{24}H_{22}N_2O_6S$	61.79 (61.60	4.75 4.87	6.00 6.16)	2.41 (3H, s), 3.78 (3H, s), 3.85 (3H, s), 4.61 (2H, s), 5.14 (2H, s), 6.85—6.92 (1H, m), 6.98—7.07 (4H, m), 7.25—7.36 (3H, m)[b)]
7e	54	181—182 EA–EE	$C_{24}H_{22}N_2O_6S \cdot 0.5H_2O$	60.62 (60.76	4.88 4.74	5.89 5.87)	2.40 (3H, s), 3.72 (3H, s), 3.79 (3H, s), 4.63 (2H, s), 5.17 (2H, s), 6.85—6.92 (3H, m), 7.02 (2H, d, *J*=8.8 Hz), 7.23—7.36 (3H, m)[b)]
7f	81	193—195 E	$C_{24}H_{22}N_2O_6S \cdot 0.1H_2O$	61.55 (61.26	4.78 4.83	5.98 5.90)	2.40 (3H, s), 3.72 (3H, s), 3.79 (3H, s), 4.62 (2H, s), 5.12 (2H, s), 6.91 (2H, d, *J*=8.8 Hz), 7.03 (2H, d, *J*=8.8 Hz), 7.30 (2H, d, *J*=6.3 Hz), 7.35 (2H, d, *J*=6.3 Hz)[b)]
7g	87	239—240 E	$C_{24}H_{22}N_2O_5S$	63.99 (63.92	4.92 4.97	6.22 6.29)	2.38 (3H, s), 2.48 (3H, s), 3.82 (3H, s), 4.91 (2H, s), 5.21 (2H, s), 6.88—7.27 (8H, m)[c)]
7h	59	196—197 E	$C_{23}H_{19}FN_2O_5S$	60.78 (60.73	4.21 4.24	6.16 6.06)	2.49 (3H, s), 3.84 (3H, s), 4.91 (2H, s), 5.29 (2H, s), 6.94 (2H, d, *J*=8.8 Hz), 7.05—7.31 (6H, m)[b)]
7i	71	260—262 E	$C_{24}H_{19}N_3O_5S \cdot 0.1H_2O$	62.22 (61.92	4.17 4.23	9.07 8.90)	2.42 (3H, s), 3.79 (3H, s), 4.61 (2H, s), 5.39 (2H, s), 7.00—7.93 (8H, m)[b)]
7j	58	190—191 E	$C_{23}H_{19}N_3O_7S$	57.38 (57.10	3.98 3.78	8.73 8.91)	2.45 (3H, s), 3.78 (3H, s), 4.61 (2H, s), 5.56 (2H, s), 7.00 (2H, d, *J*=8.8 Hz), 7.34 (2H, d, *J*=8.8 Hz), 7.26—8.23 (4H, m)[b)]
7k	87	185—189 EA–H	$C_{24}H_{22}N_2O_5S_2 \cdot 0.1EtOAc$	58.93 (58.62	5.29 5.36	4.90 4.63)	2.50 (3H, s), 2.53 (3H, s), 3.83 (3H, s), 4.92 (2H, s), 5.35 (2H, m), 6.89—7.32 (9H, m)[c)]
7l	67	216—217.5 EE–H	$C_{24}H_{22}N_2O_6S \cdot 0.1H_2O$	61.55 (61.39	4.78 4.79	5.98 6.00)	2.24 (3H, s), 3.75 (3H, s), 3.84 (3H, s), 4.62 (2H, s), 5.13 (2H, s), 6.85—7.13 (5H, m), 7.22—7.45 (3H, m)[b)]
7m	79	252—255 EA	$C_{23}H_{20}N_2O_5S \cdot 0.1H_2O$	63.03 (62.80	4.65 4.71	6.39 6.32)	2.46 (3H, s), 3.85 (3H, s), 4.62 (2H, s), 5.16 (2H, s), 6.85—6.92 (1H, m), 7.02—7.07 (2H, m), 7.25—7.51 (6H, m)[b)]
9	100	204—206 EA–H	$C_{24}H_{23}N_3O_5S$	61.92 (61.66	4.98 5.02	9.03 8.82)	2.45 (3H, s), 2.78 (3H, s), 3.81 (3H, s), 4.65 (2H, s), 5.07 (2H, s), 5.40 (1H, s), 6.53—6.65 (2H, m), 6.82 (1H, d, *J*=7.2 Hz), 7.03 (2H, d, *J*=8.4 Hz), 7.17 (1H, t, *J*=7.2 Hz), 7.35 (2H, d, *J*=8.4 Hz)[b)]
11a	67	223—224 E	$C_{24}H_{22}N_2O_6S_2$	57.82 (57.67	4.45 4.59	5.62 5.59)	2.45 (3H, s), 2.83 (3H, s), 3.81 (3H, s), 4.65 (2H, s), 5.24, 5.49 (each 1H, ABq, *J*=16.7 Hz), 7.05 (2H, d, *J*=8.8 Hz), 7.27 (1H, d, *J*=7.6 Hz), 7.36 (2H, d, *J*=8.8 Hz), 7.54 (1H, t, *J*=7.6 Hz), 7.64 (1H, t, *J*=7.6 Hz), 7.97 (1H, d, *J*=7.6 Hz)[b)]
11b	96	142—145 EA–H	$C_{24}H_{22}N_2O_7S_2 \cdot 0.5EtOAc$	55.90 (55.73	4.69 4.75	5.01 5.06)	2.47 (3H, s), 3.43 (3H, s), 3.80 (3H, s), 4.65 (2H, s), 5.67 (2H, s), 7.03 (2H, d, *J*=8.8 Hz), 7.26 (1H, d, *J*=8.8 Hz), 7.36 (2H, d, *J*=8.8 Hz), 7.61—7.73 (2H, m), 8.05 (1H, d, *J*=8.8 Hz)[b)]
16a	84	221—222 EA–EE	$C_{24}H_{22}N_2O_6S \cdot 0.1H_2O$	61.55 (61.35	4.78 4.94	5.98 5.77)	2.46 (3H, s), 3.78 (3H, s), 3.85 (3H, s), 4.62 (2H, s), 5.15 (2H, s), 6.86—6.97 (4H, m), 7.02—7.06 (2H, m), 7.27—7.39 (2H, m)[b)]
16b	73	186—187 E	$C_{26}H_{26}N_2O_5S_2 \cdot 0.5H_2O$	60.10 (60.10	5.24 5.12	5.39 5.41)	2.47 (3H, s), 2.57 (3H, s), 2.84 (2H, t, *J*=6.7 H), 3.25 (3H, s), 3.56 (2H, t, *J*=6.7 H), 4.65 (2H, s), 5.21 (2H, s), 6.97—7.42 (8H, m)[b)]
20a	72	244—246 EA–H	$C_{23}H_{20}N_2O_5S_2$	56.78 (56.71	4.56 4.29	5.76 5.55)	2.43 (3H, s), 2.57 (3H, s), 4.65 (2H, s), 5.21 (2H, s), 6.84 (2H, d, *J*=8.8 Hz), 6.99 (1H, d, *J*=7.9 Hz), 7.12—7.45 (5H, m), 9.76 (1H, s)[b)]
20b	78	185—188 E	$C_{26}H_{26}N_2O_5S_2 \cdot 0.5H_2O$	60.10 (60.34	5.24 5.05	5.39 5.40)	0.99 (3H, t, *J*=7.3 Hz), 1.74 (2H, sext, *J*=7.3 Hz), 2.44 (3H, s), 2.57 (3H, s), 3.96 (2H, d, *J*=7.3 Hz), 4.64 (2H, s), 5.21 (2H, s), 6.97—7.44 (8H, m)[b)]
20c	72	178—180 E	$C_{27}H_{28}N_2O_5S_2$	61.81 (61.83	5.38 5.46	5.34 5.22)	0.98 (3H, t, *J*=7.3 Hz), 1.49 (2H, sext, *J*=7.3 Hz), 1.78 (2H, quint, *J*=7.3 Hz), 2.50 (3H, s), 2.53 (3H, s), 3.98 (2H, t, *J*=7.3 Hz), 4.92 (2H, s), 5.34 (2H, s), 6.88—7.32 (8H, m)[b)]
20d	79	142—144 EA–EE–H	$C_{25}H_{24}N_2O_6S_2 \cdot 0.5H_2O$	57.57 (57.84	4.83 4.69	5.37 5.36)	2.43 (3H, s), 2.55 (3H, s), 3.38 (3H, s), 4.63 (2H, s), 5.19 (2H, s), 5.21 (2H, s), 6.96—7.17 (4H, m), 7.28—7.42 (4H, m)[b)]
20e	79	152—154 EA–EE–H	$C_{25}H_{24}N_2O_5S_3$	56.80 (56.52	4.58 4.62	5.30 5.29)	2.18 (3H, s), 2.43 (3H, s), 2.55 (3H, s), 4.63 (2H, s), 5.20 (2H, s), 5.30 (2H, s), 6.96—7.18 (4H, m), 7.29—7.42 (4H, m)[b)]
20f	78	285—290(d) E	$C_{26}H_{24}N_2O_6S_2 \cdot 0.5CH_2Cl_2$	56.12 (55.85	4.44 4.51	4.93 4.70)	2.17 (3H, s), 2.44 (3H, s), 2.57 (3H, s), 4.32 (2H, s), 4.87 (2H, s), 5.17 (2H, s), 6.95—7.37 (8H, m)[b)]
23	50	197—199 E	$C_{27}H_{26}N_2O_5S \cdot 0.1H_2O$	61.84 (61.64	5.04 5.09	5.34 5.32)	0.94 (3H, t, *J*=7.2 Hz), 1.66 (2H, q, *J*=7.2 Hz), 2.55 (3H, s), 2.58 (3H, s), 3.02 (2H, t, *J*=7.2 Hz), 4.66 (2H, s), 5.24 (2H, s), 7.02 (1H, d, *J*=7.7 Hz), 7.16 (1H, t, *J*=7.7 Hz), 7.32—7.45 (2H, m), 7.58 (2H, d, *J*=8.2 Hz), 8.03 (2H, d, *J*=8.2 Hz)[b)]
27	70	>300 E	$C_{23}H_{21}N_3O_4S_2 \cdot 2.8HCl$	48.49 (48.32	4.21 4.22	7.37 7.11)	2.41 (3H, s), 2.56 (3H, s), 4.40 (2H, s), 5.16 (2H, s), 5.38 (2H, br s), 6.60 (2H, d, *J*=8.6 Hz), 6.95—7.45 (6H, m)[b)]
28	73	274—275 E	$C_{26}H_{25}N_3O_5S_2 \cdot 0.5H_2O$	58.63 (58.71	4.92 4.90	7.89 7.79)	1.08 (3H, t, *J*=7.5 Hz), 2.34 (2H, q, *J*=7.5 Hz), 2.46 (3H, s), 2.57 (3H, s), 4.65 (2H, s), 5.20 (2H, s), 6.98 (1H, d, *J*=7.6 Hz), 7.15 (1H, t, *J*=7.6 Hz), 7.31—7.43 (4H, m), 7.68 (2H, d, *J*=8.6 Hz), 10.02 (1H, s)[b)]

Table 6. (continued)

Compd.	Yield (%)	mp (°C) Recryst. solvent[a]	Formula	Analysis (%) Calcd (Found) C	H	N	^{1}H-NMR δ
31	100	>300 EA	$C_{25}H_{25}N_3O_6S_2 \cdot 1.6H_2O$	53.96 (53.76	5.11 5.00	7.44 7.34)	2.54 (3H, s), 3.36 (3H, s), 3.88 (2H, s), 4.45 (2H, s), 5.21 (2H, s), 5.22 (2H, s), 6.99—7.15 (4H, m), 7.29—7.40 (4H, m)[b]
32a	58	205—212 EA–H	$C_{27}H_{27}N_3O_7S_2 \cdot 1.8H_2O$	53.86 (53.92	5.12 4.90	6.98 6.71)	1.78 (3H, s), 2.55 (3H, s), 3.37 (3H, s), 4.36 (2H, s), 4.37—4.40 (4H, m), 5.19 (2H, s), 5.22 (2H, s), 7.00—7.16 (4H, m), 7.34—7.41 (4H, m), 7.86—7.87 (1H, m)[b]
32b	62	170—175 M–EE	$C_{28}H_{29}N_3O_7S_2 \cdot 0.8H_2O$	56.23 (56.20	5.16 5.27	7.03 7.21)	1.13 (3H, t, J=7.5 Hz), 2.21 (2H, q, J=7.5 Hz), 2.53 (3H, s), 3.47 (3H, s), 4.52 (2H, d, J=6.6 Hz), 4.94 (2H, s), 5.18 (2H, s), 5.35 (2H, s), 7.02—7.34 (6H, m), 7.50 (2H, d, J=8.8 Hz)[c]
32c	69	170—172 M	$C_{29}H_{31}N_3O_7S_2 \cdot 0.5H_2O$	57.41 (57.26	5.32 5.43	6.93 6.75)	0.92 (3H, t, J=7.4 Hz), 1.57—1.78 (2H, m), 2.13—2.17 (2H, m), 2.53 (3H, s), 3.47 (3H, s), 4.52 (2H, d, J=6.6 Hz), 4.95 (2H, s), 5.18 (2H, s), 5.35 (2H, s), 7.01—7.35 (6H, m), 7.50 (2H, d, J=8.5 Hz)[c]
32d	96	177—179 M	$C_{30}H_{33}N_3O_7S_2 \cdot H_2O$	57.22 (57.26	5.60 5.35	6.67 6.86)	0.89 (3H, t, J=7.3 Hz), 1.26—1.38 (2H, m), 1.54—1.64 (2H, m), 2.17 (2H, t, J=7.7 Hz), 2.53 (3H, s), 3.47 (3H, s), 4.52 (2H, d, J=6.4 Hz), 4.95 (2H, s), 5.18 (2H, s), 5.36 (2H, s), 7.02—7.34 (6H, m), 7.50 (2H, d, J=8.5 Hz)[c]
32e	46	215—218 EA–H	$C_{32}H_{29}N_3O_7S_2 \cdot 2.5H_2O$	56.79 (56.51	5.06 4.79	6.21 6.25)	2.54 (3H, s), 3.35 (3H, s), 4.37 (2H, s), 4.60 (2H, s), 5.19 (4H, s), 7.03—7.11 (4H, m), 7.28—7.50 (7H, m), 7.71 (2H, d, J=8.5 Hz), 8.56 (1H, t, J=6.6 Hz)[b]
32f	84	202—204 M	$C_{28}H_{30}N_4O_7S_2$	56.17 (55.97	5.05 5.27	9.36 9.38)	1.05 (3H, t, J=7.2 Hz), 2.52 (2H, s), 3.07 (2H, q, J=7.2Hz), 3.47 (3H, s), 4.45 (2H, s), 4.88 (2H, s), 5.18 (2H, s), 5.35 (2H, s), 7.02—7.34 (6H, m), 7.48 (2H, d, J=8.6 Hz)[c]
32g	71	204—206 EA–EE	$C_{26}H_{27}N_3O_8S_3$	51.56 (51.76	4.49 4.69	6.94 6.88)	2.55 (3H, s), 2.89 (3H, s), 3.38 (3H, s), 4.30 (2H, d, J=5.4 Hz), 4.67 (2H, s), 5.23 (4H, s), 6.93 (1H, t, J=5.4 Hz), 6.98—7.00 (1H, m), 7.09—7.17 (3H, m), 7.31—7.43 (4H, m)[b]
32h	80	125—128 EA–H	$C_{27}H_{29}N_3O_8S_3 \cdot H_2O$	50.85 (51.15	4.90 4.78	6.59 6.54)	1.33 (3H, t, J=7.4 Hz), 2.53 (3H, s), 2.96 (2H, q, J=7.4 Hz), 3.48 (3H, s), 4.35 (2H, d, J=6.6 Hz), 4.92 (2H, s), 5.19 (2H, s), 5.36 (2H, s), 6.05 (1H, t, J=6.6 Hz), 7.01—7.37 (8H, m)[c]
32i	93	123—124 EA–H	$C_{28}H_{31}N_3O_8S_3 \cdot 0.5H_2O$	52.32 (52.21	5.02 4.87	6.54 6.51)	0.98 (3H, t, J=7.2 Hz), 1.77—1.78 (2H, m), 2.86—2.92 (2H, m), 2.53 (3H, s), 3.48 (3H, s), 4.35 (2H, d, J=6.8 Hz), 4.92 (2H, s), 5.19 (2H, s), 5.36 (2H, s), 6.04—6.05 (1H, m), 7.00—7.18 (5H, m), 7.32—7.37 (3H, m)[c]
32j	52	128—129 IPE	$C_{31}H_{29}N_3O_8S_3 \cdot 0.5H_2O$	55.02 (54.85	4.47 4.44	6.21 6.31)	2.54 (3H, s), 3.40 (3H, s), 4.19 (2H, d, J=5.4 Hz), 4.52 (2H, s), 5.13 (2H, s), 5.24 (2H, s), 6.87—7.55 (15H, m)[b]
34	93	200—204 EA–EE	$C_{27}H_{29}N_3O_8S_3 \cdot 2.5H_2O$	48.78 (48.60	5.16 4.95	6.32 6.27)	2.54 (3H, s), 2.55 (3H, s), 2.84 (3H, s), 3.38 (3H, s), 4.39 (2H, s), 4.56 (2H, s), 5.21 (4H, s), 7.03—7.15 (4H, m), 7.28—7.44 (4H, m)[b]

a) Recrystallization solvent: EA=ethyl acetate, EE=ethyl ether, E=ethanol, H=hexane, IPE=isopropyl ether, M=methanol. *b*) in DMSO-d_6. *c*) in $CDCl_3$.

was acidified with 2 N HCl (20 ml, 40 mmol) and concentrated *in vacuo* (removal of EtOH). The precipitate was collected by filtration, dried, and recrystallized from EtOH to give **5a** (6.18 g, 96%) as colorless needles, mp 164—165 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7.2 Hz), 2.45 (3H, s), 3.85 (3H, s), 4.26 (2H, q, J=7.2 Hz), 4.78 (2H, s), 6.95 (2H, d, J=8.8 Hz), 7.31 (2H, d, J=8.8 Hz), 10.58 (1H, s). IR (KBr): 2914, 1742, 1713, 1655, 1605, 1568, 1528, 1499, 1450 cm^{-1}. *Anal.* Calcd for $C_{18}H_{18}N_2O_5S$: C, 57.74; H, 4.85; N, 7.48. Found: C, 57.78; H, 5.03; N, 7.45.

Compounds **5b**, **d**, and **13** were prepared by a similar procedure to that used for the preparation of **5a**, in 81, 86, and 88% yields, respectively. **5b**: mp 83—84 °C (from ether–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, t, J=7.1 Hz), 2.35 (3H, s), 3.83 (3H, s), 4.24 (2H, q, J=7.1 Hz), 4.77 (2H, s), 6.96—7.05 (2H, m), 7.25—7.43 (2H, m), 10.47 (1H, s). *Anal.* Calcd for $C_{18}H_{18}N_2O_5S \cdot 0.1H_2O$: C, 57.47; H, 4.88; N, 7.45. Found: C, 57.43; H, 4.90; N, 7.33. **5d**: mp 119—120 °C (from EtOH). ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7.1 Hz), 2.49 (3H, s), 4.26 (2H, q, J=7.1 Hz), 4.79 (2H, s), 7.35—7.47 (5H, m), 10.58 (1H, br s). *Anal.* Calcd for $C_{17}H_{16}N_2O_4S \cdot 0.1H_2O$: C, 58.91; H, 4.94; N, 7.90. Found: C, 58.98; H, 4.72; N, 8.09. **13**: mp 229—230 °C (from EtOH). ^{1}H-NMR ($CDCl_3$) δ: 1.21 (3H, t, J=.1 Hz), 2.35 (3H, s), 4.14 (2H, q, J=7.1 Hz), 4.57 (2H, s), 6.74 (1H, s), 12.35 (1H, br s). *Anal.* Calcd for $C_{11}H_{12}N_2O_4S \cdot 0.5H_2O$: C, 47.65; H, 4.73; N, 10.10. Found: C, 47.90; H, 4.43; N, 10.07.

Ethyl 1-(2-Methoxybenzyl)-6-(4-methoxyphenyl)-5-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetate (6d) A mixture of **5a** (0.60 g, 1.60 mmol), 2-methoxybenzyl chloride (0.51 g, 3.26 mmol), and K_2CO_3 powder (0.45 g, 3.26 mmol) in *N*,*N*-dimethylformamide (DMF, 7 ml) was stirred at room temperature for 15 h. After evaporation of the solvent *in vacuo*, the residue was diluted with water and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). The solution was concentrated *in vacuo* and the residue purified by flash column chromatography (EtOAc–hexane, 1 : 4 then 3 : 7). The resulting crystalline product was recrystallized from ether–hexane to give **6d** (0.57 g, 72%) as colorless crystals, mp 141—142 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7.2 Hz), 2.49 (3H, s), 3.83 (3H, s), 3.87 (3H, s), 4.25 (2H, q, J=7.2 Hz), 4.83 (2H, s), 5.24 (2H, s), 6.86—6.96 (4H, m), 7.09—7.14 (1H, m), 7.22—7.31 (3H, m). IR (KBr): 2982, 1756, 1710, 1665, 1607, 1562, 1535, 1477 cm^{-1}. *Anal.* Calcd for $C_{26}H_{26}N_2O_6S$: C, 63.14; H, 5.30; N, 5.66. Found: C, 63.01; H, 5.48; N, 5.48.

Compounds **6a—c**, **6e—m**, and **22** were prepared by a similar procedure to that used for the preparation of **6d** and their physicochemical data are listed in Table 5.

1-(2-Methoxybenzyl)-6-(4-methoxyphenyl)-5-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetic Acid (7d) To a solution of **6d** (0.35 g, 0.71 mmol) in MeOH (4 ml) and tetrahydrofuran (THF, 8 ml) was added 1 N NaOH (3.6 ml, 3.6 mmol). After being stirred at room temperature for 1.5 h, the mixture was acidified with 1 N HCl (4 ml, 4 mmol) and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). After evaporation of the solvent *in vacuo*, the residue was crystallized from ether–hexane to give **7d** (0.31 g, 93%) as colorless crystals, mp 216—218 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.41 (3H, s), 3.78 (3H, s), 3.85 (3H, s), 4.61 (2H, s), 5.14 (2H, s), 6.85—6.92 (1H, m), 6.98—7.07 (4H, m), 7.25—7.36 (3H, m). IR (KBr): 2966, 1731, 1702, 1659, 1607, 1562, 1535, 1479 cm^{-1}. *Anal.* Calcd for $C_{24}H_{22}N_2O_6S$: C, 61.79; H, 4.75; N, 6.00. Found: C, 61.60; H, 4.87; N, 6.16.

Compounds **7a—c**, **7e—m**, **9**, **11a**, **b**, **16a**, **b**, **20a—f**, **23**, **27**, **28**, **31**, **32a—j**, and **34** were prepared by a similar procedure to that used for the

Table 7. Physicochemical Data of Ethyl 5-Substitutedmethyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetates

Compd.	Yield (%)	mp (°C) Recryst. solvent[a]	Formula	Analysis (%) Calcd (Found) C	H	N	^{1}H-NMR (in $CDCl_3$) δ
30a	87	175—177 EA–H	$C_{29}H_{31}N_3O_7S_2$	58.28 (57.99	5.23 5.28	7.03 6.93)	1.32 (3H, t, J=7.2 Hz), 1.96 (3H, s), 2.53 (3H, s), 3.47 (3H, s), 4.28 (2H, q, J=7.2 Hz), 4.51 (2H, d, J=6.6 Hz), 4.88 (2H, s), 5.18 (2H, s), 5.35 (2H, s), 5.75 (1H, m), 7.02—7.34 (6H, m), 7.49 (2H, d, J=8.7 Hz)
30b	85	168—169 EA–H	$C_{30}H_{33}N_3O_7S_2 \cdot 0.3H_2O$	58.39 (58.38	5.49 5.55	6.81 6.92)	1.13 (3H, t, J=7.5 Hz), 1.32 (3H, t, J=7.1 Hz), 2.20 (2H, q, J=7.5 Hz), 2.53 (3H, s), 3.47 (3H, s), 4.27 (2H, q, J=7.1 Hz), 4.52 (2H, d, J=6.6 Hz), 4.87 (2H, s), 5.18 (2H, s), 5.35 (2H, s), 7.02—7.15 (4H, m), 7.26—7.34 (2H, m), 7.51 (2H, d, J=8.8 Hz)
30c	83	159—162 EA–H	$C_{31}H_{35}N_3O_7S_2 \cdot 0.5H_2O$	58.66 (58.74	5.72 5.54	6.62 6.55)	0.92 (3H, t, J=7.3 Hz), 1.32 (3H, t, J=7.2 Hz), 1.70—1.71 (1H, m), 2.15 (2H, t, J=7.3 Hz), 2.53 (3H, s), 3.47 (3H, s), 4.27 (2H, q, J=7.2 Hz), 4.52 (2H, d, J=6.6 Hz), 4.88 (2H, s), 5.18 (2H, s), 5.35 (2H, s), 7.01—7.35 (6H, m), 7.50 (2H, d, J=8.5 Hz)
30d	96	163—165 EA–H	$C_{32}H_{37}N_3O_7S_2$	60.07 (59.87	5.83 5.82	6.57 6.69)	0.90 (3H, t, J=7.3 Hz), 1.26—1.38 (5H, m), 1.52—1.62 (2H, m), 2.17 (2H, t, J=7.7 Hz), 2.52 (2H, s), 3.47 (3H, s), 4.27 (2H, q, J=7.3 Hz), 4.52 (2H, d, J=6.6 Hz), 4.86 (2H, s), 5.18 (2H, s), 5.35 (2H, s), 7.02—7.35 (6H, m), 7.50 (2H, d, J=8.6 Hz)
30e	90	Amorphous	$C_{34}H_{33}N_3O_7S_2 \cdot 0.2H_2O$	61.56 (61.52	5.08 4.97	6.33 6.37)	1.31 (3H, t, J=7.2 Hz), 2.53 (3H, s), 3.48 (3H, s), 4.28 (2H, q, J=7.2 Hz), 4.73 (2H, d, J=6.6 Hz), 4.91 (2H, s), 5.20 (2H, s), 5.36 (2H, s), 7.01—7.14 (4H, m), 7.31—7.48 (5H, m), 7.58 (2H, d, J=8.5 Hz), 7.79 (2H, d, J=7.1 Hz), 8.28 (1H, t J=6.6 Hz)
30g	100	115—118 EA–H	$C_{28}H_{31}N_3O_8S_3 \cdot 0.1C_6H_{14}$	53.48 (53.66	5.08 5.20	6.54 6.37)	1.33 (3H, t, J=7.1 Hz), 2.53 (3H, s), 2.87 (3H, s), 3.48 (3H, s), 4.27 (2H, q, J=7.1 Hz), 4.37 (2H, d, J=6.8 Hz), 4.85 (2H, s), 5.19 (2H, s), 5.36 (2H, s), 6.07 (1H, t, J=6.8 Hz), 7.01—7.18 (4H, m), 7.27—7.36 (4H, m)
30h	86	153—155 EA–H	$C_{29}H_{33}N_3O_8S_3$	53.77 (53.58	5.13 5.22	6.49 6.24)	1.30—1.35 (6H, m), 2.53 (3H, s), 2.95 (2H, q, J=7.4 Hz), 3.48 (3H, s), 4.27 (2H, q, J=7.2 Hz), 4.35 (2H, d, J=6.6 Hz), 4.85 (2H, s), 5.19 (2H, s), 5.36 (2H, s), 6.07 (1H, t, J=6.6 Hz), 7.01—7.08 (4H, m), 7.29—7.37 (4H, m)
30i	85	122—123 EA–H	$C_{30}H_{35}N_3O_8S_3$	54.45 (54.19	5.33 5.31	6.35 6.18)	0.98 (3H, t, J=7.2 Hz), 1.32 (3H, t, J=7.2 Hz), 1.77—1.78 (2H, m), 2.53 (3H, s), 2.88—2.89 (2H, m), 3.48 (3H, s), 4.27 (2H, q, J=7.2 Hz), 4.35 (2H, d, J=6.9 Hz), 4.85 (2H, s), 5.19 (2H, s), 5.36 (2H, s), 6.06 (1H, t, J=6.9 Hz), 7.01—7.37 (8H, m)
30j	83	Amorphous					1.34 (3H, t, J=7.2 Hz), 2.52 (3H, s), 3.50 (3H, s), 4.26—4.33 (4H, m), 4.82 (2H, s), 5.21 (2H, s), 5.26 (2H, s), 6.63 (1H, t, J=6.8 Hz), 6.97 (1H, d, J=7.2 Hz), 7.08 (2H, d, J=9.0 Hz), 7.14—7.20 (1H, m), 7.28—7.60 (7H, m), 7.66 (2H, d, J=7.8 Hz)

a) Recrystallization solvent: EA=ethyl acetate, H=hexane.

preparation of **7d** and their physicochemical data are listed in Table 6.

Ethyl 6-(4-Methoxyphenyl)-5-methyl-1-(2-methylaminobenzyl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetate (8) A mixture of **6j** (0.60 g, 1.2 mmol) and iron powder (0.36 g, 6.1 mmol) in acetic acid (25 ml) was stirred at 80 °C for 2 h. After cooling, the mixture was filtered through Celite and the filtrate concentrated *in vacuo*. The residue was diluted with saturated $NaHCO_3$ and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$), concentrated *in vacuo*, and the residue purified by flash column chromatography (EtOAc–hexane, 3 : 7 then 4 : 6). The resulting crystalline product was recrystallized from EtOAc–hexane to afford the 2-aminobenzyl derivative (0.445 g, 80%) as a colorless crystalline powder, mp 138—140 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7.1 Hz), 2.47 (3H, s), 3.85 (3H, s), 4.25 (2H, q, J=7.1 Hz), 4.47 (2H, br s), 4.82 (2H, s), 5.16 (2H, s), 6.63—6.73 (2H, m), 6.96 (2H, d, J=8.8 Hz), 7.13 (1H, t, J=7.4 Hz), 7.28—7.36 (3H, m). IR (KBr): 1754, 1705, 1636, 1562, 1528, 1502 cm^{-1}. FAB-MS *m/e*: 480 (MH^+).

To a solution of the 2-aminobenzyl derivative (0.30 g, 0.62 mmol) in THF (5 ml) was added acetic formic anhydride [prepared from acetic anhydride (1 ml, 10.6 mmol) and formic acid (0.5 ml, 13.3 mmol) according to the reported procedure[28]] at −20 °C. The mixture was stirred at the same temperature for 30 min. The mixture was then concentrated *in vacuo* to give a white powder which was dissolved in THF (5 ml). To the solution was added dropwise borane–methyl sulfide complex (0.25 ml, 2.5 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 30 min, and then gently refluxed for 2 h. After cooling, MeOH (1 ml) was added at 0 °C and the mixture was stirred at room temperature for 1 h. The mixture was then treated with ethanolic HCl to attain pH≤2, and the resulting mixture was gently refluxed for 0.5 h. After addition of MeOH (20 ml), the mixture was concentrated *in vacuo*. The residue was diluted with saturated $NaHCO_3$ and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). The solution was concentrated *in vacuo* and the residue purified by flash column chromatography (EtOAc–hexane, 1 : 4) to afford **8** (0.16 g, 52%) as a white solid. Recrystallization from EtOAc–hexane gave colorless crystals, mp 188—189 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, t, J=7.2 Hz), 2.46 (3H, s), 2.82 (3H, s), 3.85 (3H, s), 4.26 (2H, q, J=7.2 Hz), 4.83 (2H, s), 5.15 (2H, s), 6.59—6.69 (2H, m), 6.95 (2H, d, J=8.8 Hz), 7.21—7.36 (4H, m). IR (KBr): 3396, 1742, 1696, 1647, 1607, 1570, 1535 cm^{-1}. FAB-MS *m/e*: 494 (MH^+). *Anal.* Calcd for $C_{26}H_{25}N_3O_6S$: C, 63.27; H, 5.51; N, 8.51. Found: C, 63.20; H, 5.69; N, 8.63.

Ethyl 6-(4-Methoxyphenyl)-5-methyl-1-(2-methylsulfinylbenzyl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetate (10a) To an ice-cooled solution of **6k** (0.35 g, 0.69 mmol) in CH_2Cl_2 (10 ml) was added dropwise a solution of *m*-chloroperbenzoic acid (50% purity, 0.26 g, 0.75 mmol) in CH_2Cl_2 (10 ml). After 30 min at 0 °C, the mixture was diluted with saturated $NaHCO_3$ and extracted with CH_2Cl_2. The extract was washed with brine and dried ($MgSO_4$). The solution was concentrated *in vacuo* and the residue purified by flash column chromatography (CH_2Cl_2–MeOH, 20 : 1 then 10 : 1) to give **10a** (0.26 g, 72%) as a pale yellow amorphous powder, mp 90—95 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7.1 Hz), 2.50 (3H, s), 2.83 (3H, s), 3.83 (3H, s), 4.25 (2H, q, J=7.1 Hz), 4.82 (2H, s), 5.19, 5.49 (each 1H, ABq, J=16.5 Hz), 6.93 (2H, d, J=8.8 Hz), 7.23—7.28 (3H, m), 7.47 (1H, t, J=8.8 Hz), 7.57 (1H, t, J=8.8 Hz), 8.05 (1H, d, J=8.8 Hz). IR (KBr): 1748, 1709, 1665, 1609, 1564, 1535, 1477 cm^{-1}. *Anal.* Calcd for $C_{26}H_{26}N_2O_6S_2 \cdot 0.2H_2O$: C, 58.89; H, 5.01; N, 5.28. Found: C, 58.89; H, 5.04; N, 5.22.

Compound **10b** was prepared in 67% yield from **6k** by essentially the same procedure (using 2 eq of *m*-chloroperbenzoic acid) described for the preparation of **10a**, as pale yellow crystals (from EtOAc–hexane), mp 141—

144 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J=7.1 Hz), 2.53 (3H, s), 3.26 (3H, s), 3.84 (3H, s), 4.23 (2H, q, J=7.1 Hz), 4.79 (2H, s), 5.64 (2H, s), 6.94 (2H, d, J=8.9 Hz), 7.22—7.32 (3H, m), 7.48—7.64 (2H, m), 8.11 (1H, d, J=7.6 Hz). IR (KBr): 1742, 1705, 1661, 1533, 1477, 1377 cm^{-1}. *Anal.* Calcd for $C_{26}H_{26}N_2O_7S_2$: C, 57.55; H, 4.83; N, 5.16. Found: C, 57.64; H, 4.69; N, 5.16.

Ethyl 6-Bromo-1-(2-methoxybenzyl)-5-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetate (14a) A mixture of **13** (0.27 g, 1.01 mmol) and NBS (0.20 g, 1.11 mmol) in $CHCl_3$ (30 ml) was heated under reflux for 2.5 h. After cooling, the mixture was diluted with water and extracted with $CHCl_3$. The extract was washed with brine and dried ($MgSO_4$). After evaporation of the solvent *in vacuo*, the residue was purified by flash column chromatography (EtOAc–hexane, 3 : 7 then 4 : 6) to give the 6-bromo derivative (0.31 g, 90%) as pale yellow solid. Recrystallization from EtOAc–hexane afforded colorless fine needles, mp 192—193 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.33 (3H, t, J=7.1 Hz), 2.39 (3H, s), 4.28 (2H, q, J=7.1 Hz), 4.75 (2H, s), 10.41 (1H, br s). FAB-MS *m/e*: 347 (MH^+, ^{79}Br), 349 (MH^+, ^{81}Br).

To a mixture of the 6-bromo derivative (0.30 g, 0.86 mmol), K_2CO_3 powder (0.18 g, 1.30 mmol), and KI powder (72 mg, 0.43 mmol) in DMF (7 ml) was added a solution of 2-methoxybenzyl chloride (0.16 g, 1.02 mmol) in DMF (3 ml). After being stirred at room temperature for 16 h, the mixture was concentrated *in vacuo*. The residue was diluted with water and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). After removal of the solvent *in vacuo*, the residue was purified by flash column chromatography (EtOAc–hexane, 1 : 4) to give **14a** (0.38 g, 94%) as a white solid. Recrystallization from EtOAc–ether–hexane afforded colorless crystals, mp 124—126 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, t, J=7.2 Hz), 2.43 (3H, s), 3.88 (3H, s), 4.24 (2H, q, J=7.2 Hz), 4.80 (2H, s), 5.17 (2H, s), 6.89—6.94 (2H, m), 7.10—7.13 (1H, m), 7.26—7.32 (1H, m). IR (KBr): 3422, 2972, 1748, 1707, 1669, 1560, 1501, 1475 cm^{-1}. *Anal.* Calcd for $C_{19}H_{19}BrN_2O_5S \cdot 0.1C_6H_{14}$: C, 49.46; H, 4.32; N, 5.89. Found: C, 49.39; H, 4.09; N, 6.09.

Compound **14b** was prepared in 68% yield from **13** by the same procedure described for the preparation of **14a** as colorless crystals (from EtOAc–hexane), mp 150—151 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7.1 Hz), 2.44 (3H, s), 2.55 (3H, s), 4.24 (2H, q, J=7.1 Hz), 4.82 (2H, s), 5.29 (2H, s), 6.97—7.34 (4H, m). IR (KBr): 1746, 1707, 1669, 1475 cm^{-1}. *Anal.* Calcd for $C_{19}H_{19}BrN_2O_4S_2$: C, 47.21; H, 3.96; N, 5.80; S, 13.27. Found: C, 47.39; H, 3.99; N, 5.86; S, 13.25.

Ethyl 1-(2-Methoxybenzyl)-6-(3-methoxyphenyl)-5-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetate (15a) To a mixture of **14a** (0.26 g, 0.56 mmol), 3-methoxyphenylboronic acid (0.12 g, 0.68 mmol), and 2 M Na_2CO_3 (1.40 ml, 2.80 mmol) in 1,2-dimethoxyethane (10 ml) was added tetrakis(triphenylphosphine)palladium (0) (65 mg, 0.056 mmol) under an argon atmosphere. The mixture was heated under reflux with vigorous stirring under an argon atmosphere for 5 h. After cooling, the reaction mixture was diluted with EtOAc and filtered through Celite. The filtrate was diluted with water and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). After evaporation of the solvent *in vacuo*, the residue was purified by flash column chromatography (EtOAc–hexane, 1 : 4) to give **15a** (0.22 g, 80%) as a pale yellow amorphous solid. Crystallization from EtOAc–ether–hexane afforded colorless crystals, mp 140—141 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7.1 Hz), 2.54 (3H, s), 3.82 (3H, s), 3.87 (3H, s), 4.25 (2H, q, J=7.1 Hz), 4.83 (2H, s), 5.25 (2H, s), 6.86—6.96 (5H, m), 7.10—7.14 (1H, m), 7.24—7.33 (2H, m). IR (KBr): 3438, 2976, 1748, 1709, 1661, 1593, 1562, 1531, 1495, 1473 cm^{-1}. *Anal.* Calcd for $C_{26}H_{26}N_2O_6S$: C, 63.14; H, 5.30; N, 5.66; S, 6.48. Found: C, 63.17; H, 5.35; N, 5.60; S, 6.58.

Compound **15b** was prepared in 54% yield from **14b** by the same procedure described for the preparation of **15a** as a colorless crystalline powder (from EtOAc–hexane), mp 126—128 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7.2 Hz), 2.51 (3H, s), 2.54 (3H, s), 2.89 (2H, t, J=6.9 Hz), 3.35 (3H, s), 3.61 (2H, t, J=6.9 Hz), 4.26 (2H, q, J=7.2 Hz), 4.86 (2H, s), 5.34 (2H, s), 7.05—7.40 (8H, m). IR (KBr): 1754, 1709, 1663, 1477 cm^{-1}. *Anal.* Calcd for $C_{28}H_{30}N_2O_5S_2$: C, 62.43; H, 5.61; N, 5.20. Found: C, 62.32; H, 5.39; N, 5.09.

Ethyl 6-(4-Hydroxyphenyl)-5-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetate (17) To an ice-cooled mixture of anhydrous aluminum chloride (2.90 g, 21.7 mmol) and dimethyl disulfide (2.45 ml, 27.2 mmol) in CH_2Cl_2 (60 ml) was added dropwise a solution of **5a** (2.0 g, 5.34 mmol) in CH_2Cl_2 (40 ml) under a nitrogen atmosphere during 30 min. After stirring at room temperature for 20 h, the mixture was poured into ice-water and the resulting suspension was concentrated *in vacuo*. The concentrate was diluted with water and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). The solution was concentrated *in vacuo*, and the residue was purified by flash column chromatography (EtOAc–hexane, 4 : 6 then 1 : 1) to give **17** (1.64 g, 85%) as a white solid, mp 240—242 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.22 (3H, t, J=7.1 Hz), 2.37 (3H, s), 4.15 (2H, q, J=7.1 Hz), 4.59 (2H, s), 6.85 (2H, d, J=8.6 Hz), 7.26 (2H, d, J=8.6 Hz), 9.73 (1H, s), 12.39 (1H, s). IR (KBr): 3356, 2992, 1720, 1690, 1667, 1611, 1593, 1568, 1537, 1502 cm^{-1}. *Anal.* Calcd for $C_{17}H_{16}N_2O_5S \cdot 0.1H_2O$: C, 56.38; H, 4.51; N, 7.73. Found: C, 56.28; H, 4.48; N, 7.64.

Ethyl 6-(4-Acetoxyphenyl)-5-methyl-1-(2-methylthiobenzyl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetate (18) To a solution of **17** (6.0 g, 16.6 mmol) in pyridine (100 ml) was added acetic anhydride (32 ml, 0.339 mol). After stirring at room temperature for 3 h, the mixture was concentrated *in vacuo*. The residue was diluted with aqueous HCl and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). The solution was concentrated *in vacuo* to give a pale orange syrup, which was dissolved in DMF (60 ml). To this solution were added K_2CO_3 powder (4.60 g, 33.3 mmol) and a solution of 2-methylthiobenzyl chloride (5.80 g, 33.6 mmol) in DMF (20 ml), and the reaction mixture stirred at room temperature for 13 h. The mixture was concentrated *in vacuo* and the residue was diluted with water. The resulting mixture was extracted with EtOAc and the extract washed with brine and dried ($MgSO_4$). After evaporation of the solvent *in vacuo*, the residue was purified by flash column chromatography (EtOAc–hexane, 1 : 3 then 3 : 7) to give **18** (6.02 g, 67%) as an orange amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (3H, t, J=7.2 Hz), 2.31 (3H, s), 2.52 (3H, s), 2.53 (3H, s), 4.26 (2H, q, J=7.2 Hz), 4.85 (2H, s), 5.34 (2H, s), 7.01—7.18 (4H, m), 7.23—7.38 (4H, m). IR (KBr): 2982, 1765, 1752, 1707, 1665, 1564, 1533, 1475 cm^{-1}.

Ethyl 6-(4-Hydroxyphenyl)-5-methyl-1-(2-methylthiobenzyl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetate (19a) To a solution of **18** (6.0 g, 11.1 mmol) in MeOH (150 ml) and THF (200 ml) was added a solution of K_2CO_3 (3.10 g, 22.4 mmol) in H_2O (70 ml). After stirring at room temperature for 40 min, the mixture was acidified with 1 N HCl (50 ml) at 0 °C and concentrated *in vacuo*. The concentrate was diluted with water and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). After evaporation of the solvent *in vacuo*, the residue was crystallized from EtOAc–isopropyl ether to give **19a** (4.33 g, 78%) as colorless crystals, mp 177—178 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, t, J=7.2 Hz), 2.45 (3H, s), 2.52 (3H, s), 4.28 (2H, q, J=7.2 Hz), 4.87 (2H, s), 5.28 (2H, s), 5.75 (1H, s), 6.78 (2H, d, J=8.6 Hz), 6.97—7.14 (4H, m), 7.21—7.34 (2H, m). IR (KBr): 3346, 2978, 1752, 1700, 1651, 1613, 1591, 1564, 1535, 1481 cm^{-1}. *Anal.* Calcd for $C_{25}H_{24}N_2O_5S_2 \cdot 0.1H_2O$: C, 60.25; H, 4.89; N, 5.62; S, 12.87. Found: C, 60.09; H, 4.66; N, 5.57; S, 12.97.

Ethyl 6-(4-Methoxymethoxyphenyl)-5-methyl-1-(2-methylthiobenzyl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetate (19d) To an ice-cooled solution of **19a** (0.50 g, 1.01 mmol) in DMF (4 ml) was added sodium hydride (60% in oil, 43 mg, 1.08 mmol) under a nitrogen atmosphere. After stirring at 0 °C for 30 min, chloromethyl methyl ether (0.15 ml, 1.97 mmol) was added to the mixture. The mixture was then stirred at room temperature for 3 h and concentrated *in vacuo*. The residue was diluted with aqueous ammonium chloride and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$), evaporated *in vacuo*, and the residue purified by flash column chromatography (EtOAc–hexane, 1 : 3) to give **19d** (0.46 g, 85%) as a white amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7.1 Hz), 2.50 (3H, s), 2.53 (3H, s), 3.48 (3H, s), 4.25 (2H, q, J=7.1 Hz), 4.85 (2H, s), 5.19 (2H, s), 5.34 (2H, s), 7.01—7.18 (4H, m), 7.22—7.35 (4H, m). IR (KBr): 2964, 1750, 1711, 1665, 1564, 1533, 1477 cm^{-1}.

Compounds **19b**, **c**, **e**, **f** were prepared from **19a** by a similar procedure to that used for the preparation of **19d**, in 84, 85, 77, and 48% yields, respectively. **19b**: mp 122—123 °C (from EtOAc–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, t, J=7.3 Hz), 1.31 (3H, t, J=7.1 Hz), 1.82 (2H, sext, J=7.3 Hz), 2.50 (3H, s), 2.53 (3H, s), 3.93 (2H, t, J=6.5 Hz), 4.26 (2H, q, J=7.1 Hz), 4.85 (2H, s), 5.34 (2H, s), 6.88—7.32 (8H, m). *Anal.* Calcd for $C_{28}H_{30}N_2O_5S_2$: C, 62.43; H, 5.61; N, 5.20. Found: C, 62.24; H, 5.68; N, 5.06. **19c**: mp 85—88 °C (from EtOAc–hexane). ^{1}H-NMR ($CDCl_3$) δ: 0.98 (3H, t, J=7.7 Hz), 1.31 (3H, t, J=7.1 Hz), 1.49 (2H, sext, J=7.7 Hz), 1.78 (2H, quint, J=7.7 Hz), 2.50 (3H, s), 2.53 (3H, s), 3.97 (2H, t, J=7.7 Hz), 4.25 (2H, q, J=7.1 Hz), 4.85 (2H, s), 5.34 (2H, s), 6.90 (2H, d, J=8.8 Hz), 7.00—7.37 (6H, m). *Anal.* Calcd for $C_{29}H_{32}N_2O_5S_2$: C, 63.02; H, 5.84; N, 5.07. Found: C, 63.17; H, 5.94; N, 4.98. **19e**: mp 110—111 °C (from EtOAc–ether–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7.1 Hz), 2.26 (3H, s), 2.50 (3H, s), 2.53 (3H, s), 4.25 (2H, q, J=7.1 Hz), 4.85 (2H, s), 5.16 (2H, s), 5.34 (2H, s), 6.94—7.18 (4H, m), 7.23—7.36 (4H, m). *Anal.* Calcd for $C_{27}H_{28}N_2O_5S_3$: C, 58.25; H, 5.07; N, 5.03; S, 17.28. Found: C, 58.20; H,

5.22; N, 4.95; S, 17.07. **19f**: mp 127—128 °C (from EtOAc–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.31 (3H, t, J=7.1 Hz), 2.29 (3H, s), 2.50 (3H, s), 2.53 (3H, s), 4.25 (2H, q, J=7.1 Hz), 4.56 (2H, s), 4.85 (2H, s), 5.33 (2H, s), 6.87—7.32 (8H, m). *Anal.* Calcd for $C_{28}H_{28}N_2O_6S_2$: C, 60.85; H, 5.11; N, 5.07. Found: C, 60.69; H, 5.05; N, 4.80.

Ethyl 6-(4-Butyrylphenyl)-5-methyl-2,4-dioxo-1,2,3,4-tetrahydrothieno-[2,3-*d*]pyrimidine-3-acetate (21) To an ice-cooled mixture of **5d** (0.39 g, 1.10 mmol) in nitrobenzene (10 ml) were added successively aluminum chloride (0.63 g, 4.75 mmol) and butyryl chloride (0.21 ml, 2.20 mmol). The reaction mixture was then stirred at 50 °C for 6 h. The mixture was poured into ice-water and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). After removal of the solvent *in vacuo*, the residue was purified by flash column chromatography (EtOAc–hexane, 3 : 1) to give **21** (0.05 g, 14%) as a pale yellow amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 1.03 (3H, t, J=7.2 Hz), 1.32 (3H, t, J=7.2 Hz), 1.77 (2H, sext, J=7.2 Hz), 2.54 (3H, s), 2.98 (2H, t, J=7.2 Hz), 4.27 (2H, q, J=7.2 Hz), 4.78 (2H, s), 7.51 (2H, d, J=8.4 Hz), 8.02 (2H, d, J=8.4 Hz), 10.20 (1H, s). FAB-MS *m/e*: 445 (MH^+).

Ethyl 5-Methyl-6-(4-nitrophenyl)-2,4-dioxo-1,2,3,4-tetrahydrothieno-[2,3-*d*]pyrimidine-3-acetate (24) To an ice-cooled solution of **5d** (2.20 g, 6.39 mmol) in concentrated sulfuric acid (12 ml) was added dropwise a solution of sodium nitrate (0.55 g, 6.47 mmol) in concentrated sulfuric acid (15 ml) over 10 min. The mixture was stirred at 0 °C for 1 h and then poured into ice-water and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). After evaporation of the solvent *in vacuo*, the residue was purified by flash column chromatography (EtOAc–hexane–CH_2Cl_2, 1 : 2 : 2) to give **24** (1.30 g, 52%) as a yellow solid. Recrystallization from EtOAc–hexane afforded yellow crystals, mp 277—280 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.33 (3H, t, J=7.2 Hz), 2.56 (3H, s), 4.28 (2H, q, J=7.2 Hz), 4.79 (2H, s), 7.57 (2H, d, J=8.8 Hz), 8.30 (2H, d, J=7.2 Hz), 10.66 (1H, br s). IR (KBr): 1748, 1663, 1522, 1460, 1348 cm^{-1}. *Anal.* Calcd for $C_{17}H_{15}N_3O_6S \cdot 0.4H_2O$: C, 51.48; H, 4.01; N, 10.59. Found: C, 51.64; H, 3.79; N, 10.61.

Ethyl 6-(4-Aminophenyl)-5-methyl-1-(2-methylthiobenzyl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetate (25) A mixture of **24** (3.30 g, 8.47 mmol), 2-methylthiobenzyl chloride (1.76 g, 10.2 mmol), K_2CO_3 powder (1.76 g, 12.7 mmol), and KI powder (0.28 g, 1.69 mmol) in DMF (100 ml) was stirred at room temperature for 48 h. The mixture was concentrated *in vacuo*, and the residue was diluted with water and extracted with EtOAc. The extract was washed with brine and dried (Na_2SO_4). After removal of the solvent *in vacuo*, the residue was purified by flash column chromatography (CH_2Cl_2) to afford the 2-methylthiobenzyl derivative (2.80 g, 69%) as a yellow amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, t, J=7.2 Hz), 2.54 (3H, s), 2.60 (3H, s), 4.26 (2H, q, J=7.2 Hz), 4.85 (2H, s), 5.36 (2H, s), 7.01—7.36 (4H, m), 7.51 (2H, d, J=8.9 Hz), 8.25 (2H, d, J=8.9 Hz).

A mixture of the 2-methylthiobenzyl derivative (3.0 g, 5.71 mmol), concentrated hydrochloric acid (2.0 ml), and iron powder (1.2 g, 20.4 mmol) in EtOH (40 ml) was heated under reflux with vigorous stirring for 30 min. After cooling, the mixture was filtered through Celite and the filtrate was concentrated *in vacuo*. The residue was diluted with saturated $NaHCO_3$ and extracted with EtOAc. The extract was washed with brine and dried (Na_2SO_4). The solution was concentrated *in vacuo*, and the residue was recrystallized from EtOAc–isopropyl ether to give **25** (2.08 g, 74%) as colorless prisms, mp 172—173 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7.1 Hz), 2.49 (3H, s), 2.53 (3H, s), 3.78 (2H, br s), 4.25 (2H, q, J=7.1 Hz), 4.84 (2H, s), 5.32 (2H, s), 6.67 (2H, d, J=8.5 Hz), 7.03—7.32 (6H, m). IR (KBr): 3372, 1752, 1702, 1657, 1609, 1537, 1479 cm^{-1}. *Anal.* Calcd for $C_{25}H_{25}N_3O_4S_2$: C, 60.59; H, 5.08; N, 8.48. Found: C, 60.56; H, 4.93; N, 8.49.

Ethyl 5-Methyl-1-(2-methylthiobenzyl)-2,4-dioxo-6-(4-propionylamino-phenyl)-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetate (26) To a solution of **25** (0.30 g, 0.61 mmol) in CH_2Cl_2 (10 ml) were added triethylamine (0.10 ml, 0.73 mmol) and propionyl chloride (0.11 ml, 1.22 mmol). After stirring at room temperature for 1.5 h, the mixture was diluted with aqueous ammonium chloride and extracted with CH_2Cl_2. The extract was washed with brine and dried ($MgSO_4$). After evaporation of the solvent *in vacuo*, the residue was purified by flash column chromatography (EtOAc–hexane–CH_2Cl_2, 5 : 5 : 1). The crude product was crystallized from EtOAc–hexane to give **26** (0.25 g, 74%) as colorless crystals, mp 218—219 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.22—1.34 (6H, m), 2.41 (2H, q, J=7.5 Hz), 2.51 (3H, s), 2.53 (3H, s), 4.26 (2H, q, J=7.1 Hz), 4.85 (2H, s), 5.32 (2H, s), 7.00—7.56 (9H, m). IR (KBr): 1707, 1661, 1537, 1479 cm^{-1}. *Anal.* Calcd for $C_{28}H_{29}N_3O_5S_2$: C, 60.96; H, 5.30; N, 7.62. Found: C, 60.82; H, 5.08; N, 7.78.

Ethyl 5-Aminomethyl-6-(4-methoxymethoxyphenyl)-1-(2-methylthio-benzyl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetate (29) To a solution of **19d** (6.10 g, 11.30 mmol) in CCl_4 (200 ml) were added NBS (2.11 g, 11.85 mmol) and 2,2′-azobis(isobutyronitrile) (0.19 g 1.13 mmol) and the reaction mixture heated under reflux for 2 h. After cooling, insoluble material was removed by filtration and the filtrate diluted with water and extracted with $CHCl_3$. The extract was washed with brine and dried ($MgSO_4$). After evaporation of the solvent *in vacuo*, the residue was purified by flash column chromatography (EtOAc–hexane, 1 : 3 then 3 : 7). The crude product was crystallized from EtOAc–ether–hexane to afford the 5-bromomethyl derivative (5.73 g, 82%) as a white crystalline powder, mp 108—110 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (3H, t, J=7.1 Hz), 2.53 (3H, s), 3.49 (3H, s), 4.26 (2H, q, J=7.1 Hz), 4.79 (2H, s), 4.88 (2H, s), 5.20 (2H, s), 5.35 (2H, s), 7.04—7.19 (4H, m), 7.24—7.35 (2H, m), 7.45 (2H, d, J=9.0 Hz).

A mixture of the 5-bromomethyl derivative (1.26 g, 2.03 mmol) and potassium phthalimide (0.44 g, 2.13 mmol) in DMF (30 ml) was stirred at room temperature for 4 h. The mixture was concentrated *in vacuo*, and the residue was diluted with water and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). After evaporation of the solvent *in vacuo*, the residue was purified by flash column chromatography (EtOAc–hexane, 3 : 7 then 1 : 1). Crystallization from EtOAc–ether gave the 5-phthalimidomethyl derivative (1.05 g, 75%) as white crystals, mp 180—182 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J=7.1 Hz), 2.49 (3H, s), 3.37 (3H, s), 4.22 (2H, q, J=7.1 Hz), 4.81 (2H, s), 4.91 (2H, s), 5.22 (2H, s), 5.31 (2H, s), 6.70 (2H, d, J=8.8 Hz), 7.04—7.32 (4H, m), 7.57—7.66 (4H, m).

To a mixture of the 5-phthalimidomethyl derivative (0.95 g, 1.38 mmol) in EtOH (50 ml) was added hydrazine monohydrate (0.34 ml, 6.92 mmol) and the reaction mixture stirred at 80 °C for 16 h. After cooling, the mixture was concentrated *in vacuo* and the residue was diluted with saturated $NaHCO_3$. The resulting mixture was then extracted with CH_2Cl_2. The extract was washed with brine and dried ($MgSO_4$). After removal of the solvent *in vacuo*, the residue was purified by flash column chromatography ($CHCl_3$–MeOH, 20 : 1) to give **29** (0.64 g, 84%) as a white solid. Recrystallization from EtOAc–hexane afforded a white crystalline powder, mp 114—116 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (3H, t, J=7.1 Hz), 2.53 (3H, s), 3.48 (3H, s), 3.92 (2H, s), 4.26 (2H, q, J=7.1 Hz), 4.86 (2H, s), 5.19 (2H, s), 5.35 (2H, s), 7.01—7.35 (8H, m). IR (KBr): 1705, 1671, 1584, 1531 cm^{-1}. *Anal.* Calcd for $C_{27}H_{29}N_3O_6S_2$: C, 58.36; H, 5.26; N, 7.56. Found: C, 58.22; H, 5.34; N, 7.54.

Ethyl 5-(3-Ethylureidomethyl)-6-(4-methoxymethoxyphenyl)-1-(2-methylthiobenzyl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetate (30f) A mixture of **29** (0.30 g, 0.54 mmol) and ethyl isocyanate (45 μl, 0.57 mmol) in pyridine (5 ml) was stirred at room temperature for 2 h. After evaporation of the solvent *in vacuo*, the residue was diluted with aqueous ammonium chloride and extracted with CH_2Cl_2. The extract was washed with brine and dried ($MgSO_4$). After evaporation of the solvent *in vacuo*, the residue was recrystallized from EtOAc–hexane to give **30f** (0.33 g, 98%) as a colorless crystalline powder, mp 207—208 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.08 (3H, t, J=7.1 Hz), 1.32 (3H, t, J=7.1 Hz), 2.52 (3H, s), 3.11—3.20 (2H, m), 3.47 (3H, s), 4.16 (1H, t, J=5.8 Hz), 4.26 (2H, q, J=7.1 Hz), 4.46 (2H, m), 4.86 (2H, s), 5.18 (2H, s), 5.35 (2H, s), 6.07 (1H, t, J=6.8 Hz), 7.01—7.34 (6H, m), 7.51 (2H, d, J=8.6 Hz). IR (KBr): 1756, 1711, 1665, 1630, 1562, 1535 cm^{-1}. *Anal.* Calcd for $C_{30}H_{34}N_4O_7S_2$: C, 57.49; H, 5.47; N, 8.94. Found: C, 57.24; H, 5.63; N, 8.99.

Ethyl 6-(4-Methoxymethoxyphenyl)-5-methylsulfonylaminomethyl-1-(2-methylthiobenzyl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetate (30g) To an ice-cooled solution of **29** (0.80 g, 1.44 mmol) in CH_2Cl_2 (20 ml) was added triethylamine (0.22 ml, 1.58 mmol) and methanesulfonyl chloride (0.12 ml, 1.55 mmol). After stirring at 0 °C for 30 min, the mixture was diluted with brine and extracted with CH_2Cl_2. The extract was washed with brine and dried ($MgSO_4$). After evaporation of the solvent *in vacuo*, the residue was purified by flash column chromatography (EtOAc–CH_2Cl_2–MeOH, 5 : 1 : 0 then 10 : 2 : 1) to give **30g** (0.91 g, 100%) as a white solid. Recrystallization from EtOAc–hexane afforded white crystals, mp 115—118 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.33 (3H, t, J=7.1 Hz), 2.53 (3H, s), 2.87 (3H, s), 3.48 (3H, s), 4.27 (2H, q, J=7.1 Hz), 4.37 (2H, d, J=6.8 Hz), 4.85 (2H, s), 5.19 (2H, s), 5.36 (2H, s), 6.07 (1H, t, J=6.8 Hz), 7.01—7.18 (4H, m), 7.27—7.36 (4H, m). IR (KBr): 3274, 2960, 1744, 1709, 1657, 1607, 1557, 1533, 1485 cm^{-1}. *Anal.* Calcd for $C_{28}H_{31}N_3O_8S_3 \cdot 0.1C_6H_{14}$: C, 53.48; H, 5.08; N, 6.54; S, 14.97. Found: C, 53.66; H, 5.20; N, 6.37; S, 15.09.

Compounds **30a—e**, **h—j** were prepared by a similar procedure to that

used for the preparation of **30g** and their physicochemical data are listed in Table 7.

Ethyl 6-(4-Methoxymethoxyphenyl)-5-(*N*-methyl-*N*-methylsulfonylaminomethyl)-1-(2-methylthiobenzyl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetate (33) To an ice-cooled solution of **30g** (0.23 g, 0.36 mmol) in DMF (4 ml) was added sodium hydride (60% in oil, 15 mg, 0.38 mmol) under a nitrogen atmosphere. After stirring at 0 °C for 30 min, iodomethane (0.12 ml, 1.93 mmol) was added dropwise to the mixture, which was then stirred at 0 °C for 30 min and at room temperature for 2 h. After evaporation of the solvent *in vacuo*, the residue was diluted with aqueous ammonium chloride and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). The solution was concentrated *in vacuo*, and the residue purified by flash column chromatography (EtOAc–hexane, 4 : 6 then 9 : 11) to give **33** (0.21 g, 89%) as a white amorphous powder. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (3H, t, J=7.2 Hz), 2.54 (3H, s), 2.66 (3H, s), 2.87 (3H, s), 3.47 (3H, s), 4.25 (2H, q, J=7.2 Hz), 4.65 (2H, s), 4.85 (2H, s), 5.19 (2H, s), 5.37 (2H, s), 7.03—7.08 (3H, m), 7.13—7.18 (1H, m), 7.29—7.40 (4H, m). IR (KBr): 2930, 1750, 1711, 1663, 1607, 1562, 1535, 1477 cm^{-1}. FAB-MS *m/e*: 648 (MH^+).

Potassium Salt of 6-(4-Methoxymethoxyphenyl)-5-methylsulfonylaminomethyl-1-(2-methylthiobenzyl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-3-acetic Acid (32g) To a solution of **32g** (0.13 g, 0.215 mmol) in EtOH (7 ml)–THF (3 ml) was added a solution of $KHCO_3$ (21.5 mg, 0.215 mmol) in H_2O (1.5 ml) at 70 °C. After stirring at 70 °C for 2 min, the mixture was stirred at room temperature for 15 min. After evaporation of the solvent *in vacuo*, the residue was recrystallized from EtOAc–ether to afford the potassium salt of **32g** (0.123 g, 89%) as a white powder, mp 243—247 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.55 (3H, s), 2.88 (3H, s), 3.38 (3H, s), 4.22 (2H, s), 4.29 (2H, d, J=4.6 Hz), 5.19 (2H, s), 5.23 (2H, s), 6.92 (1H, br s), 7.01—7.15 (4H, m), 7.29—7.42 (4H, m). IR (KBr): 3500, 3256, 2932, 1698, 1659, 1599, 1531, 1479 cm^{-1}. FAB-MS *m/e*: 644 (MH^+). *Anal.* Calcd for $C_{26}H_{26}KN_3O_8S_3 \cdot 0.5H_2O$: C, 47.84; H, 4.17; N, 6.44. Found: C, 47.86; H, 4.30; N, 6.53.

Potassium salts of **20d**, **20e**, **32h**, and **32i** were prepared by a similar procedure to that used for the preparation of the potassium salt of **32g**, in 86, 91, 78, and 98% yields, respectively.

Potassium Salt of **20d**: mp 192—198 °C (from EtOAc–EtOH). ^{1}H-NMR (DMSO-d_6) δ: 2.43 (3H, s), 2.55 (3H, s), 3.37 (3H, s), 4.17 (2H, s), 5.14 (2H, s), 5.21 (2H, s), 6.99—7.15 (4H, m), 7.26—7.40 (4H, m). *Anal.* Calcd for $C_{25}H_{23}KN_2O_6S_2 \cdot 0.5H_2O$: C, 53.65; H, 4.32; N, 5.01. Found: C, 53.39; H, 4.18; N, 4.80.

Potassium Salt of **20e**: mp 149—155 °C (from EtOAc–EtOH). ^{1}H-NMR (DMSO-d_6) δ: 2.17 (3H, s), 2.43 (3H, s), 2.55 (3H, s), 4.17 (2H, s), 5.14 (2H, s), 5.29 (2H, s), 6.99—7.15 (4H, m), 7.26—7.40 (4H, m). *Anal.* Calcd for $C_{25}H_{23}KN_2O_5S_3 \cdot 0.4C_2H_6O \cdot 1.6H_2O$: C, 50.47; H, 4.69; N, 4.56. Found: C, 50.22; H, 4.93; N, 4.86.

Potassium Salt of **32h**: mp 155—160 °C (from EtOAc–EtOH–ether). ^{1}H-NMR (DMSO-d_6) δ: 1.16 (3H, t, J=7.2 Hz), 2.55 (3H, s), 2.98 (2H, q, J=7.2 Hz), 3.38 (3H, s), 4.20—4.28 (4H, m), 5.19—5.23 (4H, m), 6.87—7.42 (9H, m). *Anal.* Calcd for $C_{27}H_{28}KN_3O_8S_3 \cdot 0.5H_2O$: C, 48.63; H, 4.38; N, 6.30. Found: C, 48.84; H, 4.66; N, 6.37.

Potassium Salt of **32i**: mp 148—153 °C (from EtOAc–EtOH–ether). ^{1}H-NMR (DMSO-d_6) δ: 0.91 (3H, t, J=7.3 Hz), 1.59 (2H, sext, J=7.3 Hz), 2.55 (3H, s), 2.92 (2H, t, J=7.3 Hz), 3.38 (3H, s), 4.24—4.29 (4H, m), 5.19 (2H, s), 5.23 (2H, s), 6.85—7.15 (5H, m), 7.29—7.42 (4H, m). *Anal.* Calcd for $C_{28}H_{30}KN_3O_8S_3 \cdot 0.5H_2O$: C, 49.39; H, 4.59; N, 6.17. Found: C, 49.33; H, 4.84; N, 6.28.

Expression of Human ET_A and ET_B Receptors in Sf9 Cells Human ET_A cDNA[33] and ET_B cDNA[34] were cloned from human placenta mRNA using reverse transcription and polymerase chain reaction methods. Human ET_A and ET_B cDNAs were subcloned individually into a transfer vector, pBlueBacIII (Invitrogen). The transfer vectors harboring ET_A and ET_B cDNA were co-transfected into Sf9 cells separately with AcMNPV genomic DNA to generate the recombinant baculovirus. Human ET_A and ET_B receptors were expressed in Sf9 cells infected by the recombinant baculovirus as described previously.[35] Sf9 cell membranes were prepared according to the protocol previously reported.[36]

Inhibitory Effect on the Specific Binding of [^{125}I] ET-1 to the Cloned Human ET_A and ET_B Receptors The receptor binding experiments were performed as described previously.[37] Briefly, Sf9 cell membranes (1.4 μg protein/ml for the ET_A receptor and 0.7 μg protein/ml for the ET_B receptor) suspended in binding assay buffer (20 mM Tris, 2 mM EGTA, 5 mM magnesium acetate, 0.1% bovine serum albumin, 0.5 mM phenylmethylsulfonyl fluoride, 20 μg/ml leupeptin, 4 μg/ml E-64, 1 μg/ml pepstatin A, and 0.03% sodium azide, pH 7.2) were incubated with 100 pM [^{125}I]ET-1 (Amersham) and various concentrations of a test compound at 25 °C for 1 h. The mixture was diluted with chilled assay buffer supplemented with 0.05% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) and filtered through a glassfiber filter GF/F (Whatmann). Radioactivity on the filter was counted in a γ-counter to determine bound [^{125}I]ET-1. The concentration of a test compound inducing 50% inhibition of the specific binding (IC_{50} value) was derived by fitting the data into a pseudo Hill equation:

$$\log[\%SPB/(100-\%SPB)] = n[\log C - \log(IC_{50})]$$

where %SPB is specific binding as a percentage of maximum specific binding, n is a pseudo Hill constant, and C is the concentration of a test compound.

Inhibitory Effect on Constriction of Ring Preparations of Porcine Coronary Artery Induced by ET-1 and of Vein Preparations Induced by Sarafotoxin S6c The vasoconstriction assay was performed according to a procedure analogous to that described previously.[20] Ring preparations of porcine coronary artery or vein (3 mm diameter) were placed in an organ bath filled with Krebs solution gassed with 95% O_2—5% CO_2 at 37 °C and allowed to stand for 1.5 h at a resting load of 2 g tension for arteries or 0.5 g tension for veins. The preparations were constricted with 60 mM KCl for 10 min and then washed and allowed to stand for 1 h at the resting load. After pre-treatment of test compounds at various concentrations or vehicle [0.1% dimethyl sulfoxide (DMSO)] for 30 min, artery preparations constricted with ET-1 at 3 nM and vein preparations constricted with sarafotoxin S6c at 1 nM were used to determine the inhibitory effects of test compounds.

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