Discovery of a Thieno[2,3-*d*]pyrimidine-2,4-dione Bearing a *p*-Methoxyureidophenyl Moiety at the 6-Position: A Highly Potent and Orally Bioavailable Non-Peptide Antagonist for the Human Luteinizing Hormone-Releasing Hormone Receptor

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We have previously disclosed the first potent and orally effective non-peptide antagonist for the human luteinizing hormone-releasing hormone (LHRH) receptor, a thieno[2,3-*b*]pyridin-4-one derivative, T-98475 (**1**). Extensive research on developing non-peptide LHRH antagonists has been carried out by employing a strategy of replacing the thienopyridin-4-one nucleus with other heterocyclic surrogates. We describe herein the design and synthesis of a series of thieno-[2,3-*d*]pyrimidine-2,4-dione derivatives containing a biaryl moiety, which led to the discovery of a highly potent and orally active non-peptide LHRH antagonist, 5-(*N*-benzyl-*N*-methylaminomethyl)-1-(2,6-difluorobenzyl)-6-[4-(3-methoxyureido)phenyl]-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (**9k**: TAK-013). Compound **9k** showed high binding affinity and potent in vitro antagonistic activity for the human receptor with half-maximal inhibition concentration (IC_{50}) values of 0.1 and 0.06 nM, respectively. Oral administration of **9k** caused almost complete suppression of the plasma LH levels in castrated male cynomolgus monkeys at a 30 mg/kg dose with sufficient duration of action (more than 24 h). The results demonstrated that the thienopyrimidine-2,4-dione core is an excellent surrogate for the thienopyridin-4-one and that thienopyrimidine-2,4-diones and thienopyridin-4-ones constitute a new class of potent and orally bioavailable LHRH receptor antagonists. Furthermore, molecular modeling studies indicate that the unique methoxyurea side chain of **9k** preferentially forms an intramolecular hydrogen bond between the aniline NH and the methoxy oxygen atom. The hydrogen bond will shield the hydrogen bonding moieties from the solvent and reduce the desolvation energy cost. It is therefore speculated that the intramolecular hydrogen bond resulting from judicious incorporation of an oxygen atom into the terminal alkyl group of the urea may increase the apparent lipophilicity to allow increased membrane permeability and consequently to improve the oral absorption of **9k** in monkeys. On the basis of its profile, compound **9k** has been selected as a candidate for clinical trials and it is expected that it will provide a new class of potential therapeutic agents for the clinical treatment of a variety of sex-hormone-dependent diseases.

Introduction

Luteinizing hormone-releasing hormone (LHRH, also known as gonadotropin-releasing hormone (GnRH)), discovered by Schally et al. in 1971,[1] is a decapeptide amide that plays a pivotal role in modulating reproductive functions. It is secreted from the hypothalamus in a pulsatile pattern and binds to the LHRH receptor on the anterior pituitary. The activation of this G-protein-coupled receptor causes the biosynthesis and release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which regulate gonadal steroidogenesis and gametogenesis.

Several peptidic LHRH agonists,[2] which ultimately suppress gonadal steroid production and achieve a condition called "biochemical castration"[3,4] through a receptor down-regulation mechanism, have been approved for the clinical treatment of a variety of endocrine-based diseases such as prostate cancer, breast cancer, endometriosis, uterine leiomyoma, and precocious puberty.[5,6] One of the major drawbacks of these LHRH agonists is the initial gonadal hormone surge, the "flare effect", which tends to exacerbate the symptoms of the above conditions. In contrast, LHRH antagonists are expected to suppress gonadotropins from the onset of administration.[4] In fact, recent clinical studies have demonstrated that peptidic LHRH antagonists directly lowered the steroid hormone levels without the adverse flare effect.[4,7] However, peptidic LHRH antagonists still suffer from insufficient oral bioavailability because of

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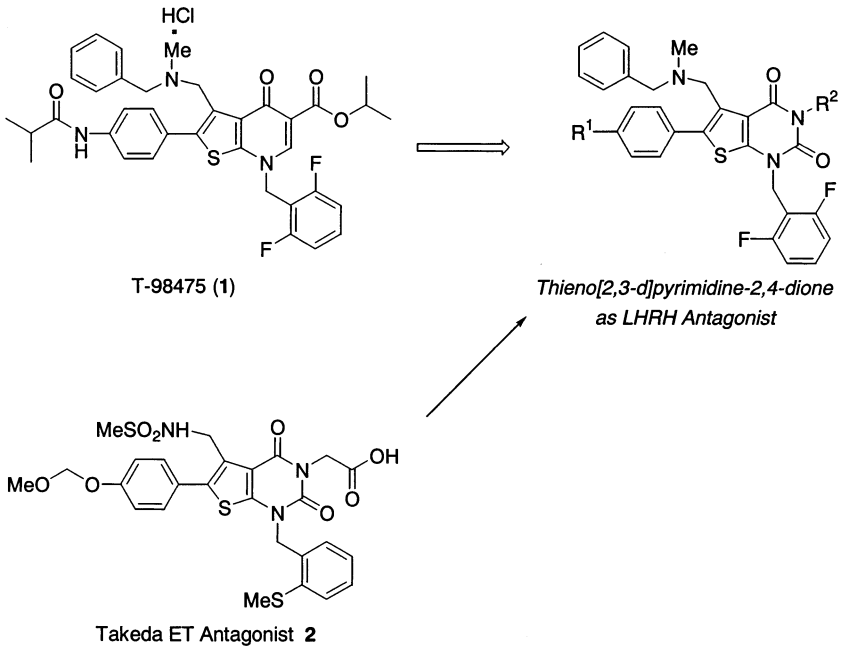


Figure 1.

being peptidic in nature, and therefore, potent and orally effective small-molecule LHRH antagonists could be superior drugs for clinical applications in the above conditions.

To date, four different classes of non-peptide LHRH antagonists have been reported: 2-phenylthienopyridin-4-ones[8] from our laboratory, more recently, 3-phenylquinolones[9] and 2-phenylindoles[10] from Merck, and 2-phenylpyrrolopyrimid-7-ones[11] from Neurocrine Biosciences. In our previous paper, we have described the discovery of the first potent and orally active non-peptide LHRH receptor antagonist T-98475 (**1**), a thieno[2,3-*b*]pyridin-4-one derivative, and the initial structure−activity relationship (SAR) of this class[8] (Figure 1). Our approach for developing non-peptide LHRH antagonists is based on the introduction of crucial functional groups for receptor binding into a bicyclic scaffold, which mimics a type II β-turn involving residues 5−8 (Tyr-Gly-Leu-Arg) of LHRH that has been proposed as the putative bioactive conformation.[6] Although T-98475 (**1**) showed excellent in vitro LHRH antagonistic activities, the in vivo antagonism of **1** when administered orally was not as effective as we had expected,[8] which may be due to the inadequate oral bioavailability conferred by the nature of its substituents.

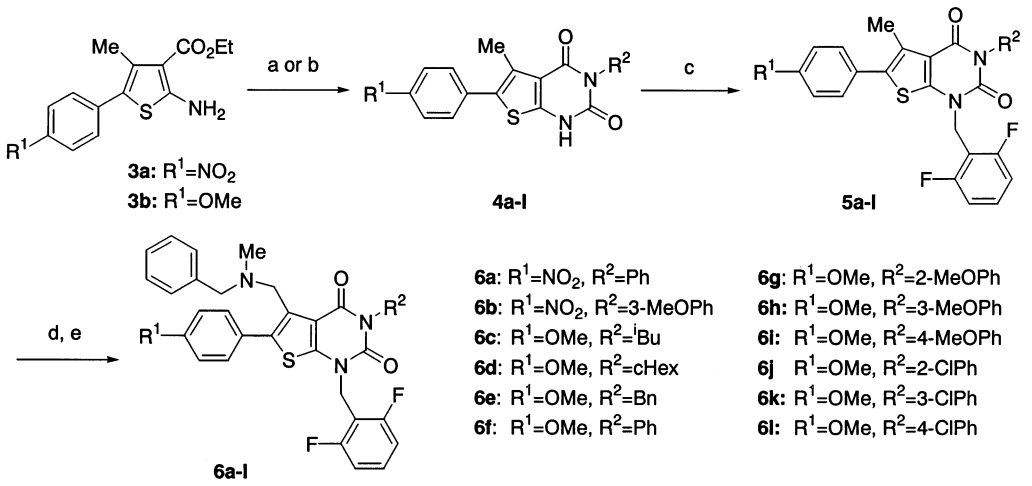
The observation prompted us to conduct an extensive research program to identify small-molecule LHRH antagonists with improved in vivo efficacy. The following two strategies were employed: (i) further optimization of each substituent of T-98475 (**1**), e.g., chemical modification of the ester moiety; (ii) replacement of the thienopyridin-4-one scaffold with other suitable heterocyclic surrogates. The former strategy of investigating T-98475-related compounds led to the identification of highly potent thienopyridin-4-ones bearing the *p*-hydroxyalkylamidophenyl and ketone moieties at the 2- and 5-position, respectively.[12] Simultaneously, we turned our attention back to the previous work from this laboratory on the non-peptide endothelin (ET) antagonist **2**.[13] Compound **2** was discovered according to essentially the same strategy described here, i.e., using the 6-phenylthieno[2,3-*d*]pyrimidine-2,4-dione core as a scaffold for non-peptide ET antagonists. In addition, the thienopyrimidine-2,4-dione core could possess important functional groups participating in ligand−receptor interaction at the corresponding positions of the thienopyridin-4-one nucleus. On the basis of the latter strategy, we therefore used the thienopyrimidine-2,4-dione core as a scaffold for the LHRH antagonist and conducted synthetic studies on this surrogate.

In this article, we describe the synthesis of the thieno-[2,3-*d*]pyrimidine-2,4-dione derivatives and their biological activity. This study revealed that the thienopyrimidine-2,4-dione nucleus was an excellent surrogate for the thienopyridin-4-one and culminated in the discovery of a highly potent and orally bioavailable non-peptide LHRH antagonist, a thienopyrimidine-2,4-dione bearing the unique *p*-(3-methoxyureido)phenyl group at the 6-position (**9k**: TAK-013). Furthermore, molecular modeling studies on **9k** that indicate the beneficial effect of the methoxyureido group on the oral absorption in monkeys are also described.

Chemistry

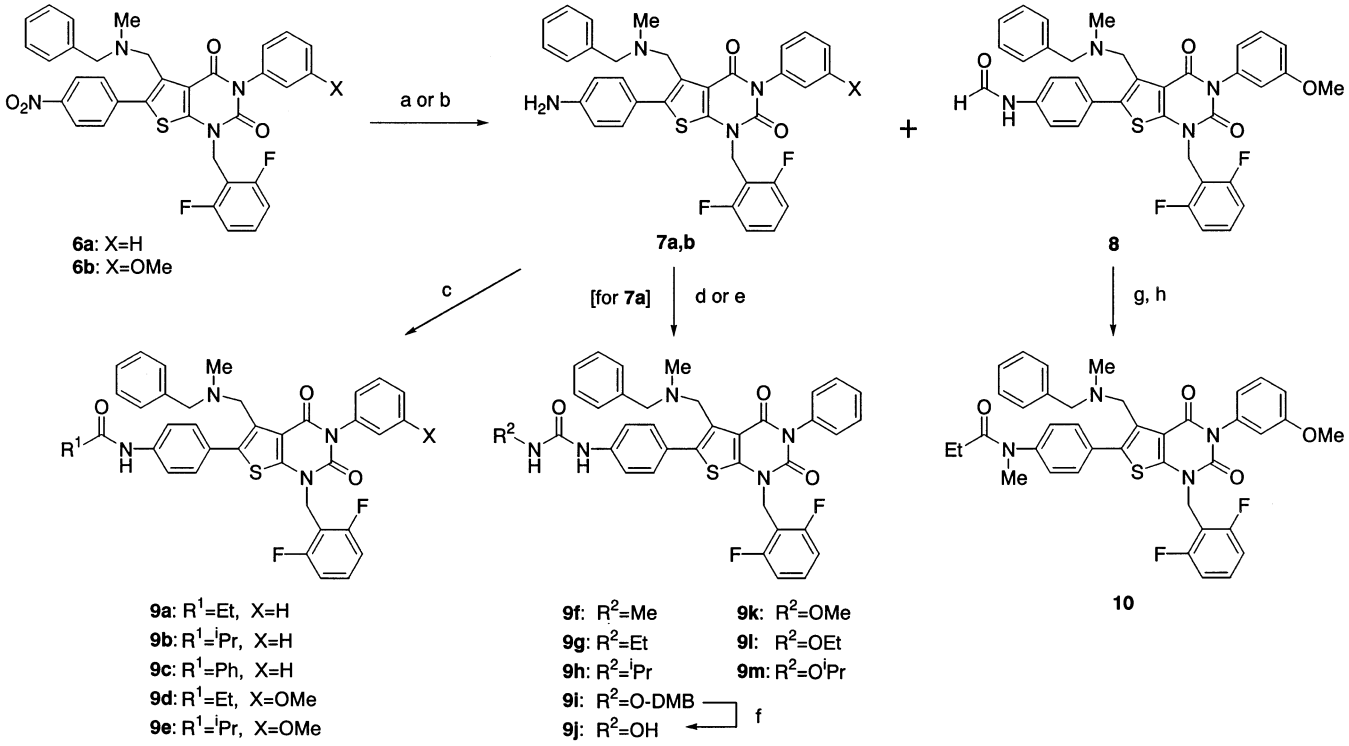
The thieno[2,3-*d*]pyrimidine-2,4-dione derivatives examined in this study were synthesized by the general procedure shown in Scheme 1. The 2-aminothiophenes **3a**,**b** prepared from the corresponding phenylacetones using the previously reported Gewald's procedure[14] were reacted with a variety of isocyanates. The resulting ureas were cyclized under basic conditions to afford the thienopyrimidine-2,4-diones **4a**−**l**. Isobutyl isocyanate, used in the preparation of **4c**, was obtained by the Curtius rearrangement reaction of isovaleric acid with diphenylphosphoryl azide (DPPA) and was used without purification. Difluorobenzylation of **4a**−**l** at the 1-position, followed by radical bromination of the 5-methyl group with *N*-bromosuccinimide (NBS), furnished the intermediary bromomethyl analogues, which were con-

Scheme 1[a]



[a] Reagents: (a) (1) R^2NCO, pyridine, (2) NaOMe, MeOH or NaOEt, EtOH; (b) (1) isovaleric acid, DPPA, Et_3N, toluene, (2) NaOMe, MeOH; (c) 2,6-difluorobenzyl chloride, K_2CO_3, KI, DMF; (d) NBS, AIBN, chlorobenzene; (e) *N*-benzylmethylamine, $^iPr_2NEt$, DMF.

Scheme 2[a]

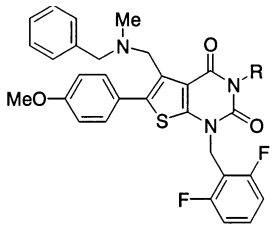


[a] Reagents: (a) H_2, Pd−C, HCl−Et_2O, HCO_2H (for the preparation of **7a**); (b) H_2, Pd−C, HCO_2H (for the preparation of **7b** and **8**); (c) R^1COCl, Et_3N, CH_2Cl_2; (d) (1) CDI, Et_3N, CH_2Cl_2, (2) alkylamine or *O*-alkylhydroxylamine, Et_3N (if necessary), CH_2Cl_2 or THF; (e) ethyl isocyanate, pyridine; (f) TFA, CH_2Cl_2; (g) $BH_3{\cdot}Me_2S$, THF; (h) EtOCOCl, Et_3N, CH_2Cl_2.

verted to compounds **6a**−**l** by introduction of the *N*-benzylmethylamino moiety.

Chemical modification of the para substituent on the 6-phenyl ring was performed according to the synthetic route depicted in Scheme 2. Catalytic hydrogenation of the nitro compound **6b** in formic acid produced the requisite aniline **7b** together with the undesired formylated byproduct **8**. This problem was overcome by addition of excess anhydrous hydrogen chloride to trap **7b** as an inactive hydrochloride. Thus, compound **7a** was successfully obtained from **6a** by reduction with palladium−charcoal in the presence of hydrogen chloride. Compounds **7a**,**b** were then converted to the amide, urea, and urea-related derivatives: the amides **9a**−**e**, alkylureas **9f**−**i**, hydroxyurea **9j**, and alkoxyureas **9k**−**m**. First, compounds **9a**−**e** and **9g** were prepared by reaction of **7a**,**b** with the appropriate acyl chlorides or ethyl isocyanate under basic conditions. Second, compounds **9f**,**h**,**i** and **9k**−**m** were obtained by a two-step methodology, i.e., reaction of **7a** with 1,1′-carbonyldiimidazole (CDI) to give the intermediary imidazolide and subsequent nucleophilic substitution with the corresponding alkylamines or *O*-alkylhydroxylamines in a one-pot reaction. The 3-(2,4-dimethoxybenzyloxy)urea **9i** was deprotected by treatment with trifluoroacetic acid (TFA) to afford the hydroxyurea **9j**.[15] Finally, compound **8** was reduced with a borane−methyl sulfide complex and the resulting amine was subsequently

Table 1. Physicochemical Data and Binding Affinities of Compounds **6c**−**l**



compound	R	mp (°C)	recryst solvent	formula	IC_{50} [a] (μM)
6c	^{i}Bu	165−167	EtOAc−Et_2O	$C_{33}H_{33}N_3O_3SF_2\cdot HCl$	0.4
6d	*c*Hex	145−150	CH_2Cl_2−Et_2O	$C_{35}H_{35}N_3O_3SF_2\cdot HCl\cdot 0.5H_2O$	0.7
6e	Bn	123−127	EtOAc−Et_2O	$C_{36}H_{31}N_3O_3SF_2\cdot HCl\cdot 0.5H_2O$	0.4
6f	Ph	160−162	EtOAc−Et_2O	$C_{35}H_{29}N_3O_3SF_2\cdot HCl\cdot 0.5H_2O$	0.05
6g	2-MeOPh	148−150	EtOAc−Et_2O	$C_{36}H_{31}N_3O_4SF_2\cdot HCl\cdot 1.5H_2O$	0.2
6h	3-MeOPh	160−163	EtOAc−Et_2O	$C_{36}H_{31}N_3O_4SF_2\cdot HCl\cdot 0.8H_2O$	0.02
6i	4-MeOPh	153−157	EtOAc−Et_2O	$C_{36}H_{31}N_3O_4SF_2\cdot HCl\cdot 0.5H_2O$	0.03
6j	2-ClPh	150−155	EtOAc−Et_2O	$C_{35}H_{28}N_3O_3SClF_2\cdot HCl\cdot 0.5H_2O$	0.08
6k	3-ClPh	152−157	EtOAc−Et_2O	$C_{35}H_{28}N_3O_3SClF_2\cdot HCl\cdot H_2O$	0.2
6l	4-ClPh	145−146	EtOAc−Et_2O	$C_{35}H_{28}N_3O_3SClF_2\cdot HCl\cdot H_2O$	0.2

[a] The binding affinity is reported as an IC_{50} value, which is the antagonist concentration required to inhibit the specific binding of [^{125}I]leuprorelin to LHRH receptor by 50%. Chinese hamster ovary (CHO) cells expressing human LHRH receptors were used as the source for LHRH receptors. All data are expressed as the mean of two or three determinations.

acylated with propionyl chloride to yield the *N*-methylanilide **10**.

Results and Discussion

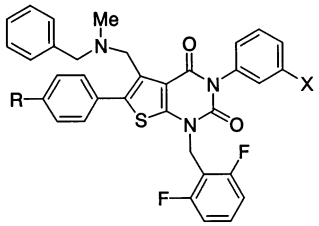
In Vitro Receptor Binding Studies. Our investigation began with an SAR study of thienopyrimidine-2,4-diones based on data from an in vitro binding assay using the cloned Chinese hamster ovary (CHO) cells expressing the human LHRH receptor and [^{125}I]leuprorelin as a ligand. The preliminary in vitro studies on T-98475-related compounds indicated that the binding affinity was dependent on the para substituent of the 2-phenyl group.[8] Accordingly, compounds possessing the *N*-benzyl-*N*-methylaminomethyl and 2,6-difluorobenzyl moieties[8,12] important for receptor binding were synthesized and evaluated for LHRH receptor binding affinity.

First, the 3-substituent of the thienopyrimidine-2,4-dione, which corresponds to the 5-ester group of T-98475 (**1**), was investigated with compounds bearing a *p*-methoxyphenyl group at the 6-position, and the results and physicochemical data are presented in Table 1. The alkyl and aralkyl analogues, e.g., isobutyl (**6c**), cyclohexyl (**6d**), and benzyl (**6e**), were found to exhibit noticeable submicromolar affinities. In contrast, aromatization of the cyclohexyl ring of **6d** (**6f**) induced a 14-fold increase in affinity, which led us to examine next the substituted phenyl derivatives. Incorporation of a methoxy group into the 3-phenyl ring (**6h**,**i**) produced modest enhancement in affinity except at the ortho position (**6g**), whereas introduction of a chlorine atom into the 3-phenyl ring (**6j**−**l**) tended to result in a deleterious effect on the activity. In view of the limited effect of the substituent on affinity in the substituted phenyl series, the *m*-methoxyphenyl and nonsubstituted phenyl groups were chosen as the 3-substituents for further investigation.

The preceding SAR studies on the thienopyridin-4-ones revealed that para substitution on the 2-phenyl ring was preferable to ortho or meta substitution.[8,12] On the basis of this finding, effects of the para substituent on the 6-phenyl ring were investigated, and the results and physicochemical data are shown in Table 2. In the *m*-methoxyphenyl series at the 3-position, nitro (**6b**) was equipotent with methoxy (**6h**); however, reduction of the nitro group of **6b** led to a boost in activity. The aniline **7b** exhibited remarkably improved affinity with an IC_{50} value of 1 nM, which was further enhanced by acylation of the amino group (**9a**−**e**). The amides **9a**,**b**,**d**,**e** exhibited subnanomolar affinities (IC_{50} = 0.2−0.4 nM), which were almost comparable to that of T-98475 (**1**) (IC_{50} = 0.2 nM). However, the benzamide **9c** was about 3-fold less potent than **9a**,**b**. In addition, N-methylation of the amide nitrogen (**10**) resulted in a marked decrease in activity. These results suggest that hydrogen donation from the aniline NH plays an important role for receptor−ligand interaction and that a spatial limitation may exist for the recognition site interacting with this part of the molecule.

Comparison between **9a**,**b** and **9d**,**e** indicates that introduction of the *m*-methoxy group into the 3-phenyl ring showed a minimal effect on the binding affinity when the 6-substituent incorporated is a *p*-acylaminophenyl moiety. Since it is well-known that the methoxyphenyl group tends to be one of the targets for metabolic enzymes, subsequent investigation into amide-related substituents of the 6-phenyl group was conducted with compounds bearing a nonsubstituted phenyl group at the 3-position. Taking into account the high affinity of the *p*-acylaminophenyl derivatives at the 6-position, it is hypothesized that the binding site accommodating this part of the molecule must be mainly hydrophilic. Thus, we envisaged that incorporation of the nitrogen and/or oxygen atoms into the amide moiety would provide more potent antagonists because of additional hydrogen bonding interaction with the LHRH receptor. Incorporation of the NH group into the amide moiety tended to increase the activity. Indeed, the alkylureas **9f**−**h** were more potent than the amides **9a**,**b**. Intriguingly, inclusion of an additional oxygen atom into the alkylurea moiety of **9f**,**g** had a beneficial effect on oral absorption compared to the alkylureas, as mentioned later, without affecting the in vitro activities. Although the hydroxyurea **9j** and isopro-

Table 2. Physicochemical Data and Binding Affinities of Compounds **6b**,**h**, **7b**, **9a**−**h**, **9j**−**m**, and **10**



compound	R	X	mp (°C)	recryst solvent	formula	IC_{50} [a] (nM)
6h	MeO	OMe	160−163	EtOAc−Et_2O	$C_{36}H_{31}N_3O_4SF_2$·HCl·0.8H_2O	20
6b	O_2N	OMe	142−144	CH_2Cl_2−Et_2O	$C_{35}H_{28}N_4O_5SF_2$·HCl·H_2O	20
7b	H_2N	OMe	162−165	EtOAc−Et_2O	$C_{35}H_{30}N_4O_3SF_2$·2.0HCl·H_2O	1
9d	EtCONH	OMe	170−175	EtOAc−Et_2O	$C_{38}H_{34}N_4O_4SF_2$·HCl·1.5H_2O	0.2
9e	^{i}PrCONH	OMe	170−173	EtOAc−Et_2O	$C_{39}H_{36}N_4O_4SF_2$·HCl·H_2O	0.3
10	EtCON(Me)	OMe	138−143	CH_2Cl_2−Et_2O	$C_{39}H_{36}N_4O_4SF_2$·HCl·1.5H_2O	20
9a	EtCONH	H	197−202	EtOAc−Et_2O	$C_{37}H_{32}N_4O_3SF_2$·HCl·H_2O	0.3
9b	^{i}PrCONH	H	185−190	EtOAc−Et_2O	$C_{38}H_{34}N_4O_3SF_2$·HCl·1.5H_2O	0.4
9c	PhCONH	H	167−169	EtOAc−Et_2O	$C_{41}H_{32}N_4O_3SF_2$·HCl·0.5H_2O	1
9f	MeNHCONH	H	175−180	CH_2Cl_2−Et_2O	$C_{36}H_{31}N_5O_3SF_2$·HCl·H_2O	0.1
9g	EtNHCONH	H	179−182	$CHCl_3$−Et_2O	$C_{37}H_{33}N_5O_3SF_2$·HCl·1.5H_2O	0.1
9h	^{i}PrNHCONH	H	172−177	EtOAc−Et_2O	$C_{38}H_{35}N_5O_3SF_2$·HCl·H_2O	0.2
9j	HONHCONH	H	180−186	MeOH−Et_2O	$C_{35}H_{29}N_5O_4SF_2$·HCl·H_2O	0.4
9k (TAK-013)	MeONHCONH	H	204−205	$CHCl_3$−Et_2O	$C_{36}H_{31}N_5O_4SF_2$	0.1
9l	EtONHCONH	H	193−197	CH_2Cl_2−Et_2O	$C_{37}H_{33}N_5O_4SF_2$·HCl·2.0H_2O	0.2
9m	^{i}PrONHCONH	H	201−204	CH_2Cl_2−Et_2O	$C_{38}H_{35}N_5O_4SF_2$·HCl·0.5H_2O	0.6

[a] The binding affinity is reported as an IC_{50} value, which is the antagonist concentration required to inhibit the specific binding of [^{125}I]leuprorelin to LHRH receptor by 50%. Chinese hamster ovary (CHO) cells expressing human LHRH receptors were used as the source for LHRH receptors. All data are expressed as the mean of two or three determinations.

poxyurea **9m** were more than 4-fold less potent than **9f**,**g**, the methoxyurea **9k** (IC_{50} = 0.1 nM) and the ethoxyurea **9l** (IC_{50} = 0.2 nM) showed comparable affinities to **9f**,**g**. With respect to the para substituent on the 6-phenyl ring, it can be seen that both the hydrogen-donating NH and terminal small alkyl (C1−C3) groups are important for high binding affinity. From the above binding assay data, the methylurea **9f**, ethylurea **9g**, and methoxyurea **9k** had the optimum LHRH receptor binding affinities, with IC_{50} values of 0.1 nM, and compounds **9g**,**k** were selected for further in vitro and in vivo evaluation.

Further in Vitro and in Vivo Studies. First, the amide **9a** and the ureas **9g**,**k** were evaluated for their binding affinities to LHRH receptors of the particular species. It can be seen from Table 3 that there are marked species specificities in receptor binding affinities similar to those observed for T-98475 (**1**).[8] Compounds **9a**,**g**,**k** exhibited more than 3- and 2000-fold selectivity for the human receptor over the monkey and rat receptors, respectively. Hence, it is reasonable that further in vitro and in vivo evaluations of these compounds are performed utilizing monkey as well as human receptors.

Second, for in vitro functional antagonism, assays for inhibition of LHRH-stimulated arachidonic acid release from CHO cells expressing the human and monkey receptors were undertaken. Compounds **9a**,**g**,**k** effectively antagonized LHRH function on CHO cells expressing the human and monkey receptors (Table 3). Moreover, species specificities in functional antagonism were observed in a manner similar to that described above for binding affinity. The in vitro antagonism of the human receptor (IC_{50} = 0.06−4 nM) exceeded that of the monkey receptor (IC_{50} = 7−300 nM), and in general, the order of the antagonistic effect virtually paralleled that of the binding affinity.

Table 3. Species Specificities of Compounds **1** and **9a**,**g**,**k** Binding to the LHRH Receptors and Inhibitory Effects of Compounds **1** and **9a**,**g**,**k** on LHRH-Stimulated Arachidonic Acid (AA) Release

	binding affinity[a] IC_{50} [c] (nM)			inhibition of AA release[b] IC_{50} [c] (nM)	
compound	human[d]	monkey[d]	rat[e]	human	monkey
1	0.2	2 (4[f])	60	0.6	nd[g]
9a	0.3	9	700	4	300
9g	0.1	0.3	200	0.07	7
9k (TAK-013)	0.1	0.6	>1000	0.06	10

[a] The binding affinity is reported as an IC_{50} value, which is the antagonist concentration required to inhibit the specific binding of [^{125}I]leuprorelin to LHRH receptor by 50%. [b] Inhibition of LHRH-stimulated arachidonic acid (AA) release from CHO cells expressing human or monkey LHRH receptors was measured to evaluate the functional LHRH antagonism of the test compounds. The IC_{50} value is the antagonist concentration required to inhibit the LHRH-stimulated AA release from CHO cells by 50%. [c] All data are expressed as the mean of two or three determinations. [d] Chinese hamster ovary (CHO) cells expressing human or monkey LHRH receptors were used as the source for LHRH receptors.[e] Rat anterior pituitaries were used as the source for LHRH receptors. [f] Monkey anterior pituitaries were used as the source for LHRH receptors (see ref 8). [g] nd = not determined.

It is widely acknowledged that urea tends to show poor oral absorption resulting from relatively low solubility and/or poor membrane permeability due to its strong hydrogen bonding ability. We therefore set out to probe oral absorption of **9a**,**g**,**k** preliminarily using cynomolgus monkeys, and the results are summarized in Table 4. As we predicted, the oral absorption of the amide **9a** in monkeys was superior to that of the ethylurea **9g** at a dose of 10 mg/kg. Surprisingly, judicious incorporation of an oxygen atom into the terminal alkyl group of **9g** provided us with the final breakthrough. Thus, the methoxyurea **9k** exhibits improved oral absorption when compared with **9g** at the

Table 4. Oral Absorption of Compounds **9a**,**g**,**k** in Cynomolgus Monkeys[a]

compound	C_{max} [b] (μM)	T_{max} [c] (h)	AUC_{0-6} [d] (μM·h)
9a	0.63	3	2.5
9g	0.063	3	0.27
9k (TAK-013)[e]	0.21	6	0.85

[a] Compounds **9a**,**g**,**k** (10 mg/kg) suspended in 0.5% methylcellulose were orally administered to cynomolgus monkeys (female, 4−8 years old, $n = 3$). [b] Maximum plasma concentration after 10 mg/kg oral dosing. [c] Time to C_{max}. [d] Area under the concentration−time curve for 0−6 h after 10 mg/kg oral dosing. [e] TAK-013 hydrochloride was used (see Experimental Section).

same dosage. These results suggest that the methoxyurea **9k** is a promising compound for exerting a potent in vivo efficacy when administered orally.

Finally, the in vivo antagonism of compound **9k** was investigated for the suppression of plasma LH concentrations in castrated male cynomolgus monkeys (Table 5). As we had anticipated, oral administration of a 30 mg/kg dose of the methoxyurea **9k** caused almost complete suppression of plasma LH levels (11% of pretreatment at 24 h) in monkeys. The suppressive effect of **9k** at a 30 mg/kg dose (po) was more potent than that of T-98475 (**1**) at a 60 mg/kg dose (po), and **9k** exhibited longer duration of action than **1**.[8] It should be noted that exposure to **9k** even at a 10 mg/kg dose (po) resulted in effective suppression (approximately 20% of pretreatment from 8 to 24 h). In these experiments the maximum plasma concentrations of **9k** were 0.34 μM (reached 6 h after administration) and 0.18 μM (reached 4 h after administration) at 30 and 10 mg/kg doses, respectively. As a consequence, the methoxyurea **9k** potently antagonized the elevated LHRH function induced by castration in male cynomolgus monkeys and the suppressive effects lasted for more than 24 h at both 30 and 10 mg/kg po doses. These data clearly demonstrate that compound **9k** is a potent and orally active LHRH antagonist. The potent in vivo antagonism of **9k** may result from its potent in vitro activity and/or excellent pharmacokinetic profiles. Furthermore, the large difference between in vitro antagonistic activities of the methoxyurea **9k** toward human and monkey receptors implies that a far smaller amount of **9k** would be sufficient to suppress plasma LH levels in humans.

Molecular Modeling Studies. Incorporation of an oxygen atom into the methylurea moiety of **9f** (**9k**) resulted in respectable improvement of the oral absorption in monkeys. During the conformational analysis of **9k**, using high-temperature molecular dynamics calculation, it was observed that the cis conformer of the methoxyurea was more populated than the trans conformer (see Table 6 for the definition of the cis and trans conformers). Both the molecular mechanics calculations and ab initio calculations confirmed that the cis conformer is more stable than the trans conformer by about 6−7 kcal/mol, as shown in Table 6. The stability of the cis conformer of the methoxyurea can be attributed to the intramolecular hydrogen bond between the aniline NH and the methoxy oxygen atom. This intramolecular hydrogen bond shields the hydrogen bonding moieties from the solvent and will reduce the desolvation energy cost during intestinal absorption. It is widely acknowledged that urea is one of the strongest hydrogen bonding functional groups,[16] which renders compounds with the urea moiety poorly bioavailable when administered orally. In fact, when the urea NH was capped with aliphatic groups, improvements in oral bioavailability were reported for somatostatin agonists[17] and β_3-adrenergic receptor agonists.[18] Ashwood et al. have recently described that the intramolecular hydrogen bond increased the penetration of the central nervous system by a neurokinin-1 receptor antagonist.[19] Since a urea moiety can contribute to the stabilization of the drug−receptor interaction through its strong hydrogen bonding properties, it is often tempting to introduce it into lead compounds in order to improve their affinities toward the target receptors or enzymes. As demonstrated here, we have attained improvement in both the in vitro activities and oral absorption through judicious incorporation of the unique methoxyurea moiety at the para position of the 6-phenyl ring. When the urea NH participates in the critical hydrogen bonding interaction, as inferred here, capping of the urea NH may not be tolerated. Introduction of a properly designed intramolecular hydrogen bond is a versatile strategy for improving oral absorption without deteriorating the potency of binding toward the target proteins of lead compounds.

Conclusion

Starting from T-98475 (**1**), we have replaced the thieno[2,3-*b*]pyridin-4-one core with a thieno[2,3-*d*]-pyrimidine-2,4-dione scaffold to attempt to develop a new non-peptide LHRH antagonist. Prominent improvements in both in vitro activities and oral absorption for this class have been accomplished through introduction of the unique methoxyurea moiety into the para position of the 6-phenyl ring. Consequently, we have succeeded in the discovery of a highly potent and orally bioavailable non-peptide antagonist for the human LHRH receptor, the methoxyurea **9k** (TAK-013). Compound **9k** showed high binding affinity and potent in vitro antagonistic activity for the human receptor, with IC_{50} values of 0.1 and 0.06 nM, respectively. The methoxyurea **9k** exhibited improved oral absorption in monkeys compared to the ethylurea **9g**, and oral administration of **9k** produced almost complete suppression of plasma LH levels in castrated male cynomolgus monkeys at a 30 mg/kg dose, with sufficient duration of action (more than 24 h). The SAR of compounds described here demonstrated that the thienopyrimidine-2,4-dione nucleus is an excellent surrogate for the thienopyridin-4-one and that both the *p*-acylamino- and *p*-ureidophenyl groups at the 6-position and the 3-phenyl ring play important roles in potent antagonistic activity. Furthermore, molecular modeling studies suggest that **9k** forms an intramolecular hydrogen bond between the aniline NH and the methoxy oxygen atom, which shields the hydrogen bonding moieties from the solvent and results in increasing apparent lipophilicity. On the basis of this finding, we speculate that the presence of the intramolecular hydrogen bond may increase the membrane permeability of the alkoxyurea during intestinal absorption, which ultimately causes the improved oral absorption of **9k** in monkeys.

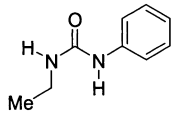
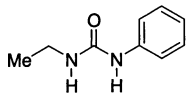
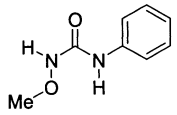
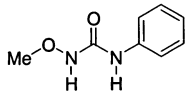
From these biochemical and pharmacological results, compound **9k** (TAK-013) has been selected as a candidate for clinical trials and it is expected that the methoxyurea **9k** will provide a new class of useful therapeutic agents for the treatment of sex-hormone-

Table 5. Time Course of Plasma LH Concentrations in Castrated Male Cynomolgus Monkeys after Oral Administration of Compound **9k** at 10 and 30 mg/kg Doses

	LH concentration[a] (mU/mL)					
	at 0 h	at 2 h	at 4 h	at 8 h	at 24 h	at 48 h
vehicle	1.13 ± 0.20	0.99 ± 0.17	0.89 ± 0.13	0.97 ± 0.17	0.93 ± 0.16	0.85 ± 0.14
	(100.0 ± 0.0)	(87.5 ± 3.9)	(80.0 ± 3.2)	(85.5 ± 0.4)	(82.2 ± 0.2)	(75.8 ± 2.3)
dose = 10 mg/kg	1.01 ± 0.18	0.74 ± 0.17	0.37 ± 0.12	0.19 ± 0.06	0.18 ± 0.01	0.76 ± 0.17
	(100.0 ± 0.0)	(72.9 ± 3.5)	(35.7 ± 4.9)	(18.4 ± 2.8)	(19.7 ± 4.1)	(74.7 ± 5.3)
dose = 30 mg/kg	0.91 ± 0.10	0.55 ± 0.10	0.25 ± 0.03	0.13 ± 0.02	0.10 ± 0.04	0.71 ± 0.15
	(100.0 ± 0.0)	(59.5 ± 5.7)	(27.7 ± 0.6)	(14.5 ± 2.2)	(10.8 ± 4.6)	(76.7 ± 10.6)

[a] Cynomolgus monkeys (male, 4−9 years old) were castrated more than 6 months prior to the examination. Compound **9k** (10 or 30 mg/kg, 3 mL/kg, $n = 3$ for each group) suspended in 0.5% methylcellulose containing 1.2% citric acid, or 0.5% methylcellulose containing 1.2% citric acid alone (3 mL/kg, $n = 3$), was administered orally. Figures in parentheses are percentages of LH concentration at the indicated times, when LH concentration at 0 h (pretreatment) is taken as 100% for each group. Values shown are the mean ± SEM.

Table 6. Energy Differences between the Cis (**I**) and Trans (**II**) Conformers of *N*-Ethyl-*N*′-phenylurea and *N*-Methoxy-*N*′-phenylurea[a]

	Discover (CVFF)		DMol	
	E [b] (kcal/mol)	$\Delta E_{cis-trans}$ [c]	E [d] (kcal/mol)	$\Delta E_{cis-trans}$ [c]
N-ethyl-*N*′-phenylurea				
I 	−13.08		−2970.24	
		1.71		−0.67
II 	−14.79		−2969.57	
N-methoxy-*N*′-phenylurea				
I 	−9.50		−2723.95	
		−5.75		−7.42
II 	−3.75		−2716.53	

[a] The cis (**I**) and trans (**II**) conformers were constructed using Insight II and subjected to either energy minimization by Discover, using the CVFF force field, or geometry optimization by DMol. [b] Internal energy obtained by Discover for each conformer after minimization. [c] Energy difference between the two conformers as calculated by subtracting the energy value of the trans conformer from that of the cis conformer. [d] Final binding energy obtained by DMol for each conformer after geometry optimization.

dependent pathologies. Further detailed pharmacological studies on **9k** are in progress, and the results will be presented elsewhere.

Experimental Section

Chemistry. General Procedures. All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. The proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a JEOL JNM-LA300 (300 MHz) spectrometer. Chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard. All J values are given in hertz. The infrared (IR) spectra were measured on a JASCO FTIR spectrometer. FAB mass spectra were recorded on a JEOL JMS-HX110. Elemental analyses were within ±0.4% of theoretical values and were determined at Takeda Analytical Research Laboratories, Osaka. Reagents and solvents were obtained from commercial sources and used without further purification. Flash chromatography was performed with Merck silica gel 60 (art. 9385; 230−400 mesh), and reaction progress was determined by thin-layer chromatography (TLC) analysis on silica gel 60 F_{254} plates (Merck). Visualization was done with UV light (254 nm) or iodine. Yields are of purified compounds and were not optimized.

Ethyl 2-Amino-4-methyl-5-(4-nitrophenyl)thiophene-3-carboxylate (3a). A mixture of 4-nitrophenylacetone (39.0 g, 0.22 mol), ethyl cyanoacetate (25.1 g, 0.22 mol), ammonium acetate (3.36 g, 0.04 mmol), and acetic acid (10.0 mL, 0.17 mol) in toluene (100 mL) was heated under reflux for 20 h, while water was azeotropically removed using Dean−Stark apparatus. After the mixture was cooled to room temperature, the mixture was concentrated in vacuo. The residue was diluted with saturated $NaHCO_3$ 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 (CH_2Cl_2−hexane, 2:4 to 4:1) to give a red liquid (43.6 g), which was dissolved in EtOH (130 mL). To the solution were added sulfur powder (5.6 g, 0.18 mol) and diethylamine (18 mL, 0.18 mol), and the resulting mixture was stirred at 65 °C for 2 h. After removal of the solvent in vacuo, the residue was diluted with brine and extracted with $CHCl_3$. The extract was washed with brine and dried ($MgSO_4$). The solution was concentrated in vacuo, and the residue was recrystallized from $CHCl_3$ to afford **3a** (32.9 g, 67%) as dark-orange crystals: mp 168−170 °C. ^{1}H NMR ($CDCl_3$): δ 1.39 (3H, t, $J = 7.1$ Hz), 2.40 (3H, s), 4.34 (2H, q, $J = 7.1$ Hz), 6.27 (2H, br), 7.48 (2H, d, $J = 8.7$ Hz), 8.23 (2H, d, $J = 8.7$ Hz). IR (KBr): 3446, 3324, 1667, 1580, 1545, 1506, 1491, 1475, 1410, 1332 cm^{-1}. FAB-MS m/z: 306 (M + H). Anal. ($C_{14}H_{14}N_2O_4S$) C, H, N, S.

Ethyl2-Amino-5-(4-methoxyphenyl)-4-methylthiophene-3-carboxylate (3b). Compound **3b** was prepared in 40% yield from 4-methoxyphenylacetone by a method similar to that described for **3a**, 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), 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/z*: 291 (M + H). Anal. ($C_{15}H_{17}NO_3S$) C, H, N, S.

5-Methyl-6-(4-nitrophenyl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4a). A mixture of **3a** (40.0 g, 0.13 mol) and phenyl isocyanate (17.0 mL, 0.16 mol) in pyridine (240 mL) was stirred at 45 °C for 2 h. The mixture was concentrated in vacuo, and the residual solid was suspended in MeOH (400 mL). To this suspension was added sodium methoxide (28% in MeOH solution, 63.2 g, 0.33 mol). After being stirred at room temperature for 6 h, the mixture was acidified with 2 N HCl (200 mL) at 0 °C. After evaporation of the solvent in vacuo, the yellow precipitate was collected by filtration, washed with H_2O, and dried over P_2O_5 in vacuo to give **4a** (50.4 g, 100%) as a yellow powder: mp >300 °C. ^{1}H NMR (DMSO-d_6): δ 2.50 (3H, s), 7.30 (2H, d, J = 6.9 Hz), 7.42−7.51 (3H, m), 7.77 (2H, d, J = 8.7 Hz), 8.31 (2H, d, J = 8.7 Hz), 12.50 (1H, s). IR (KBr): 1715, 1657, 1593, 1510 cm^{-1}. Anal. ($C_{19}H_{13}N_3O_4S\cdot0.5H_2O$) C, H, N.

3-(3-Methoxyphenyl)-5-methyl-6-(4-nitrophenyl)thieno-[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4b). Compound **4b** was prepared in 77% yield from **3a** and 3-methoxyphenyl isocyanate by a method similar to that described for **4a**, as pale-yellow plates: mp >300 °C. ^{1}H NMR ($CDCl_3$): δ 2.50 (3H, s), 3.78 (3H, s), 6.87 (1H, d, J = 8.1 Hz), 6.92 (1H, s), 7.00 (1H, d, J = 8.1 Hz), 7.38 (1H, t, J = 8.1 Hz), 7.77 (2H, d, J = 8.7 Hz), 8.31 (2H, d, J = 8.7 Hz), 12.48 (1H, s). IR (KBr): 1717, 1661, 1593, 1510, 1429 cm^{-1}. Anal. ($C_{20}H_{15}N_3O_5S$) C, H, N.

3-Isobutyl-6-(4-methoxyphenyl)-5-methylthieno[2,3-*d*]-pyrimidine-2,4(1*H*,3*H*)-dione (4c). A mixture of isovaleric acid (1.15 mL, 10.0 mmol), diphenylphosphoryl azide (DPPA; 2.83 g, 10.3 mmol), and triethylamine (1.45 mL, 10.0 mmol) in toluene (15 mL) was heated under reflux for 1.5 h. After the mixture was cooled to below 40 °C, a solution of **3b** (2.0 g, 6.85 mmol) in toluene (5 mL) was added and the resulting mixture was heated under reflux for another 4 days. The reaction mixture was diluted with brine 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, 1:6) to give the urea derivative as a white solid. To a solution of the solid in MeOH (30 mL) was added sodium methoxide (28% in MeOH solution, 3.93 g, 20.4 mmol). After being stirred at room temperature for 16 h, the mixture was acidified with 1 N HCl (22 mL) at 0 °C. The resulting mixture was concentrated in vacuo, and the precipitate was collected by filtration, washed with H_2O, and dried over P_2O_5 in vacuo. Recrystallization from EtOH afforded **4c** (1.61 g, 70%) as colorless needles: mp 215−216 °C. ^{1}H NMR ($CDCl_3$): δ 0.96 (6H, d, J = 6.8 Hz), 2.13−2.22 (1H, m), 2.50 (3H, s), 3.85−3.87 (5H, m), 6.96 (2H, d, J = 8.8 Hz), 7.33 (2H, d, J = 8.8 Hz), 9.50 (1H, s). IR (KBr): 1711, 1657, 1537, 1499, 1458 cm^{-1}. FAB-MS *m/z*: 345 (M + H). Anal. ($C_{18}H_{20}N_2O_3S$) C, H, N.

3-Cyclohexyl-6-(4-methoxyphenyl)-5-methylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4d). Compound **4d** was prepared in 84% yield from **3b** and cyclohexyl isocyanate by a method similar to that described for **4a**, as colorless needles: mp 275−276 °C. ^{1}H NMR ($CDCl_3$): δ 1.23−1.88 (8H, m), 2.41−2.55 (5H, m), 3.84 (3H, s), 4.83−4.91 (1H, m), 6.96 (2H, d, J = 8.7 Hz), 7.32 (2H, d, J = 8.7 Hz), 9.94 (1H, s). IR (KBr): 1709, 1640, 1497 cm^{-1}. Anal. ($C_{20}H_{22}N_2O_3S\cdot0.1H_2O$) C, H, N.

3-Benzyl-6-(4-methoxyphenyl)-5-methylthieno[2,3-*d*]-pyrimidine-2,4(1*H*,3*H*)-dione (4e). Compound **4e** was prepared in 92% yield from **3b** and benzyl isocyanate by a method similar to that described for **4a**, as a colorless powder: mp 241−242 °C. ^{1}H NMR ($CDCl_3$): δ 2.50 (3H, s), 3.85 (3H, s), 5.21 (2H, s), 6.96 (2H, d, J = 8.7 Hz), 7.25−7.33 (5H, m), 7.52 (2H, d, J = 8.7 Hz), 10.07 (1H, s). IR (KBr): 1705, 1649, 1497 cm^{-1}. Anal. ($C_{21}H_{18}N_2O_3S$) C, H, N.

6-(4-Methoxyphenyl)-5-methyl-3-phenylthieno[2,3-*d*]-pyrimidine-2,4(l*H*,3*H*)-dione (4f). Compound **4f** was prepared in 98% yield from **3b** and phenyl isocyanate by a method similar to that described for **4a**, as colorless crystals: mp >300 °C. ^{1}H NMR ($CDCl_3$): δ 2.47 (3H, s), 3.86 (3H, s), 6.98 (2H, d, J = 8.7 Hz), 7.25−7.53 (7H, m), 8.92 (1H, s). IR (KBr): 1719, 1659, 1607 cm^{-1}. Anal. ($C_{20}H_{16}N_2O_3S$) C, H, N.

3-(2-Methoxyphenyl)-6-(4-methoxyphenyl)-5-methylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4g). Compound **4g** was prepared in 81% yield from **3b** and 2-methoxyphenyl isocyanate by a method similar to that described for **4a**, as a colorless powder: mp 257−258 °C. ^{1}H NMR ($CDCl_3$): δ 2.46 (3H, s), 3.82 (3H, s), 3.86 (3H, s), 6.95−7.09 (4H, m), 7.21−7.42 (4H, m). IR (KBr): 1711, 1655, 1605, 1570, 1531 cm^{-1}. Anal. ($C_{21}H_{18}N_2O_4S\cdot0.1H_2O$) C, H, N.

3-(3-Methoxyphenyl)-6-(4-methoxyphenyl)-5-methylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4h). Compound **4h** was prepared in 93% yield from **3b** and 3-methoxyphenyl isocyanate by a method similar to that described for **4a**, as a colorless powder: mp >300 °C. ^{1}H NMR ($CDCl_3$): δ 2.47 (3H, s), 3.82 (3H, s), 3.86 (3H, s), 6.81−7.01 (5H, m), 7.32−7.45 (3H, m), 8.54 (1H, s). IR (KBr): 1719, 1659, 1607 cm^{-1}. Anal. ($C_{21}H_{18}N_2O_4S\cdot0.2H_2O$) C, H, N.

3-(4-Methoxyphenyl)-6-(4-methoxyphenyl)-5-methylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4i). Compound **4i** was prepared in 99% yield from **3b** and 4-methoxyphenyl isocyanate by a method similar to that described for **4a**, as a pale-yellow powder: mp >300 °C. ^{1}H NMR ($CDCl_3$): δ 2.47 (3H, s), 3.84 (3H, s), 3.86 (3H, s), 6.95−7.04 (4H, m), 7.19−7.35 (4H, m), 8.67 (1H, s). IR (KBr): 1717, 1653, 1609, 1516, 1497 cm^{-1}. Anal. ($C_{21}H_{18}N_2O_4S$) C, H, N.

3-(2-Chlorophenyl)-6-(4-methoxyphenyl)-5-methylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4j). Compound **4j** was prepared in 95% yield from **3b** and 2-chlorophenyl isocyanate by a method similar to that described for **4a**, as colorless crystals: mp 285−286 °C. ^{1}H NMR ($CDCl_3$): δ 2.45 (3H, s), 3.87 (3H, s), 6.98 (2H, d, J = 8.7 Hz), 7.29−7.40 (5H, m), 7.54 (1H, d, J = 8.4 Hz), 10.07 (1H, s). IR (KBr): 1711, 1657, 1607, 1564, 1531 cm^{-1}. Anal. ($C_{20}H_{15}N_2O_3SCl$) C, H, N.

3-(3-Chlorophenyl)-6-(4-methoxyphenyl)-5-methylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4k). Compound **4k** was prepared in 97% yield from **3b** and 3-chlorophenyl isocyanate by a method similar to that described for **4a**, as a colorless powder: mp >300 °C. ^{1}H NMR ($CDCl_3$): δ 2.37 (3H, s), 3.81 (3H, s), 7.05 (2H, d, J = 8.7 Hz), 7.29−7.32 (1H, m), 7.39 (2H, d, J = 8.7 Hz), 7.47−7.52 (3H, m). IR (KBr): 1717, 1657, 1497 cm^{-1}. Anal. ($C_{20}H_{15}N_2O_3SCl$) C, H, N.

3-(4-Chlorophenyl)-6-(4-methoxyphenyl)-5-methylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4l). Compound **4l** was prepared in 95% yield from **3b** and 4-chlorophenyl isocyanate by a method similar to that described for **4a**, as a colorless powder: mp >300 °C. H NMR ($CDCl_3$): δ 2.36 (3H, s), 3.79 (3H, s), 7.03 (2H, d, J = 8.4 Hz), 7.33 (2H, d, J = 8.4 Hz), 7.38 (2H, d, J = 8.4 Hz), 7.53 (2H, d, J = 8.4 Hz), 12.35 (1H, s). IR (KBr): 1721, 1663, 1609, 1497 cm^{-1}. Anal. ($C_{20}H_{15}N_2O_3SCI$) C, H, N.

1-(2,6-Difluorobenzyl)-5-methyl-6-(4-nitrophenyl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (5a). A mixture of **4a** (50.4 g, 0.13 mol), 2,6-difluorobenzyl chloride (25.9 g, 0.16 mol), K_2CO_3 (27.5 g, 0.20 mol), and KI (11.0 g, 66.4 mmol) in DMF (800 mL) was stirred at room temperature for 4 h. The mixture was concentrated in vacuo, and the residue was partitioned between $CHCl_3$ and H_2O. The aqueous phase was separated and extracted with $CHCl_3$. The combined extracts were washed with brine and dried ($MgSO_4$). The solution was concentrated in vacuo, and the residue was recrystallized from EtOH to afford **5a** (60.7 g, 90%) as yellow crystals: mp 280−282 °C. ^{1}H NMR ($CDCl_3$): δ 2.57 (3H, s), 5.38 (2H, s), 6.94 (2H, t, J = 8.1 Hz), 7.42−7.58 (8H, m), 8.29 (2H, d, J = 8.8 Hz). IR (KBr): 1719, 1669, 1524, 1473 cm^{-1}. Anal. ($C_{26}H_{17}N_3O_2SF_2$) C, H, N.

1-(2,6-Difluorobenzyl)-3-(3-methoxyphenyl)-5-methyl-6-(4-nitrophenyl)thieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (5b). Compound **5b** was prepared in 84% yield from **4b** by a method similar to that described for **5a**, as a yellow solid: mp 231−234 °C. ^{1}H NMR ($CDCl_3$): δ 2.57 (3H, s), 3.83 (3H, s), 5.38 (2H, s), 6.80−7.01 (5H, m), 7.29−7.46 (2H, m),

7.56 (2H, d, J = 8.8 Hz), 8.29 (2H, d, J = 8.8 Hz). IR (KBr): 1719, 1678, 1595, 1522, 1491 cm^{-1}. Anal. ($C_{27}H_{19}N_3O_5SF_2$) C, H, N.

1-(2,6-Difluorobenzyl)-3-isobutyl-6-(4-methoxyphenyl)-5-methylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (5c). Compound **5c** was prepared in 73% yield from **4c** by a method similar to that described for **5a**, as colorless needles: mp 121−122 °C. ^{1}H NMR ($CDCl_3$): δ 0.96 (6H, d, J = 6.8 Hz), 2.18−2.24 (1H, m), 2.49 (3H, s), 3.84 (3H, s), 3.91 (2H, d, J = 6.8 Hz), 5,34 (2H, s), 6.87−6.95 (4H, m), 7.26−7.29 (3H, m). IR (KBr): 1698, 1659, 1533, 1470 cm^{-1}. Anal. ($C_{25}H_{24}N_2O_3SF_2\cdot 0.1H_2O$) C, H, N.

3-Cyclohexyl-1-(2,6-difluorobenzyl)-6-(4-methoxyphenyl)-5-methylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (5d). Compound **5d** was prepared in 73% yield from **4d** by a method similar to that described for **5a**, as colorless needles: mp 207−208 °C. ^{1}H NMR ($CDCl_3$): δ 1.25−1.86 (8H, m), 2.43−2.57 (5H, m), 3.83 (3H, s), 4.87−4.95 (1H, m), 5.30 (2H, s), 6.86−6.95 (4H, m), 7.26−7.29 (3H, m). IR (KBr): 1705, 1659, 1628, 1609, 1566, 1539 cm^{-1}. Anal. ($C_{27}H_{26}N_2O_3SF_2$) C, H, N.

3-Benzyl-1-(2,6-difluorobenzyl)-6-(4-methoxyphenyl)-5-methylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (5e). Compound **5e** was prepared in 82% yield from **4e** by a method similar to that described for **5a**, as a colorless powder: mp 161−163 °C. ^{1}H NMR ($CDCl_3$): δ 2.46 (3H, s), 3.81 (3H, s), 5.26 (2H, s), 5.35 (2H, s), 6.86−6.94 (4H, m), 7.21−7.34 (6H, m), 7.51 (2H, d, J = 8.4 Hz). IR (KBr): 1709, 1661, 1535, 1473 cm^{-1}. Anal. ($C_{28}H_{22}N_2O_3SF_2$) C, H, N.

1-(2,6-Difluorobenzyl)-6-(4-methoxyphenyl)-5-methyl-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (5f). Compound **5f** was prepared in 87% yield from **4f** by a method similar to that described for **5a**, as colorless needles: mp 291−292 °C. ^{1}H NMR ($CDCl_3$): δ 2.47 (3H, s), 3.85 (3H, s), 5.36 (2H, s), 6.88−6.96 (4H, m), 7.29−7.32 (5H, m), 7.43−7.54 (3H, m). IR (KBr): 1717, 1665, 1626, 1560, 1531, 1508 cm^{-1}. Anal. ($C_{27}H_{20}N_2O_3SF_2\cdot 0.1H_2O$) C, H, N.

1-(2,6-Difluorobenzyl)-3-(2-methoxyphenyl)-6-(4-methoxyphenyl)-5-methylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (5g). Compound **5g** was prepared in 86% yield from **4g** by a method similar to that described for **5a**, as colorless crystals: mp 205−208 °C. ^{1}H NMR ($CDCl_3$): δ 2.47 (3H, s), 3.81 (3H, s), 3.84 (3H, s), 5.37 (2H, s), 6.88−6.96 (4H, m), 7.03−7.09 (2H, m), 7.21−7.32 (4H, m), 7.40 (1H, t, J = 8.0 Hz). IR (KBr): 1717, 1680, 1605, 1524, 1506 cm^{-1}. Anal. ($C_{28}H_{22}N_2O_4SF_2$) C, H, N.

1-(2,6-Difluorobenzyl)-3-(3-methoxyphenyl)-6-(4-methoxyphenyl)-5-methylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (5h). Compound **5h** was prepared in 85% yield from **4h** by a method similar to that described for **5a**, as a colorless powder: mp 253−255 °C. ^{1}H NMR ($CDCl_3$): δ 2.47 (3H, s), 3.83 (3H, s), 3.85 (3H, s), 5.36 (2H, s), 6.81−7.00 (7H, m), 7.29−7.44 (4H, m). IR (KBr): 1719, 1671, 1609, 1535 cm^{-1}. Anal. ($C_{28}H_{22}N_2O_4SF_2$) C, H, N.

1-(2,6-Difluorobenzyl)-3-(4-methoxyphenyl)-6-(4-methoxyphenyl)-5-methylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (5i). Compound **5i** was prepared in 83% yield from **4i** by a method similar to that described for **5a**, as colorless needles: mp >300 °C. ^{1}H NMR ($CDCl_3$): δ 2.47 (3H, s), 3.84 (6H, s), 5.35 (2H, s), 6.88−6.96 (4H, m), 7.02 (2H, d, J = 9.0 Hz), 7.19 (2H, d, J = 9.0 Hz), 7.26−7.32 (3H, m). IR (KBr): 1715, 1665, 1473 cm^{-1}. Anal. ($C_{28}H_{22}N_2O_4SF_2$) C, H, N.

3-(2-Chlorophenyl)-1-(2,6-difluorobenzyl)-6-(4-methoxyphenyl)-5-methylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (5j). Compound **5j** was prepared in 83% yield from **4j** by a method similar to that described for **5a**, as a colorless powder: mp 178−182 °C. ^{1}H NMR ($CDCl_3$): δ 2.47 (3H, s), 3.85 (3H, s), 5.32 (1H, d, J = 15.9 Hz), 5.46 (1H, d, J = 15.9 Hz), 6.89−6.97 (4H, m), 7.29−7.42 (6H, m), 7.56−7.58 (1H, mn). IR (KBr): 1717, 1673, 1628, 1605, 1560, 1528 cm^{-1}. Anal. ($C_{27}H_{19}N_2O_3SClF_2$) C, H, N.

3-(3-Chlorophenyl)-1-(2,6-difluorobenzyl)-6-(4-methoxyphenyl)-5-methylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (5k). Compound **5k** was prepared in 93% yield from **4k** by a method similar to that described for **5a**, as a colorless powder: mp 278−279 °C. ^{1}H NMR ($CDCl_3$): δ 2.46 (3H, s), 3.85 (3H, s), 5.35 (2H, s), 6.89−6.97 (4H, m), 7.17−7.21 (1H, m), 7.29−7.48 (6H, m). IR (KBr): 1717, 1665, 1531, 1470 cm^{-1}. Anal. ($C_{27}H_{19}N_2O_3SClF_2$) C, H, N.

3-(4-Chlorophenyl)-1-(2,6-difluorobenzyl)-6-(4-methoxyphenyl)-5-methylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (5l). Compound **5l** was prepared in 91% yield from **4l** by a method similar to that described for **5a**, as a colorless powder: mp >300 °C. ^{1}H NMR ($CDCl_3$): δ 2.46 (3H, s), 3.84 (3H, s), 5.35 (2H, s), 6.89−6.97 (4H, m), 7.21−7.35 (5H, m), 7.48 (2H, d, J = 9.0 Hz). IR (KBr): 1715, 1665, 1470 cm^{-1}. Anal. ($C_{27}H_{19}N_2O_3SClF_2$) C, H, N.

5-(*N*-Benzyl-*N*-methylaminomethyl)-1-(2,6-difluorobenzyl)-6-(4-nitrophenyl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (6a). A mixture of **5a** (30.7 g, 60.7 mmol), *N*-bromosuccinimide (NBS; 13.0 g, 72.9 mmol), and 2,2′-azobisisobutyronitrile (AIBN; 1.20 g, 7.29 mmol) in chlorobenzene (450 mL) was stirred at 90 °C for 1.5 h. After the mixture was cooled to room temperature, the mixture was concentrated in vacuo. The residue was diluted with H_2O and extracted with CH_2Cl_2. The extract was washed with brine and dried ($MgSO_4$). The solution was concentrated in vacuo to give the crude bromomethyl compound (40.1 g) as a yellow solid: ^{1}H NMR ($CDCl_3$): δ 4.77 (2H, s), 5.38 (2H, s), 6.96 (2H, t, J = 8.1 Hz), 7.29−7.58 (6H, m), 7.79 (2H, d, J = 8.5 Hz), 8.35 (2H, d, J = 8.5 Hz). FAB-MS m/z: 584 (M + H).

To a mixture of the bromomethyl compound (40.0 g, 59.1 mmol) and *N*,*N*-diisopropylethylamine (9.94 g, 76.8 mmol) in DMF (300 mL) was added *N*-benzylmethylamine (8.58 g, 70.9 mmol). After being stirred at room temperature for 2 h, the mixture was concentrated in vacuo and the residue was diluted with saturated $NaHCO_3$ 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 (CH_2Cl_2−EtOAc−hexane, 1:1:4 to 1:1:3) to give a yellow solid. Recrystallization from EtOAc afforded **6a** (33.0 g, 88%) as yellow needles: mp 173−174 °C. ^{1}H NMR ($CDCl_3$): δ 2.15 (3H, s), 3.60 (2H, s), 3.96 (2H, s), 5.39 (2H, s), 6.95 (2H, t, J = 8.2 Hz), 7.18−7.55 (11H, m), 8.02 (2H, d, J = 9.0 Hz), 8.26 (2H, d, J = 9.0 Hz). IR (KBr): 1719, 1678, 1597, 1520 cm^{-1}. Anal. ($C_{34}H_{26}N_4O_4SF_2\cdot 0.5H_2O$) C, H, N.

Compounds **6b**−**l** were prepared by a procedure similar to that described for **6a**, and the physicochemical data are shown in Table 1 (**6c**−**l**) and Table 2 (**6b**).

6-(4-Aminophenyl)-5-(*N*-benzyl-*N*-methylaminomethyl)-1-(2,6-difluorobenzyl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (7a). A solution of **6a** (20.0 g, 32.0 mmol) and 1 M ethereal HCl (96 mL) in formic acid (140 mL) was hydrogenated over 10% palladium−charcoal (containing 50% H_2O, 2.0 g) under atomospheric pressure at room temperature for 3.5 h. The mixture was filtered though Celite and the filtrate was concentrated in vacuo. The residue was diluted with saturated $NaHCO_3$ and extracted with CH_2Cl_2. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography (CH_2Cl_2−EtOAc−MeOH, 80:20:1 to 20:5:1) to give a pale-yellow solid. Recrystallization from EtOAc−diethyl ether afforded **7a** (16.5 g, 87%) as pale-yellow crystals: mp 205−207 °C. ^{1}H NMR ($CDCl_3$): δ 2.05 (3H, s), 3.56 (2H, s), 3.83 (2H, s), 3.88 (2H, s), 5.36 (2H, s), 6.70 (2H, d, J = 8.8 Hz), 6.88−6.94 (2H, m), 7.21−7.31 (8H, m), 7.41−7.53 (5H, m). IR (KBr): 1715, 1657, 1628, 1537 cm^{-1}. Anal. ($C_{34}H_{28}N_4O_2SF_2\cdot H_2O$) C, H, N.

6-(4-Aminophenyl)-5-(*N*-benzyl-*N*-methylaminomethyl)-1-(2,6-difluorobenzyl)-3-(3-methoxyphenyl)thieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (7b) and 5-(*N*-Benzyl-*N*-methylaminomethyl)-1-(2,6-difluorobenzyl)-6-(4-formylaminophenyl)-3-(3-methoxyphenyl)thieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (8). A solution of **6b** (2.40 g, 3.66 mmol) in formic acid (30 mL) was hydrogenated over 10% palladium−charcoal (containing 50% H_2O, 240 mg) under atomospheric pressure at room temperature for 2 h. The residue obtained after a similar workup as described above was purified by flash column chromatography ($CHCl_3$−EtOAc,

1:1) to give **7b** (1.37 g, 60%) as a colorless amorphous solid and **8** (0.26 g, 11%) as a white solid.

7b. ^{1}H NMR ($CDCl_3$): δ 2.04 (3H, s), 3.56 (2H, s), 3.83 (3H, s), 3.88 (2H, s), 5.35 (2H, s), 6.71 (2H, d, J = 8.8 Hz), 6.82−7.00 (5H, m), 7.16−7.52 (9H, m). The free amine was treated with 1 M ethereal HCl to afford the hydrochloride as a white powder (from EtOAc−diethyl ether): mp 162−165 °C. IR (KBr): 1715, 1659, 1537, 1473 cm^{-1}. Anal. ($C_{35}H_{30}N_4O_3SF_2$·2.0HCl·H_2O) C, H, N.

8. Mp 213−215 °C. ^{1}H NMR ($CDCl_3$): δ 2.06 (3H, s), 3.58 (2H, s), 3.83 (3H, s), 3.90 (2H, s), 5.36 (2H, s), 6.82−7.76 (17H, m), 8.43−8.75 (1H, m). IR (KBr): 1717, 1669, 1609, 1537, 1464 cm^{-1}. Anal. ($C_{36}H_{30}N_4O_4SF_2$·1.2H_2O) C, H, N.

5-(*N*-Benzyl-*N*-methylaminomethyl)-1-(2,6-difluorobenzyl)-3-phenyl-6-(4-propionylaminophenyl)thieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (9a). To an ice-cooled solution of **7a** (10.0 g, 15.0 mmol) and triethylamine (8.35 mL, 59.9 mmol) in CH_2Cl_2 (200 mL) was added propionyl chloride (1.82 mL, 21.0 mmol). After being stirred at room temperature for 4 h, the mixture was diluted with saturated $NaHCO_3$ and extracted with $CHCl_3$. 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 ($CHCl_3$−EtOAc−MeOH, 80:20:1 to 60:40:5) to give **9a** (7.50 g, 77%) as a colorless amorphous powder. ^{1}H NMR ($CDCl_3$): δ 1.25 (3H, t, J = 7.2 Hz), 2.05 (3H, s), 2.39 (2H, q, J = 7.2 Hz), 3.56 (2H, s), 3.90 (2H, s), 5.36 (2H, s), 6.89−6.94 (2H, m), 7.15−7.59 (13H, m), 7.69 (2H, d, J = 8.8 Hz). The free amine was treated with 1 M ethereal HCl to afford the hydrochloride as a white powder (from EtOAc−diethyl ether): mp 197−202 °C. IR (KBr): 1717, 1655, 1543, 1475 cm^{-1}. Anal. ($C_{37}H_{32}N_4O_3SF_2$·HCl·H_2O) C, H, N.

Compounds **9b**−**e** were synthesized by a procedure similar to that described for **9a**, and the physicochemical data are shown in Table 2.

5-(*N*-Benzyl-*N*-methylaminomethyl)-1-(2,6-difluorobenzyl)-6-[4-(3-ethylureido)phenyl]-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (9g). To a solution of **7a** (0.70 g, 1.18 mmol) in pyridine (10 mL) was added ethyl isocyanate (0.11 mL, 1.42 mmol). After being stirred at room temperature for 18 h, the mixture was concentrated in vacuo. The residue was diluted with H_2O 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 ($CHCl_3$−EtOAc−MeOH, 80:20:1) to give **9g** (0.78 g, 100%) as a pale-yellow amorphous powder. ^{1}H NMR ($CDCl_3$): δ 1.19 (3H, t, J = 7.2 Hz), 2.06 (3H, s), 3.28−3.37 (2H, m), 3.56 (2H, s), 3.90 (2H, s), 4.62 (1H, t, J = 5.3 Hz), 5.36 (2H, s), 6.28 (1H, s), 6.92 (2H, t, J = 8.0 Hz), 7.18−7.55 (13H, m), 7.69 (2H, d, J = 8.6 Hz). The free amine was treated with 1 M ethereal HCl to afford the hydrochloride as a white powder (from $CHCl_3$−diethyl ether): mp 179−182 °C. Anal. ($C_{37}H_{33}N_5O_3SF_2$·HCl·1.5H_2O) C, H, N.

5-(*N*-Benzyl-*N*-methylaminomethyl)-1-(2,6-difluorobenzyl)-6-[4-(3-methoxyureido)phenyl]-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (9k). To an ice-cooled solution of **7a** (20.6 g, 34.7 mmol) and triethylamine (9.67 mL, 69.4 mmol) in CH_2Cl_2 (480 mL) was added 1,1′-carbonyldiimidazole (CDI; 11.3 g, 69.4 mmol). After being stirred at room temperature for 24 h, *O*-methylhydroxylammonium chloride (24.7 g, 296 mmol) and triethylamine (41.2 mL, 296 mmol) at 0 °C were added to the mixture. After being stirred at room temperature for 3 h, the reaction mixture was washed successively with saturated $NaHCO_3$, H_2O, and brine. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash column chromatography ($CHCl_3$−EtOAc−MeOH, 60:40:0 to 12:8:1) to give **9k** (16.5 g, 71%) as a colorless solid. Recrystallization from $CHCl_3$−diethyl ether afforded white needles: mp 204−205 °C. ^{1}H NMR ($CDCl_3$): δ 2.05 (3H, s), 3.57 (2H, s), 3.82 (3H, s), 3.90 (2H, s), 5.37 (2H, s), 6.92 (2H, t, J = 8.2 Hz), 7.16−7.31 (9H, m), 7.42−7.57 (5H, m), 7.63 (1H, s), 7.73 (2H, d, J = 8.8 Hz). IR (KBr): 3338, 3064, 1717, 1669, 1628, 1591, 1531, 1470 cm^{-1}. Anal. ($C_{36}H_{31}N_5O_4SF_2$) C, H, N. The free amine (TAK-013) was treated with 1 M ethereal HCl to afford the hydrochloride (TAK-013 hydrochloride) as a white powder (from MeOH−diethyl ether): mp 182−185 °C. IR (KBr): 3440, 3042, 1713, 1665, 1628, 1593, 1539, 1473 cm^{-1}. FAB-MS *m*/*z*: 668 (M + H). Anal. ($C_{36}H_{31}N_5O_4SF_2$·HCl·0.5H_2O) C, H, N.

Compounds **9f**,**h**,**l**,**m** were synthesized by a procedure similar to that described for **9k**, and the physicochemical data are shown in Table 2.

5-(*N*-Benzyl-*N*-methylaminomethyl)-1-(2,6-difluorobenzyl)-6-[4-[3-(2,4-dimethoxybenzyloxy)ureido]phenyl]-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (9i). Compound **9i** was prepared from **7a** and *O*-(2,4-dimethoxybenzyl)-hydroxylamine[15] by a method similar to that described for **9k**, as a pale-brown amorphous powder. ^{1}H NMR ($CDCl_3$): δ 2.06 (3H, s), 3.57 (2H, s), 3.80 (3H, s), 3.82 (3H, s), 3.91 (2H, s), 4.87 (2H, s), 5.37 (2H, s), 6.43−6.60 (2H, m), 6.92 (2H, t, J = 8.2 Hz), 7.20−7.31 (13H, m), 7.44−7.53 (4H, m), 8.12 (1H, s).

5-(*N*-Benzyl-*N*-methylaminomethyl)-1-(2,6-difluorobenzyl)-6-[4-(3-hydroxyureido)phenyl]-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (9j). To a solution of **9i** (2.70 g, 3.36 mmol) in CH_2Cl_2 (50 mL) was added trifluoroacetic acid (TFA; 5 mL). After being stirred at room temperature for 20 min, the mixture was diluted with saturated $NaHCO_3$ 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 ($CHCl_3$−MeOH, 10:1) to give a colorless amorphous powder. Recrystallization from $CHCl_3$−diethyl ether afforded **9j** (2.23 g, 100%) as white crystals; mp 164−165 °C. ^{1}H NMR ($CDCl_3$): δ 2.05 (3H, s), 3.46 (2H, s), 3.92 (2H, s), 5.35 (2H, s), 6.65 (1H, br), 6.90 (2H, t, J = 8.0 Hz), 7.28−7.43 (15H, m), 8.04 (1H, s), 9.73 (1H, br). The free amine was treated with 1 M ethereal HCl to afford the hydrochloride as a white powder (from MeOH−diethyl ether): mp 180−186 °C. IR (KBr): 3388, 3066, 1713, 1663, 1628, 1593, 1537, 1473 cm^{-1}. FAB-MS *m*/*z*: 654 (M + H). Anal. ($C_{35}H_{29}N_5O_4SF_2$·HCl·H_2O) C, H, N.

5-(*N*-Benzyl-*N*-methylaminomethyl)-1-(2,6-difluorobenzyl)-3-(3-methoxyphenyl)-6-[4-(*N*-methyl-*N*-propionylamino)phenyl]thieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (10). To a solution of **8** (0.73 g, 1.12 mmol) in THF (20 mL) was added dropwise a solution of borane−methyl sulfide complex (0.28 mL, 2.8 mmol) in THF (10 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h and then heated under reflux for 2 h. After the mixture was cooled, MeOH (5 mL) was added at 0 °C and the mixture was stirred for 30 min. The resulting mixture was treated with 1 M ethereal HCl to attain pH < 2 and heated at 80 °C for 1 h. After evaporation of the solvent in vacuo, the concentrate 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 was purified by flash column chromatography ($CHCl_3$−EtOAc, 10:1 to 4:1) to give the *N*-methylaniline (0.61 g, 85%) as a white powder. To an ice-cooled solution of the powder (0.20 g, 0.31 mmol) and triethylamine (43 μL, 0.31 mmol) in CH_2Cl_2 (10 mL) was added dropwise propionyl chloride (27 μL, 0.31 mmol). After being stirred at room temperature for 4 h, the mixture was diluted with saturated $NaHCO_3$ and 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 (CH_2Cl_2−EtOAc, 10:1 to 4:1) to afford **10** (0.18 g, 84% for two steps from **8**) as a colorless amorphous powder. ^{1}H NMR ($CDCl_3$): δ 1.09 (3H, t, J = 7.3 Hz), 2.10−2.20 (5H, m), 3.30 (3H, s), 3.59 (2H, s), 3.84 (3H, s), 3.94 (2H, s), 5.38 (2H, s), 6.82−7.01 (5H, m), 7.15−7.47 (9H, m), 7.83 (2H, d, J = 8.6 Hz). The free amine was treated with 1 M ethereal HCl to afford the hydrochloride as a white powder (from CH_2Cl_2−diethyl ether): mp 138−143 °C. IR (KBr): 1715, 1665, 1605, 1473 cm^{-1}. FAB-MS *m*/*z*: 695 (M + H). Anal. ($C_{39}H_{36}N_4O_4SF_2$·HCl·1.5H_2O) C, H, N.

In Vitro Binding Assays. Receptor binding assays were carried out as described previously.[8] Briefly, human LHRH receptor cDNA was cloned from a pituitary cDNA library, and

CHO cells stably expressing high levels of the recombinant human LHRH receptor were isolated. [^{125}I][Tyr^5]leuprorelin (0.12−0.15 nM) and the membrane fractions of the CHO cells (0.2 mg/mL) were incubated at 25 °C for 60 min in 0.2 mL of assay buffer A [25 mM Tris, 1 mM EDTA, 0.1% bovine serum albumin (BSA), 0.03% NaN_3, 0.25 mM phenylmethanesulfonyl fluoride, 1 μg/mL pepstatin A, 20 μg/mL leupeptin, and 100 μg/mL phosphoramidon, pH 7.5] containing various concentrations of the test compounds. The reaction was terminated by adding 2 mL of ice-cold assay buffer A, and the bound and free ligands were immediately separated by filtration through a poly(ethylenimine)-coated glass microfiber filter (Whatman, GF/F). The filter was washed twice with 2 mL of assay buffer A, and radioactivity was measured using an X-ray counter. Specific binding was determined by subtracting the nonspecific binding, which was measured in the presence of 1 μM unlabeled leuprorelin, from the total binding. The concentration of each test compound that produced 50% inhibition of the specific binding (IC_{50} value) was derived by fitting the data into a pseudo-Hill equation:

$$\log\left(\frac{\%\ \mathrm{SPB}}{100 - \%\ \mathrm{SPB}}\right) = n[\log(C) - \log(\mathrm{IC}_{50})]$$

where % SPB is the specific binding expressed as a percentage of the maximum specific binding, n is the pseudo-Hill constant, and C is the concentration of the test compound. Similarly, the binding experiments to the LHRH receptor of other species were performed as follows. The monkey LHRH receptor cDNA was cloned from a pituitary cDNA library of cynomolgus monkeys, and CHO cells stably expressing high levels of the recombinant monkey LHRH receptor were isolated as described previously.[8] The binding assays were carried out by incubating [^{125}I][Tyr^5]leuprorelin (0.12−0.13 nM) and membranes prepared from the CHO cells (0.25 mg/mL) at 25 °C for 60 min, in the presence or absence of compounds. For the binding experiments to the rat LHRH receptor, [^{125}I][Tyr^5]-leuprorelin (0.15 nM) and membranes (0.2 mg/mL) from the anterior pituitary of male Wistar rats were incubated with or without compounds at 4 °C for 90 min.[8]

In Vitro Functional Assays. LHRH-stimulated arachidonic acid release from CHO cells expressing human or monkey LHRH receptors was measured according to the previously reported protocol.[20] The receptor-expressing CHO cells were seeded into 24-well plates at a density of 4×10^4 cells/well and cultured for 1 day. The cells were then incubated with [5,6,8,9,11,12,14,15-^{3}H]arachidonic acid (11 kBq/well, NEN Lifescience Products) for 1 day and washed with Dulbecco's modified Eagle's medium (DMEM) supplemented with 20 mM HEPES and 0.2% BSA. The cells were then preincubated with the compounds at 37 °C for 60 min and the reaction was started by addition of LHRH (1 nM). After incubation at 37 °C for 40 min, radioactivity in the medium was measured with a liquid scintillation counter.

Oral Absorption in Cynomolgus Monkeys. The hydrochloride of **9k** (TAK-013 hydrochloride) was used in order to unify the experimental conditions. Compounds **9a**,**g** and **9k** (10 mg/kg) suspended in 0.5% methylcellulose were orally administered to cynomolgus monkeys (female, 4−8 years old, $n = 3$). Blood samples (heparin-plasma) were collected from a forearm vein 1, 3, and 6 h after administration. To the plasma (0.3 mL) the same volume of 5% acetonitrile containing 0.05% TFA was added, and the precipitated plasma proteins were removed by centrifugation. The compound concentrations in the supernatant were measured by reverse-phase HPLC using a TSK ODS-80TM column (4 mm × 250 mm, TOSO) with a linear gradient of acetonitrile (40−80%) containing 0.05% TFA (flow rate, 1.0 mL/min; detection, 320 nm; column temperature, 35 °C).

In Vivo Efficacy in Cynomolgus Monkeys. Cynomolgus monkeys (male, 4−9 years old) were castrated more than 6 months prior to the examination. The monkeys were trained to sit in a primate-restraining chair during administration of the compound. Compound **9k** (10 or 30 mg/kg, 3 mL/kg, $n = 3$ for each group) suspended in 0.5% methylcellulose containing 1.2% citric acid, or 0.5% methylcellulose containing 1.2% citric acid alone (3 mL/kg, $n = 3$), were administered orally. Blood samples (heparin-plasma) were collected from a femoral vein 24 h before administration and 0, 2, 4, 8, 24, and 48 h after administration. LH concentrations in the plasma were measured by bioassays using mouse testicular cells.[8]

Molecular Modeling Studies. All calculations were performed on a Silicon Graphics O2 R10000 workstation. Molecular modeling was carried out using the Insight II software package (Accelrys Inc., San Diego, CA). Molecular mechanics and ab initio calculations were performed to evaluate the energy difference between the cis and trans conformers concerning the methoxyurea part of compound **9k**. To compare the intrinsic propensities of the conformation of the urea part, only the partial structures, *N*-ethyl-*N*′-phenylurea and *N*-methoxy-*N*′-phenylurea, were considered. The cis and trans conformers were constructed using Insight II and subjected to either energy minimization by Discover using the CVFF force field or geometry optimization by DMol.

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