Journal of Medicinal Chemistry

ARTICLE

pubs.acs.org/jmc

Discovery of 1-{4-[1-(2,6-Difluorobenzyl)-5-[(dimethylamino)methyl]-3-(6-methoxypyridazin-3-yl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (TAK-385) as a Potent, Orally Active, Non-Peptide Antagonist of the Human Gonadotropin-Releasing Hormone Receptor

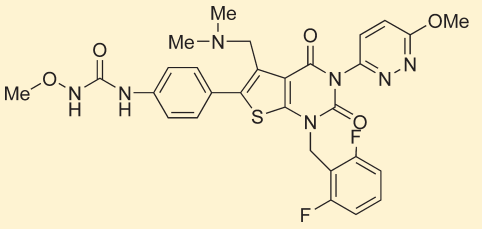
Kazuhiro Miwa,[*,†] Takenori Hitaka,[‡] Takashi Imada,[§] Satoshi Sasaki,[§] Mie Yoshimatsu,[‡] Masami Kusaka,[†] Akira Tanaka,[§] Daisuke Nakata,[§] Shuichi Furuya,[§] Satoshi Endo,[‡] Kazumasa Hamamura,[‡] and Tomoyuki Kitazaki[‡]

[†]CMC Center and [‡]Pharmaceutical Research Division, Takeda Pharmaceutical Company, Ltd., 17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532-8686, Japan

[§]Pharmaceutical Research Division, Takeda Pharmaceutical Company, Ltd., 10 Wadai, Tsukuba, Ibaraki 300-4293, Japan

Supporting Information

ABSTRACT: We previously discovered an orally active human gonadotropin-releasing hormone (GnRH) receptor antagonist, thieno[2,3-*d*]pyrimidine-2,4-dione derivative **1** (sufugolix). To reduce the cytochrome P450 (CYP) inhibitory activity and improve in vivo GnRH antagonistic activity, further optimization of this scaffold was carried out. We focused our synthetic efforts on chemical modification at the 5 and 3 positions of the thieno[2,3-*d*]pyrimidine-2,4-dione ring based on computational modeling, which resulted in the discovery of 1-{4-[1-(2,6-difluorobenzyl)-5-[(dimethylamino)methyl]-3-(6-methoxypyridazin-3-yl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (**16b**) as a highly potent and orally active GnRH antagonist. Compound **16b** showed potent in vitro GnRH antagonistic activity in the presence of fetal bovine serum (FBS) without CYP inhibition. Oral administration of **16b** maintained the suppressive effect of the plasma luteinizing hormone levels in castrated cynomolgus monkeys at a 3 mg/kg dose for more than 24 h. Compound **16b** is currently under clinical development with the code name of TAK-385.



16b

INTRODUCTION

Gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone-releasing hormone (LHRH), plays an important role in the reproductive system.[1] It is a linear decapeptide that is produced in the hypothalamus and released into the pituitary in a pulsatile manner. In the pituitary gland, GnRH binds to and activates the GnRH receptor, which belongs to the class A G-protein-coupled receptor (GRCR).[2] This activation of the GnRH receptor triggers the biosynthesis and release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which regulate gonadal steroidogenesis and gametogenesis in testes and ovaries.[3] Several peptidic GnRH agonists represented by leuprorelin[4] paradoxically suppress gonadal steroid production and subsequently achieve a condition called "chemical castration" through receptor down-regulation and/or desensitization.[5] They have been approved for the clinical treatment of a variety of endocrine-based diseases such as prostate, ovarian, and breast cancer as well as endometriosis and uterine leiomyoma.[6,7] GnRH agonists occasionally evoke an initial gonadal hormone surge attributed to overstimulation of the receptor, known as the "flare effect", leading to symptom exacerbation. In contrast, GnRH peptidic antagonists directly lower hormone levels without the concomitant flare effect.[8] However, peptidic antagonists show poor oral bioavailability and usually need to be administered by daily subcutaneous injection, intranasal spray, or suitable depot form. Therefore, orally effective non-peptide GnRH antagonists could be superior therapeutic agents for clinical applications by overcoming the above problems.[9]

Several non-peptide GnRH antagonists have been reported from various research groups;[10−19] however, only a few compounds have reached clinical trials. We have previously found three cores (**1**−**3**), thieno[2,3-*d*]pyrimidine-2,4-dione,[11b] thieno[2,3-*b*]pyridin-4-one,[11a,c] and imidazo[1,2-*a*]pyrimidine-5-one,[11d] as non-peptide GnRH receptor antagonists (Figure 1). In particular, the thieno[2,3-*d*]pyrimidine-2,4-dione core acting as a bicyclic scaffold could mimic a type II β-turn involving residues 5−8 (Tyr-Gly-Leu-Arg) of the human GnRH receptor, and structure−activity relationship (SAR) studies led to identification of **1** (sufugolix) bearing the unique *p*-(3-methoxyureido)phenyl group at the 6-position of the

Received: February 25, 2011
Published: June 09, 2011

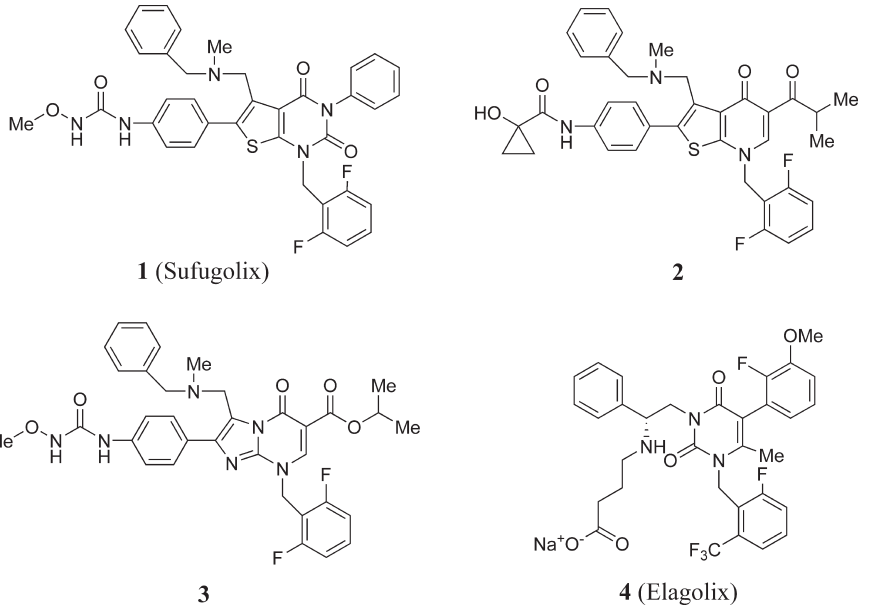


Figure 1. Representative potent small molecule GnRH receptor antagonists.

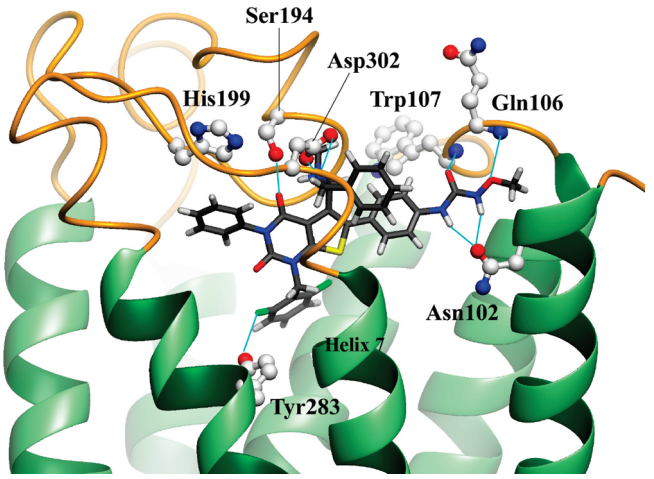


Figure 2. Binding model of sufugolix **1** in the human GnRH receptor. A three-dimensional model of the human GnRH receptor in complex with compound **1** is viewed from the extracellular side. Transmembrane helices (TM1−TM7) and extracellular loops (ECL1−ECL3) are depicted as green and orange, respectively. Compound **1** is drawn with atoms in gray (carbon), blue (nitrogen), red (oxygen), yellow (sulfur), and light green (fluorine). The receptor residues participating in the specific interactions with **1** are shown as ball-and-stick representation with similar coloring to **1**. The putative intermolecular hydrogen bonds are depicted as cyan lines. This figure was produced using MOLMOL.[25]

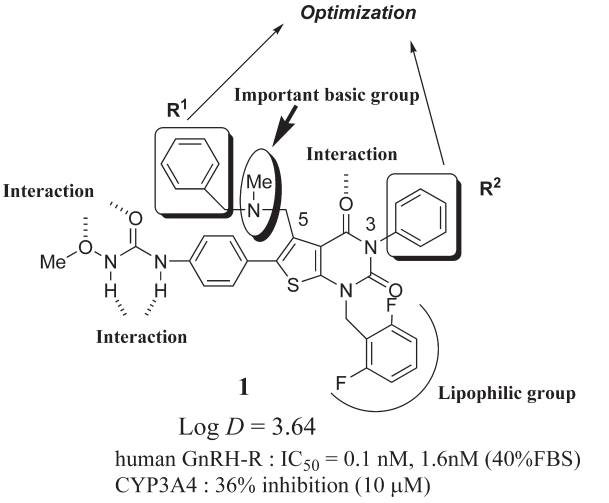


Figure 3. Chemical modification of sufugolix **1**.

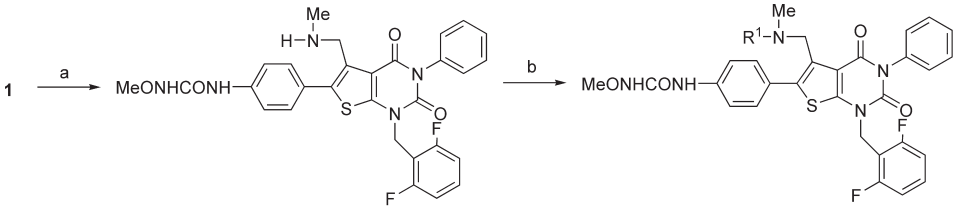
core ring.[11b] Compound **1** showed good in vitro and in vivo GnRH antagonistic activities[20] and therefore was selected as a first clinical candidate. Recently, compound **4** (elagolix, Figure 1), based on a series of uracil derivatives, demonstrated suppression of LH in castrated macaques by oral administration[13] and entered clinical studies for the treatment of endometriosis.

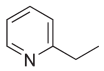
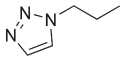
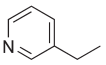
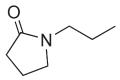
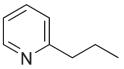
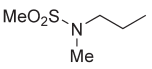
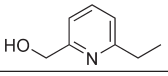
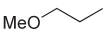
Inhibition of the cytochrome P450 (CYP) 3A4 enzyme is well-known to potentially induce drug−drug interactions. Our early thieno[2,3-*d*]pyrimidine-2,4-dione derivatives had a common issue of CYP3A4 inhibition. In a series of derivatives based on this scaffold, CYP3A4 inhibition of the selected candidate **1** was weak but still remained (36% inhibition at 10 μM). Additionally, binding affinity of **1** was reduced significantly in the presence of fetal bovine serum (FBS) (IC_{50} = 1.6 nM), in comparison with results in the absence of FBS (IC_{50} = 0.1 nM). Therefore, our goal was to discover and develop an orally active GnRH antagonist with potent activity, even in the presence of FBS, and with no CYP3A4 inhibition.

We judged that our thieno[2,3-*d*]pyrimidine-2,4-dione derivatives with potent GnRH anatagonistic activities had high log *D* values, which resulted in the potent CYP3A4 inhibitory activities observed. Thus, we speculated that decreasing the log *D* values for our compounds could reduce CYP3A4 inhibition. In order to reduce the lipophilicity of **1** while retaining high GnRH anatagonistic activity, we adopted a strategy using the molecular modeling of **1** interacting with human GnRH receptor (Figure 2). Our model was constructed based on the crystal structure of rhodopsin,

dx.doi.org/10.1021/jm200216q | *J. Med. Chem.* 2011, 54, 4998–5012

Scheme 1[a]



No.	R^1	X	Yield (%)	No.	R^1	X	Yield (%)
6a		Cl	73	**6e**		Cl	41
6b		Cl	67	**6f**		OMs	60
6c		OMs	56	**6g**		Cl	67
6d		Br	63	**6h**		Cl	89

[a] Reagents: (a) 10% Pd/C, H_2, MeOH, 96%; (b) R^1-X, $^iPr_2NEt$, KI, DMF.

which is a member of the largest subfamily constituting ~90% of all GPCRs. According to this model, the terminal methoxyureido group at the 6-position of thieno[2,3-*d*]pyrimidine-2,4-dione ring interacts with both Gln106 and Asn102 of the GnRH receptor, resulting in high binding affinity.[11b] Other important interactions were found as follows: a hydrophobic interaction between the 2,6-difluorobenzyl group at the 1-position and Tyr283, and a hydrogen-bonding interaction between the basic tertiary amine at the 5-position and Asp302. These two interactions of the 2,6-difluorobenzyl and *N*-methylamino groups with GnRH receptor also contributed to the high binding affinity (Figure 3). In contrast, the two phenyl rings (R^1 and R^2) at the 5 and 3 positions of the thieno[2,3-*d*]pyrimidine-2,4-dione ring do not appear to make important interactions with the receptor in our model, and we predicted that these regions had steric space available for the introduction of various sizes of substituents. Thus, our synthetic efforts focused on optimization of these phenyl rings at R^1 and R^2 in **1** to avoid CYP3A4 inhibition and enhance antagonistic activity, and we examined the introduction of substituents more polar than phenyl at these positions.

On the other hand, during our studies, Betz et al. published detailed mutagenesis data on the human GnRH receptor.[21] Their data clearly showed the importance of Asp302 and aromatic residues such as Tyr283/284 in the binding of **1**. They also indicated that a cluster of residues in the transmembrane helix 7 form the binding site of **1**. These observations are consistent with our binding model presented in Figure 2. Although they argued that the methoxyureido part of **1** interacts with Asp302, their data did not exclude the possibility of the interaction with Asn102. We think that the binding site of the methoxyureido part of **1** remains to be elucidated. In the meantime, our hypothetical binding model was used to gain insight into the design of non-peptide GnRH antagonists as described here.

In this article, we report the discovery of a highly potent and orally active GnRH antagonist **16b** (TAK-385) as a clinical candidate without CYP3A4 inhibition and with improved in vivo efficacy.

CHEMISTRY

Modification at the 5-position of the thieno[2,3-*d*]pyrimidine-2,4-dione ring was carried out according to Scheme 1 starting from **1** in two steps. Thus, reductive debenzylation using palladium–carbon under hydrogen atmosphere and subsequent alkylation with various halides or mesylates provided the corresponding R^1-substituted derivatives **6a**–**h**.

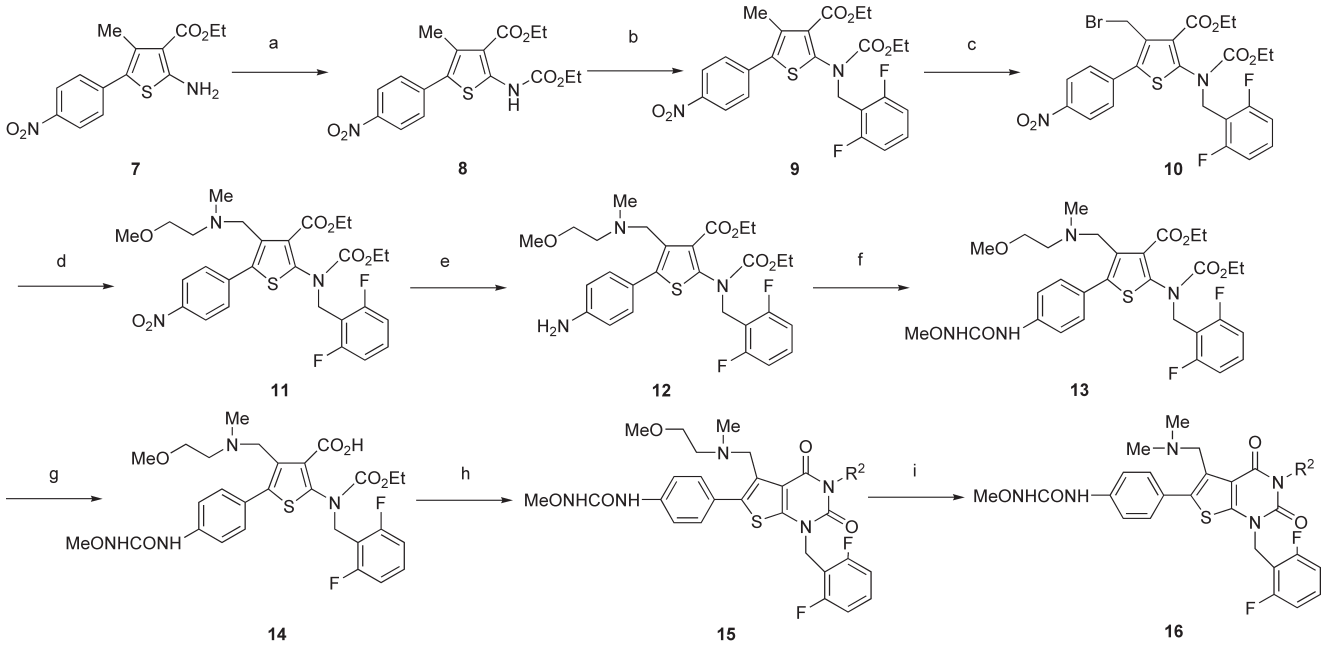
The synthesis of R^2-substituted compounds **15** and **16** is outlined in Scheme 2. Ethoxycarbonylation of 2-aminothiophene **7**[11b] followed by alkylation with 2,6-difluorobenzylchloride gave N,N-disubstituted amine **9**. Bromination of **9** with *N*-bromosuccinimide (NBS) and 2,2′-azobis(isobutyronitrile) (AIBN) proceeded at the 3-methyl group, and the resulting bromomethyl derivative **10** was reacted with *N*-(2-methoxyethyl)methylamine to give tertiary amine **11**. Hydrogenation of nitro compound **11** was then carried out. Aniline **12** was converted to urea **13** by a two-step methodology. Thus, reaction of **12** with 1,1′-carbonyldiimidazole (CDI) afforded the intermediary imidazolide, and subsequent nucleophilic substitution with *N*-methoxyamine hydrochloride gave **13** in a one-pot reaction. Next, selective alkaline hydrolysis of ethyl ester yielded thiophene-3-carboxylic acid **14**. Condensation reaction of **14** with various amines using diethyl phosphorocyanidate (DEPC) followed by intramolecular cyclization under basic conditions using sodium methoxide provided the corresponding thieno[2,3-*d*]pyrimidine-2,4-dione derivatives **15a**–**j**. Finally, the (2-methoxyethyl)(methyl)aminomethyl group at the 5-position of **15i** and **15j** was converted to a dimethylaminomethyl group in two steps. Thus, reaction of **15i** and **15j** with 1-chloroethylchloroformate afforded the intermediary quaternary ammonium salts, and subsequent nucleophilic substitution with dimethylamine gave the 5-dimethylaminomethyl derivatives **16a** and **16b**, respectively.

RESULTS AND DISCUSSION

In Vitro Activities and CYP3A4 Inhibition. As a primary assay for establishing SAR studies, all synthesized compounds

dx.doi.org/10.1021/jm200216q | *J. Med. Chem.* 2011, 54, 4998–5012

Scheme 2[a]



No.	R^2	Yied (%)
15a	OMe	58
15b	OEt	82
15c	OH	25
15d	OH	42
15e	N	33
15f	Me, N	71

No.	R^2	Yied (%)
15g	F, N	46
15h	OMe, N	62
15i	OMe, N	71
15j	OMe, N, N	69

No.	R^2	Yied (%)
16a	OMe, N	24
16b	OMe, N, N	44

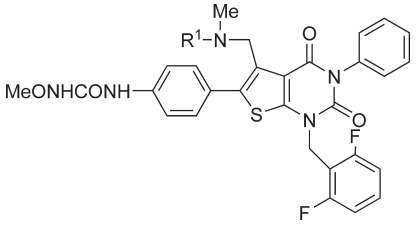
[a] Reagents: (a) $ClCO_2Et$, toluene, 94%; (b) 2,6-difluorobenzylchloride, K_2CO_3, KI, DMF, 93%; (c) NBS, AIBN, EtOAc, quant; (d) $MeOCH_2CH_2NHMe$, $^iPr_2NEt$, DMF, quant; (e) 10% Pd/C, H_2, EtOH, 97%; (f) (1) CDI, $^iPr_2NEt$, CH_2Cl_2, (2) $MeONH_2 \cdot HCl$, $^iPr_2NEt$, 89%; (g) 2 N NaOH, EtOH, 96%; (h) (1) R^2NH_2, DEPC, $^iPr_2NEt$, DMF, (2) MeONa, MeOH; (i) (1) $ClCO_2CH(Cl)CH_3$, CH_2Cl_2, (2) Me_2NH/THF, $^iPr_2NEt$, DMF.

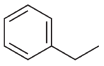
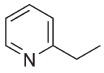
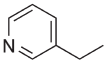
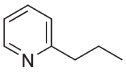
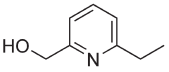
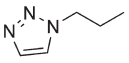
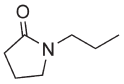
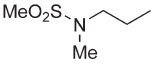
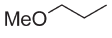
were evaluated for their in vitro binding affinities, using cloned Chinese hamster ovary (CHO) cells expressing the human GnRH receptor and [^{125}I]leuprorelin as a ligand, expressed as IC_{50}.[11a] All compounds were also tested for their ability to inhibit ligand binding to the CYP3A4 enzyme in vitro assay, expressed as the percent inhibition at 10 μM. For in vitro antagonistic activity, key compounds were evaluated for their inhibitory potencies of GnRH-stimulated arachidonic acid (AA) release from CHO cells expressing the human GnRH receptor, expressed as IC_{50}.[11a] Compound log *D* values were determined at pH 7.4 by a reported method.[22]

First, thieno[2,3-*d*]pyrimidine-2,4-diones having substituents (R^1) at the 5-(aminomethyl) group were investigated, and the results are shown in Table 1. As expected, introduction of various substituents such as pyridyl ring, amide group, or hydrophilic side chain into the R^1 region was tolerated in terms of activity. All compounds (**6a**−**h**) showed high binding affinities ($IC_{50} \leq 0.3$ nM), equivalent to sufugolix (**1**), over a wide range of log *D* (2.12−3.15). Substitution for phenyl with pyridyl brought a decrease of log *D*; however, CYP3A4 inhibition was not eliminated (**6a**, 33%; **6b**, 66% inhibition at 10 μM). Extension of the methylene linker in **6a** and incorporation of a hydroxymethyl group into the pyridyl ring of **6a** also did not reduce the CYP3A4 inhibitory activity (**6c**, 64%; **6d**, 45% inhibition at 10 μM), although their log *D* values decreased. In addition, replacement of the pyridyl ring with a triazolyl ring or cyclic carboxamide group was not effective for removal of CYP inhibition. On the other hand, introduction of an alkylsulfamoyl or methoxyethyl group resulted in the reduction of CYP3A4 inhibitory activities along with a decrease of log *D* (**6g**, 17%; **6h**, 14% inhibition at 10 μM). As a result, compounds bearing a linear hydrophilic substituent with low log *D* seem to be preferable. These results suggest that not only an increase in hydrophilicity (decreasing log *D*) but also the size and shape of the substituent in the R^1 position contribute to the reduction of

dx.doi.org/10.1021/jm200216q |*J. Med. Chem.* 2011, 54, 4998–5012

Table 1. In Vitro Activities and CYP3A4 Inhibitory Activities of Compounds 1 and 6a−h



compd	R^1	binding affinity IC_{50} (nM)[a]	inhibition of human AA release IC_{50} (nM)[b]	CYP3A4 (% inhibition, 10μM)	Log D[c]
1		0.10	0.06	36	3.64
6a		0.09	0.08	33	3.05
6b		0.2	ND[d]	66	3.15
6c		0.07	0.07	64	2.54
6d		0.10	0.24	45	2.60
6e		0.3	ND[d]	54	2.62
6f		0.09	0.21	45	2.26
6g		0.2	0.27	17	2.12
6h		0.10	0.08	14	2.27

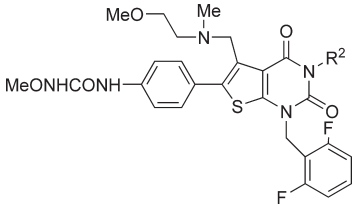
[a] Binding affinity is reported as IC_{50}, which is the antagonist concentration required to inhibit the specific binding of [^{125}I]leuprorelin to human GnRH receptor by 50%. CHO cells expressing human GnRH receptor were used as the source for GnRH receptor. All data are expressed as the mean of two or three determinations. [b] Inhibition of GnRH-stimulated AA release from CHO cells expressing human GnRH receptor was measured to evaluate the GnRH antagonistic activities of the test compounds. The IC_{50} is the antagonist concentration required to inhibit the GnRH-stimulated AA release from CHO cells by 50%. All data are expressed as the mean of the two or three determinations. [c] Measured at pH 7.4. [d] ND = not determined.

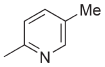
CYP3A4 inhibitory activity. Compound **6h** also showed potent antagonistic activity compared with **1** (**6h**, IC_{50} = 0.08 nM).

Next, the effect of modifying the 3-substituent (R^2) on the thieno[2,3-*d*]pyrimidine-2,4-dione ring of **6h** was investigated, and the results are shown in Table 2. Compounds **15a**−**d**, bearing an alkoxy or hydroxyalkyl group at the R^2 position, maintained high binding affinities (IC_{50} = 0.2−0.3 nM). CYP3A4 inhibition for **15a**−**d** (8−30% inhibition at 10 μM) with a linear substituent was almost comparable to that for **6h**, and the lower log D (1.38) compound **15c** showed 23% CYP3A4 inhibition at 10 μM. This result suggests that a linear hydrophilic substituent at the R^2 position does not seem to contribute to a major decrease in CYP3A4 inhibition, and this encouraged us to introduce more polar aromatic heterocycles into the 3-position. Among several five- and six-membered heterocycle derivatives synthesized, pyridine derivatives were preferred for showing high binding affinities. Substitution of a phenyl group with a pyridyl ring, **15e**, provided a lower log D (1.62) and equally lower CYP3A4 inhibitory activity (10% inhibition at 10 μM) while maintaining high binding affinity (**15e**, IC_{50} = 0.2 nM). In addition, the introduction of methyl group into the para-position on the pyridyl ring of **15e** resulted in a slight increase in antagonistic activity while maintaining a lower CYP3A4 inhibitory activity. For these pyridine derivatives, when log D is below 2, CYP3A4 inhibitory activity tended to be less than 10% at 10 μM; however, antagonistic activities were generally weaker than that of **6h**. We found that the introduction

dx.doi.org/10.1021/jm200216q | *J. Med. Chem.* 2011, 54, 4998–5012

Table 2. In Vitro Activities and CYP3A4 Inhibitory Activities of Compounds 15a−g



compd	R^2	binding affinity IC_{50} (nM)[a]	inhibition of human AA release IC_{50} (nM)[b]	CYP3A4 (% inhibition, 10μM)	Log D[c]
15a		0.2	0.29	8	2.10
15b		0.2	0.37	30	2.49
15c		0.3	ND[d]	23	1.38
15d		0.3	ND[d]	11	2.26
15e		0.2	0.83	10	1.62
15f		0.10	0.18	4	1.80
15g		0.2	1.0	9	1.87

[a] Binding affinity is reported as IC_{50}, which is the antagonist concentration required to inhibit the specific binding of [^{125}I]leuprorelin to human GnRH receptor by 50%. CHO cells expressing human GnRH receptor were used as the source for GnRH receptor. All data are expressed as the mean of two or three determinations. [b] Inhibition of GnRH-stimulated AA release from CHO cells expressing human GnRH receptor was measured to evaluate the GnRH antagonistic activities of the test compounds. The IC_{50} is the antagonist concentration required to inhibit the GnRH-stimulated AA release from CHO cells by 50%. All data are expressed as the mean of the two or three determinations. [c] Measured at pH 7.4. [d] ND = not determined.

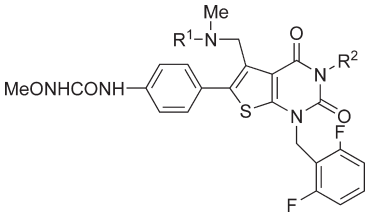
of a hydrophilic heterocyclic substituent into the 3-position led to a reduction in CYP3A4 inhibitory activity with a corresponding decrease in log D.

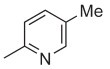
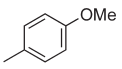
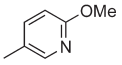
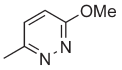
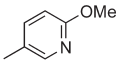
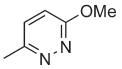
Effect of FBS on In Vitro Activities. During the course of our preclinical development program of GnRH antagonists, in parallel with the above screening we improved our in vitro assay methods in order to better mimic our in vivo evaluation system. Thus, measurements of binding affinity and antagonistic activity were carried out in the presence of 40% FBS. Unexpectedly, the binding affinity of **1** was reduced significantly in the presence of FBS (IC_{50} = 1.6 nM) in contrast to its strong affinity in the absence of FBS (IC_{50} = 0.10 nM) (Table 3). The antagonistic activity of **1** also decreased 32-fold under the influence of FBS, suggesting that the apparent antagonistic activity of **1** would not translate to our in vivo evaluation systems.

We searched for relationships between chemical structure and the extent of this FBS-induced activity decrease for our 3,5-disubstituted thieno[2,3-*d*]pyrimidine-2,4-dione series, and the results of representative compounds are shown in Table 3. The ratios of binding affinities and antagonistic activities in the presence and absence of FBS for **6h** (5.0 and 17) were improved 3- and 2-fold compared to compound **1** (ratio of 16 and 32), respectively. Pyridine derivative **15e** showed further improvement in the binding affinity ratio (ratio of 1.5), with high binding affinity even in the presence of FBS (IC_{50} = 0.30 nM). These results suggest that the decrease in the influence of FBS on GnRH binding affinities and antagonistic activities for certain compounds can be attributed to their lower lipophilicities (log D = 2.27 for **6h** and log D = 1.62 for **15e**). It is known that high lipophilicity is likely to result in high plasma protein binding; in fact, compound **1** showed 98% plasma protein binding. Therefore, the activity of the less lipophilic compounds is more effectively translated into in vitro binding affinity and antagonistic activity in the presence of FBS. As for antagonistic activity, the pyridine derivative bearing a methyl group, **15f**, showed about 2-fold improvement in activity ratio compared with **15e** (ratio of 7.2 for **15f** and 16 for **15e**). In addition, introduction of a methoxy group into the para position of the phenyl ring in **6h** led to significant improvement in antagonistic activity. Thus, the activity ratio for *p*-methoxyphenyl derivative **15h** was reduced from 17-fold to 2.5-fold and the IC_{50} of **15h** was found to be 0.43 nM in the presence of FBS. On the basis of these SAR data, we next incorporated a methoxy group into the para position of a pyridine derivative. Compound **15i** showed potent antagonistic activity (IC_{50} = 0.49 nM in the presence of FBS) with negligible CYP3A4 inhibitory activity (7% inhibition at 10 μM). Considering the improvement found for the reduction of CYP3A4 inhibition, substitution of a pyridazine ring for the pyridine ring was performed to reduce log D. The pyridazine derivative **15j** maintained high antagonistic activity (IC_{50} = 0.94 nM in the presence of FBS), even though weak CYP3A4 inhibitory activity remained (9% inhibition at 10 μM). To further reduce log D and molecular weight, conversion of the *N*-2-methoxyethyl group to the smaller *N*-methyl group at R^1 in **15i** and **15j** was carried out. Dimethylaminomethyl derivatives

dx.doi.org/10.1021/jm200216q | J. Med. Chem. 2011, 54, 4998–5012

Table 3. Binding to GnRH Receptor and Inhibitory Effects of Compounds 1, 6h, 15e,f,h−j, and 16a,b on GnRH-Stimulated Arachidonic Acid (AA) Release in the Presence or Absence of FBS



compd	R^1	R^2	binding affinity IC_{50} (nM)[a]			inhibition of human AA release IC_{50} (nM)[b]			CYP3A4 (% inhibition, 10μM)	Log D^c
			FBS (0%)	FBS (40%)	ratio[d]	FBS (0%)	FBS (40%)	ratio[d]		
1			0.10	1.6	16	0.06	1.9	32	34	3.64
6h	$MeOCH_2CH_2$-		0.10	0.50	5.0	0.08	1.4	17	14	2.27
15e	$MeOCH_2CH_2$-		0.2	0.30	1.5	0.83	13	16	10	1.62
15f	$MeOCH_2CH_2$-		0.10	0.30	3.0	0.18	1.3	7.2	4	1.80
15h	$MeOCH_2CH_2$-		0.07	0.29	4.1	0.17	0.43	2.5	17	2.24
15i	$MeOCH_2CH_2$-		0.2	0.80	4.0	0.07	0.49	7.1	7	2.34
15j	$MeOCH_2CH_2$-		0.08	0.24	3.0	0.26	0.94	3.6	9	1.70
16a	Me-		0.12	0.55	4.6	0.26	0.95	3.7	17	1.83
16b	Me-		0.08	0.33	4.1	0.33	0.82	2.5	0	1.27

[a] Binding affinity is reported as IC_{50}, which is the antagonist concentration required to inhibit the specific binding of [^{125}I]leuprorelin to human GnRH receptor by 50%, in the presence or absence of FBS. All data are expressed as the mean of two or three determinations. [b] Inhibition of GnRH-stimulated AA release from CHO cells expressing human GnRH receptor was measured to evaluate the GnRH antagonistic activities of the test compounds, in the presence or absence of FBS. The IC_{50} is the antagonist concentration required to inhibit the GnRH-stimulated AA release from CHO cells by 50%. All data are expressed as the mean of two or three determinations. [c] Measured at pH 7.4. [d] IC_{50}(40% FBS)/IC_{50}(0% FBS).

16a and **16b** showed potent binding affinities and antagonistic activities comparable to **15i** and **15j**. Of particular note was pyridazine derivative **16b**, which exhibited no CYP3A4 inhibitory activity (0%) at 10 μM with potent antagonistic activity (IC_{50} = 0.82 nM) in the presence of FBS. The elimination of CYP3A4 inhibitory activity and improvement in antagonistic activity can be attributed to its low log D (1.27) and plasma protein binding (71%). Among the compounds prepared, **16b** exhibited the best overall profile in terms of in vitro GnRH antagonistic activity and lack of CYP inhibitory activity. Therefore, this compound, **16b**, was selected as a candidate for further investigation.

In Vitro Species Specificity. Species specificities for the binding affinity and antagonistic activity of **16b** were investigated in a manner similar to that described above. The results are shown in Table 4. For the monkey receptor, **16b** exhibited strong binding affinity (IC_{50} = 0.32 nM) comparable to that for the human receptor while displaying a 30000-fold decrease for the rat receptor (IC_{50} = 9800 nM). In contrast, the antagonistic in vitro activity of **16b** with respect to the human receptor (IC_{90} = 18 nM) exceeded that for the monkey receptor (IC_{90} = 1700 nM) by 95-fold in the presence of 40% serum. Thus, compound **16b** showed much more potent activity than **1**, giving the expectation that significant suppression of LH and testosterone levels may be found in clinical studies.

As for binding specificity, **16b** displayed no significant activity in 134 different enzyme and radioligand binding assays (<60% inhibition at 10 μM).

In Vivo Studies. The pharmacokinetic profile of **16b** was evaluated after single oral administration to male cynomolgus monkeys. As shown in Table 5, C_{max}, T_{max}, and AUC_{0-24h} were 16.0 ng/mL, 2.7 h, and 90.1 ng·h/mL, respectively, at a dose of 1 mg/kg. Although the plasma concentration of **16b** at a dose of 3 mg/kg showed an increase over the dose ratio, the value does not represent a marked variability. In consideration of the in vitro

dx.doi.org/10.1021/jm200216q | J. Med. Chem. 2011, 54, 4998–5012

Table 4. Species Specificities for Binding to GnRH Receptor and Inhibitory Effects on GnRH-Stimulated AA Release of Compounds 1 and 16b

	binding affinity[a] IC_{50} (nM) (40% FBS)[b]		inhabition of AA release[a] IC_{90} (nM) (40% human or monkey serum)[c]	
	monkey[d]	rat[d]	human	monkey
1	1.6	1.3	270	13694
16b	0.33	0.32	18	1700

[a] All data are expressed as the mean of the two or three determinations. [b] Binding affinity is reported as IC_{50}, which is the antagonist concentration required to inhibit the specific binding of [^{125}I]leuprorelin to GnRH receptor by 50%, in the presence of 40% FBS. [c] Inhibition of GnRH-stimulated AA release from CHO cells expressing human or monkey GnRH receptor was measured to evaluate the GnRH antagonistic activities of the test compounds. The IC_{90} is the antagonist concentration required to inhibit the GnRH-stimulated AA release from CHO cells by 90% in the presence of 40% human or monkey serum. [d] CHO cells expressing human, monkey, or rat GnRH receptor were used as the source for the GnRH receptor.

Table 5. Oral Absorption of Compound 16b in Cynomolgus Monkeys[a]

1 mg/kg (po)			3 mg/kg (po)		
C_{max} (ng/mL)	T_{max} (h)	AUC (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)	AUC (ng·h/mL)
16.0	2.7	90.1	112.8	2.0	533.1

[a] See Experimental Section.

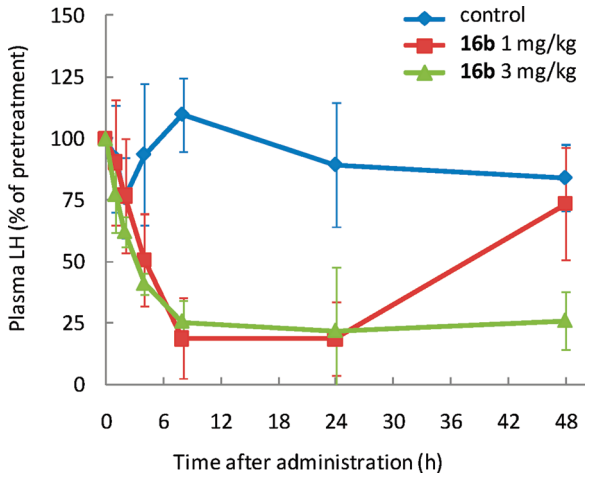


Figure 4. Time course of plasma LH concentrations in castrated cynomolgus monkeys after oral administration of compound **16b** at doses of 1 mg/kg (red square) and 3 mg/kg (green triangle). Figures are percentages of LH concentration at the indicated times, with LH concentration at 0 h (pretreatment) taken as 100% for each group.

potency of **16b**, this PK profile suggests that **16b** is a promising compound for exerting potent in vivo efficacy when administered orally.

Finally, the in vivo antagonistic activity of **16b** was evaluated in castrated cynomolgus monkeys, and the suppression of plasma LH levels is illustrated in Figure 4. As expected, a single oral administration of **16b** at doses of 1 and 3 mg/kg exhibited clear suppressive effects on circulating LH levels in monkeys, and the suppressive effects lasted for more than 24 h (1 mg/kg) and 48 h (3 mg/kg). The suppressive effect of **16b** at a 1 mg/kg dose was comparable to that of **1** at a 10 mg/kg dose, and the minimum effective dose of **16b** was 1 mg/kg, which is 10-fold lower than that of **1**.[11b] It is worth noting that exposure to **16b** at a dose as low as 1 mg/kg dose resulted in effective suppression. The potent antagonistic in vivo activity of **16b** may be attributable to its potent in vitro activity. Consequently, compound **16b** dose-dependently exerted a rapid and prolonged suppressive effect on plasma LH levels in castrated monkeys. These results clearly demonstrate that **16b** is a potent and orally active GnRH antagonist. Moreover, the large difference between in vitro antagonistic activities of **16b** toward human and monkey receptors implies that a much smaller dose of **16b** should be sufficient to suppress plasma LH levels in human.

CONCLUSION

To develop our nonpeptidic orally active GnRH antagonist, we focused on optimization at the thieno[2,3-*d*]pyrimidine-2,4-dione ring 5 and 3 positions based on computational modeling results. Introduction of polar substituents gave compounds with log *D* of 2 or lower, resulting in decreased CYP3A4 inhibition while maintaining potent GnRH antagonistic activity. In particular, a heterocycle at the 3-position was found to be an effective substituent, giving desirable levels of both reduced CYP3A4 inhibitory activity and potent GnRH antagonistic activity in the presence of FBS. Among the compounds synthesized, **16b** showed high antagonistic activity in the presence of serum without any CYP3A4 inhibition. Furthermore, **16b** exhibited a good pharmacokinetic profile and obvious suppressive effects of circulating LH levels in monkeys at a dose of 1 mg/kg. Compound **16b** was selected as a candidate for development and is under clinical study. We strongly believe that **16b** represents a novel compound that will be a useful therapeutic tool for the treatment of sex-hormone-dependent diseases.

EXPERIMENTAL SECTION

Melting points were determined on a Yanagimoto micro melting point apparatus or a SRS OptiMelt melting point apparatus and are uncorrected. ^{1}H NMR spectra were measured with a Varian Gemini-200 (200 MHz) or a Varian Mercury-300 (300 MHz) spectrometer. Chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard, and coupling constants (J) are given in hertz (Hz). Peak multiplicities and abbreviations are expressed as follows: s, singlet; d, doublet; t, triplet; dd, double doublet; dt, double triplet; q, quartet; m, multiplet; br s, broad singlet. Elemental analyses were carried out by Takeda Analytical Research Laboratories Ltd., and the results were within ±0.4% of the theoretical values. HPLC analyses were performed on an Agilent HP1100 system. Chromatographic separations were carried out on silica gel (Kieselgel 60, 63−200 mesh, Merck) or basic silica gel (Chromatorex NH-DM1020, 100−200 mesh, Fuji Silysia Chemical Ltd.) using the indicated eluents. Yields were not optimized. Purity of compounds (>95%) was established by elemental analyses and HPLC analyses. The data of elemental analysis and HPLC analyses are in Supporting Information.

1-(4-{1-(2,6-Difluorobenzyl)-5-[(methylamino)methyl]-2,4-dioxo-3-phenyl-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl}phenyl)-3-methoxyurea (5). A mixture of 1-{4-[5-{[benzyl(methyl)amino]methyl}-1-(2,6-difluorobenzyl)-2,4-dioxo-3-phenyl-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea

dx.doi.org/10.1021/jm200216q |*J. Med. Chem.* 2011, 54, 4998–5012

(**1**) (6.68 g, 10.0 mmol), 10% Pd/C (50% wet, 2.23 g), 1 N HCl (11.0 mL, 11.0 mmol), and MeOH (150 mL) was stirred under H_2 atmosphere (1 atm) at room temperature for 2 h. The mixture was filtered through Celite, and the filtrate was concentrated in vacuo. The residue was diluted with 1 N NaOH and extracted with EtOAc and THF. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was washed with Et_2O and diisopropyl ether to give **5** (5.52 g, 96%) as a white powder. ^{1}H NMR ($CDCl_3$): δ 2.35 (3H, s), 3.76 (2H, s), 3.82 (3H, s), 5.37 (2H, s), 6.92 (2H, t, J = 8.2 Hz), 7.25−7.70 (12H, m).

1-{4-[1-(2,6-Difluorobenzyl)-5-{[methyl(pyridin-2-ylmethyl)amino]methyl}-2,4-dioxo-3-phenyl-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (6a). A mixture of **5** (350 mg, 0.61 mmol), 2-chloromethylpyridine hydrochloride (149 mg, 0.91 mmol), and *N*,*N*-diisopropylethylamine (0.32 mL, 1.82 mmol) in DMF (8 mL) was stirred at room temperature for 14 h. Thereaction mixture was diluted with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The extract was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (EtOAc) to afford **6a** (297 mg, 73%) as colorless crystals, mp 181−182 °C. ^{1}H NMR ($CDCl_3$): δ 2.10 (3H, s), 3.71 (2H, s), 3.83 (3H, s), 3.99 (2H, s), 5.36 (2H, s), 6.92 (2H, t, J = 8.2 Hz), 7.00−7.10 (1H, m), 7.15 (1H, s), 7.20−7.35 (4H, m), 7.40−7.65 (9H, m), 8.40−8.50 (1H, m). IR (KBr): 1715, 1667, 1532, 1472, 735 cm^{-1}. LC−MS m/z: 669.0 [M + H$^+$], 667.0 [M + H$^-$]. Anal. ($C_{35}H_{30}F_2N_6O_4S\cdot0.5H_2O$) C, H, N.

Compounds **6b**,**d**,**e**,**g**,**h** were prepared by a procedure similar to that described for **6a**, and their physicochemical data are shown below.

1-{4-[1-(2,6-Difluorobenzyl)-5-{[methyl(pyridin-3-ylmethyl)amino]methyl}-2,4-dioxo-3-phenyl-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (6b). Yield 67%, colorless crystals, mp 184−185 °C. ^{1}H NMR ($CDCl_3$): δ 2.04 (3H, s), 3.58 (2H, s), 3.83 (3H, s), 3.92 (2H, s), 5.37 (2H, s), 6.92 (2H, t, J = 8.2 Hz), 7.05−7.70 (14H, m), 8.35−8.45 (2H, m). IR (KBr): 1713, 1669, 1532, 1464, 1329, 1238, 1032, 787 cm^{-1}. LC−MS m/z: 669.1 [M + H$^+$], 667.0 [M + H$^-$]. Anal. ($C_{35}H_{30}F_2N_6O_4S\cdot0.3H_2O$) C, H, N.

1-{4-[1-(2,6-Difluorobenzyl)-5-{[{[6-(hydroxymethyl)pyridin-2-yl]methyl}(methyl)amino]methyl}-2,4-dioxo-3-phenyl-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (6d). Yield 63%, colorless crystals, mp 178−180 °C. ^{1}H NMR ($CDCl_3$): δ 2.13 (3H, s), 3.72 (2H, s), 3.83 (3H, s), 3.97 (2H, s), 4.05−4.10 (1H, m), 4.65−4.75 (2H, m), 5.37 (2H, s), 6.93 (2H, t, J = 8.2 Hz), 6.90−7.05 (1H, m), 7.10−7.20 (2H, m), 7.20−7.70 (12H, m). IR (KBr): 1713, 1669, 1534, 1472, 1032, 789, 735 cm^{-1}. LC−MS m/z: 699.1 [M + H$^+$], 697.0 [M + H$^-$]. Anal. ($C_{36}H_{32}F_2N_6O_5S\cdot1.1H_2O$) C, H, N.

1-{4-[1-(2,6-Difluorobenzyl)-5-({(methyl)[2-(1*H*-1,2,3-triazol-1-yl)ethyl]amino}methyl)-2,4-dioxo-3-phenyl-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (6e). Yield 41%, colorless crystals, mp 194−196 °C. ^{1}H NMR ($CDCl_3$): δ 2.14 (3H, s), 2.82 (2H, t, J = 6.0 Hz), 3.80 (2H, s), 3.82 (3H, s), 4.39 (2H, t, J = 6.0 Hz), 5.37 (2H, s), 6.92 (2H, t, J = 8.2 Hz), 6.85−6.95 (1H, m), 7.14 (1H, s), 7.20−7.55 (11H, m), 7.63 (1H, s). IR (KBr): 1719, 1672, 1526, 1470, 1236, 1231, 1028, 824, 733 cm^{-1}. LC−MS m/z: 673.3 [M + H$^+$]. Anal. ($C_{33}H_{30}F_2N_8O_4S\cdot0.4H_2O$) C, H, N.

***N*-{2-[{[1-(2,6-Difluorobenzyl)-6-(4-{[(methoxyamino)carbonyl]amino}phenyl)-2,4-dioxo-3-phenyl-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-5-yl]methyl}(methyl)amino]ethyl}-*N*-methylmethanesulfonamide (6g).** Yield 67%, colorless crystals, mp 196−198 °C. ^{1}H NMR ($CDCl_3$): δ 2.12 (3H, s), 2.54 (2H, t, J = 6.3 Hz), 2.70 (6H, s), 3.16 (2H, t, J = 6.3 Hz), 3.82 (2H, s), 3.83 (3H, s), 5.38 (2H, s), 6.93 (2H, t, J = 8.1 Hz), 7.13 (1H, s), 7.25−7.65 (11H, m). IR (KBr): 1715, 1667, 1534, 1470, 1335, 1144, 1034, 791 cm^{-1}. Anal. ($C_{33}H_{34}F_2N_6O_6S_2$) C, H, N.

1-{4-[1-(2,6-Difluorobenzyl)-5-{[(2-methoxyethyl)(methyl)amino]methyl}-2,4-dioxo-3-phenyl-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (6h). Yield 89%, white crystals, mp 161−163 °C. ^{1}H NMR ($CDCl_3$): δ 2.14 (3H, s), 2.64 (2H, t, J = 5.9 Hz), 3.27 (3H, s), 3.41 (2H, t, J = 5.9 Hz), 3.83 (5H, s), 5.37 (2H, s), 6.93 (2H, t, J = 8.2 Hz), 7.12−7.63 (12H, m). IR (KBr): 1709, 1663, 1560, 1522 cm^{-1}. LC−MS m/z: 636.0 [M + H$^+$], 633.9 [M + H$^-$]. Anal. ($C_{32}H_{31}F_2N_5O_5S\cdot0.5H_2O$) C, H, N.

1-{4-[1-(2,6-Difluorobenzyl)-5-({methyl[2-(2-oxopyrrolidin-1-yl)ethyl]amino}methyl)-2,4-dioxo-3-phenyl-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (6f). To an ice-cooled solution of 1-(hydroxyethyl)pyrrolidine-2-one (0.50 g, 3.85 mmol) and triethylamine (0.58 mL, 4.16 mmol) in THF (5 mL) was added methanesulfonyl chloride (0.27 mL, 3.49 mmol). After being stirred at room temperature for 1 h, the mixture was diluted with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The extract was dried ($MgSO_4$) and concentrated in vacuo. The residue was dissolved in DMF (8 mL), and to this solution were added **5** (404 mg, 0.7 mmol), *N*,*N*-diisopropylethylamine (0.24 mL, 1.38 mmol), and potassium iodide (465 mg, 2.80 mmol). After being stirred at 50 °C for 24 h, the mixture was diluted with water and extracted with EtOAc. The extract was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (EtOAc−MeOH, 40:1) to afford **6f** (290 mg, 60%) as colorless crystals, mp 134−136 °C. ^{1}H NMR ($CDCl_3$): δ 1.70−1.85 (2H, m), 2.14 (3H, s), 2.21 (2H, t, J = 8.1 Hz), 2.54 (2H, t, J = 6.2 Hz), 3.19 (2H, t, J = 7.0 Hz), 3.29 (2H, t, J = 6.2 Hz), 3.77 (2H, s), 3.83 (3H, s), 5.38 (2H, s), 6.93 (2H, t, J = 8.1 Hz), 7.18 (1H, s), 7.25−7.7 (11H, m). IR (KBr): 1715, 1672, 1530, 1470, 1323, 1238, 1032, 735 cm^{-1}. LC−MS m/z: 689.1 [M + H$^+$], 687.0 [M + H$^-$]. Anal. ($C_{35}H_{34}F_2N_6O_5S\cdot0.5H_2O$) C, H, N.

1-{4-[1-(2,6-Difluorobenzyl)-5-{[methyl(2-pyridin-2-ylethyl)amino]methyl}-2,4-dioxo-3-phenyl-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (6c). Compound **6c** was prepared by a procedure similar to that described for **6f** in 56% yield as colorless crystals, mp 159−160 °C. ^{1}H NMR ($CDCl_3$): δ 2.20 (3H, s), 2.85 (4H, s), 3.82 (5H, s), 5.37 (2H, s), 6.93 (2H, t, J = 8.2 Hz), 6.95−7.10 (2H, m), 7.14 (1H, s), 7.20−7.55 (11H, m), 7.60 (1H, s), 8.43 (1H, d, J = 4.0 Hz). IR (KBr): 1717, 1667, 1530, 1470, 1331, 1236, 1030, 735 cm^{-1}. LC−MS m/z: 683.1 [M + H$^+$], 681.0 [M + H$^-$]. Anal. ($C_{36}H_{32}F_2N_6O_4S$) C, H, N.

Ethyl 2-[(Ethoxycarbonyl)amino]-4-methyl-5-(4-nitrophenyl)thiophene-3-carboxylate (8). To a suspension of ethyl 2-amino-4-methyl-5-(4-nitrophenyl)thiophene-3-carboxylate **7**[11b] (50.0 g, 163 mmol) in toluene (1.31 L) was added ethyl chloroformate (31.2 mL, 326 mmol), and the mixture was refluxed for 3 h. Ethyl chloroformate (31.2 mL, 326 mmol) was again added, and the mixture was heated at reflux. After 3 h, more ethyl chloroformate (31.2 mL, 326 mmol) was added and the reaction mixture was heated at reflux for an additional 6 h. After cooling, the mixture was concentrated in vacuo. Et_2O was added to the residue and the resulting precipitate was collected by filtration, washed with Et_2O, and dried to give **8** (57.8 g, 94%) as a yellow powder. ^{1}H NMR ($CDCl_3$): δ 1.35 (3H, t, J = 7.1 Hz), 1.42 (3H, t, J = 7.2 Hz), 2.42 (3H, s), 4.31 (2H, q, J = 7.1 Hz), 4.39 (2H, q, J = 7.2 Hz), 7.59 (2H, d, J = 9.0 Hz), 8.27 (2H, d, J = 9.0 Hz), 10.66 (1H, s). IR (KBr): 1740, 1665, 1597, 1557, 1533, 1516, 1352, 1257 cm^{-1}.

Ethyl 2-[(2,6-Difluorobenzyl)(ethoxycarbonyl)amino]-4-methyl-5-(4-nitrophenyl)thiophene-3-carboxylate (9). To a solution of **8** (57.3 g, 151 mmol) in DMF (1.82 L) were added KI (27.6 g, 167 mmol) and K_2CO_3 (27.1 g, 167 mmol). A solution of 2,6-difluorobenzyl chloride (27.1 g, 167 mmol) in DMF (100 mL) was then added to the mixture. After the mixture was stirred at room temperature for 44 h, the resulting precipitate was filtered off and the filtrate was concentrated in vacuo. The residue was diluted with EtOAc and water, then extracted with EtOAc. The extract was washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. Et_2O was added to the residue and the resulting precipitate was collected by filtration, washed with Et_2O, and dried to give **9** (71.1 g, 93%) as a pale yellow powder.

^{1}H NMR ($CDCl_3$): δ 1.15−1.35 (6H, m), 2.40 (3H, s), 4.15−4.29 (4H, m), 4.97 (2H, s), 6.86 (2H, t, J = 7.8 Hz), 7.25−7.32 (1H, m), 7.51 (2H, d, J = 8.8 Hz), 8.25 (2H, d, J = 8.8 Hz). IR (KBr): 1717, 1597, 1524, 1475, 1392, 1348 cm^{-1}.

Ethyl 4-(Bromomethyl)-2-[(2,6-difluorobenzyl)(ethoxycarbonyl)amino]-5-(4- nitrophenyl)thiophene-3-carboxylate (10). A mixture of **9** (50.5 g, 100 mmol), NBS (22.3 g, 125 mmol), and AIBN (1.65 g, 10.0 mmol) in EtOAc (600 mL) was refluxed for 2 h. NBS (3.56 g, 20.0 mmol) and AIBN (165 mg, 1.00 mmol) were added to the mixture, and the reaction mixture was heated at reflux for an additional 1.5 h. After cooling to room temperature, the reaction mixture was diluted with EtOAc and water and extracted with EtOAc. The extract was washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo to give crude **10** (63.0 g, quant) as an orange oil, which was used for the next step without purification. ^{1}H NMR ($CDCl_3$): δ 1.15−1.39 (6H, m), 4.09−4.39 (4H, m), 4.71 (2H, s), 4.99 (2H, s), 6.86 (2H, t, J = 7.8 Hz), 7.22−7.32 (1H, m), 7.72 (2H, d, J = 8.0 Hz), 8.32 (2H, d, J = 8.0 Hz). IR (KBr): 1725, 1628, 1522, 1475, 1379, 1348 cm^{-1}.

Ethyl 2-[(2,6-Difluorobenzyl)(ethoxycarbonyl)amino]-4-{[(2-methoxyethyl)(methyl)amino]methyl}-5-(4-nitrophenyl)thiophene-3-carboxylate (11). To a solution of **10** (157 g, 221 mmol) in DMF (1.33 L) was added a mixture of *N*,*N*-diisopropylethylamine (45.7 mL, 265 mmol) and *N*-(2-methoxylethyl)methylamine (21.4 g, 240 mmol) in DMF (50 mL). After being stirred at room temperature for 24 h, the reaction mixture was concentrated in vacuo. The residue was diluted with EtOAc and water and extracted with EtOAc. The extract was washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo to give **11** (145 g, quant) as an orange oil, which was used for the next step without purification. ^{1}H NMR ($CDCl_3$): δ 1.15−1.30 (3H, m), 1.31 (3H, t, J = 7.4 Hz), 2.11 (3H, s), 2.40−2.60 (2H, m), 3.28 (2H, s), 3.30−3.45 (2H, m), 3.68 (2H, s), 4.10−4.30 (2H, m), 4.23 (2H, q, J = 7.4 Hz), 5.01 (2H, s), 6.85 (2H, t, J = 7.6 Hz), 7.20−7.30 (1H, m), 7.69 (2H, d, J = 8.8 Hz), 8.24 (2H, d, J = 8.8 Hz).

Ethyl 5-(4-Aminophenyl)-2-[(2,6-difluorobenzyl)(ethoxycarbonyl)amino]-4-{[(2-methoxyethyl)(methyl)amino]-methyl}thiophene-3-carboxylate (12). A solution of **11** (12.4 g, 21.0 mmol) and 2 N HCl/Et_2O (21.0 mL, 42.0 mmol) in EtOH (315 mL) was hydrogenated over 10% Pd/C (50% wet, 3.73 g) under atomospheric pressure at room temperature for 1 h. The mixture was filtered through Celite, and the filtrate was diluted with saturated aqueous $NaHCO_3$ solution. The mixture was concentrated in vacuo, diluted with EtOAc and water, and extracted with EtOAc. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (EtOAc−MeOH, 40:1) to afford **12** (11.4 g, 97%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.12−1.30 (3H, m), 2.05 (3H, s), 2.39 (2H, t, J = 6.3 Hz), 3.27 (3H, s), 3.32 (3H, t, J = 6.3 Hz), 3.59 (2H, s), 3.78 (2H, s), 4.20 (2H, q, J = 7.1 Hz), 4.10−4.23 (2H, m), 5.00 (2H, s), 6.66 (2H, d, J = 8.6 Hz), 6.84 (2H, t, J = 8.2 Hz), 7.18 (2H, d, J = 8.6 Hz), 7.15−7.30 (1H, m). IR (KBr): 1717, 1626, 1609, 1472, 1406, 1300, 1246 cm^{-1}.

Ethyl 2-[(2,6-Difluorobenzyl)(ethoxycarbonyl)amino]-5-{4-[(methoxycarbamoyl)amino]phenyl}-4-{[(2-methoxyethyl)(methyl)amino]methyl}thiophene-3-carboxylate (13). To a solution of **12** (4.89 g, 8.70 mmol) in CH_2Cl_2 (113 mL) were added *N*,*N*-diisopropylethylamine (3.06 mL, 17.6 mmol) and CDI (2.82 g, 17.4 mmol). After being stirred at room temperature for 67 h, the mixture was cooled to 0 °C. To the mixture were added *O*-methylhydroxyamine hydrochloride (7.26 g, 86.9 mmol) and *N*,*N*-diisopropylethylamine (15.6 mL, 89.6 mmol), and the mixture was stirred at room temperature for 19 h and then was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$ solution. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$. The combined extracts were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc) to afford **13** (4.89 g, 89%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.19 (3H, br s), 1.30 (3H, t, J = 6.9 Hz), 2.04 (3H, s), 2.40 (2H, t, J = 6.0 Hz), 3.27 (3H, s), 3.33 (2H, t, J = 6.0 Hz), 3.60 (2H, s), 3.81 (3H, s), 4.13−4.24 (4H, m), 5.00 (2H, s), 6.84 (2H, t, J = 7.8 Hz), 7.19−7.29 (2H, m), 7.36 (2H, d, J = 8.7 Hz), 7.50 (2H, d, J = 8.7 Hz), 7.60 (1H, s). IR (KBr): 1717, 1590, 1528, 1472, 1408, 1304 cm^{-1}.

2-[(2,6-Difluorobenzyl)(ethoxycarbonyl)amino]-5-{4-{[(methoxycarbamoyl)amino]phenyl}-4-[(2-methoxyethyl)-(methyl)amino]methyl}thiophene-3-carboxylic Acid (14). 2 N NaOH (18.9 mL, 37.8 mmol) was added to a solution of **13** (4.81 g, 7.58 mmol) in EtOH (114 mL). After the mixture was stirred at 60 °C for 5 h, 1 N HCl (37.8 mL, 37.8 mmol) was added to the mixture at 0 °C and the whole was concentrated in vacuo. The residue was dissolved in EtOH and toluene, and the whole was concentrated in vacuo. The residue was diluted with dry EtOH followed by filtration, and the filtrate was concentrated in vacuo. Et_2O was added to the residue and the resulting precipitate was collected by filtration, washed with Et_2O, and dried to give **14** (4.43 g, 96%) as a pale yellow powder. ^{1}H NMR ($CDCl_3$): δ 1.17 (3H, br s), 2.45 (3H, s), 2.81 (2H, br s), 3.28 (3H, s), 3.55 (2H, t, J = 4.8 Hz), 3.82 (3H, s), 3.92 (2H, s), 4.10−4.35 (2H, m), 5.06 (2H, s), 6.82 (2H, t, J = 7.8 Hz), 7.16 (2H, d, J = 8.4 Hz), 7.22−7.35 (1H, m), 7.60 (2H, d, J = 8.4 Hz), 8.00−8.50 (2H, br). IR (KBr): 1713, 1605, 1528, 1472, 1408 cm^{-1}.

1-{4-[1-(2,6-Difluorobenzyl)-3-(2-hydroxy-2-methylpropyl)-5-{[(2-methoxyethyl)(methyl)amino]methyl}-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (15d). Under ice-cooling, to a solution of **14** (303 mg, 0.50 mmol) and 1-amino-2-methylpropan-2-ol (89.0 mg, 1.00 mmol) in DMF (4 mL) were successively added DEPC (0.15 mL, 0.99 mmol) and *N*,*N*-diisopropylethylamine (0.19 mL, 1.09 mmol), and the mixture was stirred at room temperature for 14 h. The reaction mixture was partitioned between EtOAc and water. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined extracts were washed with saturated aqueous $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (EtOAc−MeOH, 10:1) to afford the amide intermediate. The amide was dissolved in MeOH (10 mL), and MeONa (270 mg, 5.00 mmol) was added. After the mixture was stirred at room temperature for 2 h, 1 N HCl (5.0 mL, 5.0 mmol) was added to the mixture at 0 °C and the whole was concentrated in vacuo. The residue was partitioned between EtOAc and water. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined extracts were washed with saturated aqueous $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (EtOAc) and followed by recrystallization from EtOAc−Et_2O to afford **15d** (134 mg, 42%) as colorless crystals, mp 114−116 °C. ^{1}H NMR ($CDCl_3$): δ 1.28 (6H, s), 2.30 (3H, s), 2.64 (2H, t, J = 5.8 Hz), 3.30 (3H, s), 3.45 (2H, t, J = 5.8 Hz), 3.82 (5H, s), 3.99 (1H, s), 4.25 (2H, s), 5.36 (2H, s), 6.91 (2H, t, J = 8.2 Hz), 7.12 (1H, s), 7.20−7.40 (1H, m), 7.50−7.60 (4H, m), 7.61 (1H, s). LC−MS m/z: 632.1 [M + H$^+$], 630.0 [M + H$^-$]. Anal. ($C_{30}H_{35}F_2N_5O_6S\cdot0.1H_2O$) C, H, N.

Compounds **15a**−**c**,**e**−**j** were prepared by a procedure similar to that described for **15d**, and their physicochemical data are shown below.

1-{4-[1-(2,6-Difluorobenzyl)-3-(2-methoxyethyl)-5-{[(2-methoxyethyl)(methyl)amino]methyl}-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (15a). Yield 58%, pale yellow crystals, mp 165−167 °C. ^{1}H NMR ($CDCl_3$): δ 2.14 (3H, s), 2.65 (2H, t, J = 5.9 Hz), 3.30 (3H, s), 3.36 (3H, s), 3.45 (2H, t, J = 5.9 Hz), 3.66 (2H, t, J = 5.9 Hz), 3.81 (3H, s), 3.84 (2H, s), 4.30 (2H, t, J = 5.9 Hz), 5.33 (2H, s), 6.90 (2H, t, J = 8.3 Hz), 7.15 (1H, s), 7.24−7.34 (1H, m), 7.51 (2H, d, J = 9.0 Hz), 7.56 (2H, d, J = 9.0 Hz), 7.60 (1H, s). IR (KBr): 2936, 1705, 1663, 1590, 1532,

1472 cm^{-1}. LC−MS *m/z*: 618.1 [M + H$^+$], 615.9 [M + H$^-$]. Anal. ($C_{29}H_{33}F_2N_5O_6S$) C, H, N.

1-{4-[1-(2,6-Difluorobenzyl)-3-(2-ethoxyethyl)-5-{[(2-methoxyethyl)(methyl)amino]methyl}-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (15b). Yield 82%, pale yellow crystals, mp 151−152 °C. ^{1}H NMR ($CDCl_3$): δ 1.15 (3H, t, *J* = 6.9 Hz), 2.14 (3H, s), 2.66 (2H, t, *J* = 6.0 Hz), 3.30 (3H, s), 3.45 (2H, t, *J* = 6.0 Hz), 3.54 (2H, q, *J* = 6.9 Hz), 3.69 (2H, t, *J* = 6.0 Hz), 3.81 (3H, s), 3.84 (2H, s), 4.29 (2H, t, *J* = 6.0 Hz), 5.32 (2H, s), 6.89 (2H, t, *J* = 8.1 Hz), 7.17 (1H, s), 7.23−7.34 (1H, m), 7.52 (2H, d, *J* = 8.7 Hz), 7.57 (2H, d, *J* = 8.7 Hz), 7.60 (1H, s). IR (KBr): 2975, 1705, 1663, 1590, 1532, 1472 cm^{-1}. LC−MS *m/z*: 632.1 [M + H$^+$], 630.0 [M + H$^-$]. Anal. ($C_{30}H_{35}F_2N_5O_6S$) C, H, N.

1-{4-[1-(2,6-Difluorobenzyl)-3-(2-hydroxyethyl)-5-{[(2-methoxyethyl)(methyl)amino]methyl}-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (15c). Yield 25%, pale yellow amorphous powder. ^{1}H NMR ($CDCl_3$): δ 2.11 (3H, s), 2.65 (2H, t, *J* = 5.8 Hz), 3.30 (3H, s), 3.46 (2H, t, *J* = 5.8 Hz), 3.82 (5H, s), 3.90−4.00 (2H, m), 4.35 (2H, t, *J* = 5.2 Hz), 5.34 (2H, s), 6.92 (2H, t, *J* = 8.0 Hz), 7.14 (1H, s), 7.20−7.35 (1H, m), 7.50−7.65 (5H, m). LC−MS *m/z*: 604.2 [M + H$^+$]. Anal. ($C_{28}H_{31}F_2N_5O_6S\cdot 1.0H_2O$) C, H, N.

1-{4-[1-(2,6-Difluorobenzyl)-5-{[(2-methoxyethyl)(methyl)amino]methyl}-2,4-dioxo-3-(pyridin-2-yl)-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (15e). Yield 33%, colorless crystals, mp 196−198 °C. ^{1}H NMR ($CDCl_3$): δ 2.15 (3H, s), 2.62 (2H, t, *J* = 5.9 Hz), 3.26 (3H, s), 3.41 (2H, t, *J* = 5.9 Hz), 3.80 (3H, s), 3.81 (2H, br s), 5.34 (2H, br s), 6.91 (2H, t, *J* = 8.1 Hz), 7.24−7.40 (4H, m), 7.53 (2H, d, *J* = 8.4 Hz), 7.62 (2H, d, *J* = 8.4 Hz), 7.65 (1H, s), 7.88 (1H, dt, *J* = 1.5, 7.8 Hz), 8.67−8.69 (1H, m). IR (KBr): 1717, 1674, 1591, 1530, 1460, 1329 cm^{-1}. LC−MS *m/z*: 637.0 [M + H$^+$], 634.9 [M + H$^-$]. Anal. ($C_{31}H_{30}F_2N_6O_5S$) C, H, N.

1-{4-[1-(2,6-Difluorobenzyl)-5-{[(2-methoxyethyl)(methyl)amino]methyl}-3-(5-methylpyridin-2-yl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (15f). Yield 71%, pale yellow amorphous powder. ^{1}H NMR ($CDCl_3$): δ 2.14 (3H, s), 2.39 (3H, s), 2.62 (2H, t, *J* = 5.7 Hz), 3.26 (3H, s), 3.41 (2H, t, *J* = 5.7 Hz), 3.77 (2H, br s), 3.80 (3H, s), 5.26 (1H, br s), 5.38 (1H, br s), 6.91 (2H, t, *J* = 8.3 Hz), 7.23−7.34 (2H, m), 7.42 (1H, s), 7.53 (2H, d, *J* = 8.7 Hz), 7.62 (2H, d, *J* = 8.7 Hz), 7.66 (1H, s), 7.66−7.69 (1H, m), 8.48 (1H, d, *J* = 2.4 Hz). IR (KBr): 2938, 1717, 1675, 1586, 1530, 1462 cm^{-1}. LC−MS *m/z*: 651.0 [M + H$^+$], 649.0 [M + H$^-$]. Anal. ($C_{32}H_{32}F_2N_6O_5S\cdot 0.5H_2O$) C, H, N.

1-{4-[1-(2,6-Difluorobenzyl)-3-(5-fluoropyridin-2-yl)-5-{[(2-methoxyethyl)(methyl)amino]methyl}-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (15g). Yield 46%, pale yellow amorphous powder. ^{1}H NMR ($CDCl_3$): δ 2.13 (3H, s), 2.62 (2H, t, *J* = 5.9 Hz), 3.26 (3H, s), 3.41 (2H, t, *J* = 5.9 Hz), 3.80 (2H, br s), 3.82 (3H, s), 5.33 (2H, br s), 6.92 (2H, t, *J* = 8.3 Hz), 7.19 (1H, s), 7.28−7.38 (2H, m), 7.52−7.63 (6H, m), 8.51 (1H, d, *J* = 3.0 Hz). IR (KBr): 1715, 1674, 1586, 1530, 1462 cm^{-1}. LC−MS *m/z*: 655.0 [M + H$^+$], 653.0 [M + H$^-$]. Anal. ($C_{31}H_{29}F_3N_6O_5S$) C, H, N.

1-{4-[1-(2,6-Difluorobenzyl)-5-{[(2-methoxyethyl)(methyl)amino]methyl}-3-(4-methoxyphenyl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (15h). Yield 62%, pale brown solid. ^{1}H NMR ($CDCl_3$): δ 2.13 (3H, s), 2.64 (2H, t, *J* = 5.7 Hz), 3.27 (3H, s), 3.41 (2H, t, *J* = 5.6 Hz), 3.82−3.84 (8H, m), 5.36 (2H, s), 6.92 (2H, t, *J* = 8.1 Hz), 7.01 (2H, d, *J* = 8.7 Hz), 7.11 (1H, s), 7.19 (2H, d, *J* = 8.7 Hz), 7.26−7.33 (1H, m), 7.53−7.70 (5H, m). IR (KBr): 1716, 1661, 1625, 1588, 1500, 1456 cm^{-1}. LC−MS *m/z*: 666.3 [M + H$^+$]. Anal. ($C_{33}H_{33}F_2N_5O_6S\cdot 0.5H_2O$) C, H, N.

1-{4-[1-(2,6-Difluorobenzyl)-5-{[(2-methoxyethyl)(methyl)amino]methyl}-3-(6-methoxypyridin-3-yl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (15i). Yield 71%, colorless crystals, mp 167−169 °C. ^{1}H NMR ($CDCl_3$): δ 2.11 (3H, s), 2.64 (2H, t, *J* = 5.7 Hz), 3.27 (3H, s), 3.42 (2H, t, *J* = 5.7 Hz), 3.81 (2H, s), 3.83 (3H, s), 3.97 (3H, s), 5.37 (2H, s), 6.87 (1H, d, *J* = 8.7 Hz), 6.93 (2H, t, *J* = 8.1 Hz), 7.19 (1H, s), 7.25−7.36 (1H, m), 7.50 (1H, dd, *J* = 2.7, 8.7 Hz), 7.53−7.60 (4H, m), 7.63 (1H, s), 8.10 (1H, d, *J* = 2.7 Hz). IR (KBr): 1715, 1672, 1609, 1530, 1470 cm^{-1}. LC−MS *m/z*: 667.1 [M + H$^+$], 665.0 [M + H$^-$]. Anal. ($C_{32}H_{32}F_2N_6O_6S\cdot 0.5H_2O$) C, H, N.

1-{4-[1-(2,6-Difluorobenzyl)-5-{[(2-methoxyethyl)(methyl)amino]methyl}-3-(6-methoxypyridazin-3-yl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (15j). Yield 69%, pale yellow powder, mp 201−203 °C. ^{1}H NMR ($CDCl_3$): δ 2.13 (3H, s), 2.62 (2H, t, *J* = 5.7 Hz), 3.26 (3H, s), 3.41 (2H, t, *J* = 5.7 Hz), 3.74 (2H, br s), 3.82 (3H, s), 4.18 (3H, s), 5.32 (2H, br s), 6.92 (2H, t, *J* = 8.3 Hz), 7.12 (1H, d, *J* = 9.3 Hz), 7.29−7.35 (1H, m), 7.41 (2H, d, *J* = 9.3 Hz), 7.54 (2H, d, *J* = 9.0 Hz), 7.59 (2H, d, *J* = 8.7 Hz), 7.66 (1H, s). IR (KBr): 2936, 1717, 1674, 1591, 1530, 1460 cm^{-1}. LC−MS *m/z*: 668.1 [M + H$^+$], 665.9 [M + H$^-$]. Anal. ($C_{31}H_{31}F_2N_7O_6S$) C, H, N.

1-{4-[1-(2,6-Difluorobenzyl)-5-[(dimethylamino)methyl]-3-(6-methoxypyridin-3-yl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (16a). To a solution of **15i** (58.9 g, 88.9 mmol) in CH_2Cl_2 (1.2 L) was added dropwise 1-chloroethyl chlorocarbonate (12.5 mL, 116 mmol) at −78 °C. After the mixture was stirred at room temperature for 2 h, saturated aqueous $NaHCO_3$ solution and brine were added to the mixture, and the whole was extracted with CH_2Cl_2. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The resulting precipitates were washed with Et_2O to afford the chloromethyl intermediate, which was dissolved in DMF (400 mL). Then dimethylamine (2 M in THF, 170 mL, 340 mmol) and *N*,*N*-diisopropylethylamine (17.8 mL, 102 mmol) were added successively to the DMF solution. After the mixture was stirred at room temperature for 15 h, saturated aqueous $NaHCO_3$ solution was added to the mixture and the whole was extracted with EtOAc. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by basic silica gel column chromatography (EtOAc) followed by recrystallization from EtOAc−THF to afford **16a** (13.1 g, 24%) as colorless crystals, mp 234−236 °C. ^{1}H NMR ($CDCl_3$): δ 2.13 (6H, s), 3.68 (2H, s), 3.83 (3H, s), 3.96 (3H, s), 5.36 (2H, s), 6.8−7.0 (3H, m), 7.13 (1H, s), 7.2−7.4 (1H, m), 7.45−7.65 (6H, m), 8.10 (1H, d, *J* = 2.6 Hz). LC−MS *m/z*: 623.0 [M + H$^+$], 621.0 [M + H$^-$]. Anal. ($C_{30}H_{28}F_2N_6O_5S$) C, H, N.

1-{4-[1-(2,6-Difluorobenzyl)-5-[(dimethylamino)methyl]-3-(6-methoxypyridazin-3-yl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea (16b). Compound **16b** was prepared in 44% yield from **15j** by a procedure similar to that described for **16a** as colorless crystals, mp 228 °C (dec). ^{1}H NMR ($CDCl_3$): δ 2.15 (6H, s), 3.60−3.80 (2H, m), 3.82 (3H, s), 4.18 (3H, s), 5.35 (2H, s), 6.92 (2H, t, *J* = 8.2 Hz), 7.12 (1H, d, *J* = 8.8 Hz), 7.20−7.65 (7H, m), 7.69 (1H, s). LC−MS *m/z*: 624.0 [M + H$^+$], 621.9 [M + H$^-$]. Anal. ($C_{29}H_{27}F_2N_7O_5S$) C, H, N.

In Vitro Binding Assays. Receptor binding assays were carried out as described previously.[11a] Briefly, human GnRH receptor cDNA was cloned from a pituitary cDNA library, and CHO cells stably expressing high levels of the recombinant human GnRH receptor were isolated. [^{125}I][Tyr^5]leuprorelin (2.0−2.5 nM) and the membrane fraction of the CHO cells (20 μg/well) were incubated at 25 °C for 60 min with shaking in 0.2 mL of assay buffer A [25 mM Tris, 1 mM EDTA, 0.1% bovine serum albumin (BSA), 0.25 mM phenylmethanesulfonyl fluoride, 1 μg/mL pepstatin A, 20 μg/mL leupeptin, and 10 μg/mL phosphoramidon, pH 7.5] containing various concentrations of the test compounds. The bound and free ligands were immediately separated by filtration through a poly(ethylenimine)-coated glass microfiber filter (Whatman, GF/C). The filter was washed three times 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}) was derived by fitting the data into a pseudo-Hill equation:

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

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 compounds. Similarly, the binding experiments to the GnRH receptor of monkey and rat were performed.

In Vitro Functional Assays. GnRH-stimulated arachidonic acid release from CHO cells expressing human or monkey GnRH receptors was measured according to the previously reported protocol.[23] 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, PerkinElmer Inc.) overnight 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 15 min, and the reaction was started by addition of GnRH (1 nM). After incubation at 37 °C for 45 min, radioactivity in the medium was measured with a liquid scintillation counter. IC_{50} and IC_{90} inhibitory concentrations of each compound were calculated by logistic regression analysis.

Measurement of CYP3A4 Inhibitory Activity. Inhibitory activity of test compounds of CYP3A4 was evaluated by incubating 100 μmol/L testosterone with 10 nmol/L CYP3A4 derived from CYP3A4-expressing human B-lymphoblastoid cells (BD Biosciences) in the presence of 10 μmol/L test compound. The incubation mixture was allowed to stand for 30 min at 37 °C. The concentration of 6β-hydroxytestosterone was measured by HPLC system equipped with a UV detector.

Oral Absorption in Cynomolgus Monkeys. Compound **16b** (1 or 3 mg) suspended in 0.5% methylcellulose (containing 0.6% citric acid) was orally administered to fasted cynomolgus monkeys (male, 5−7 years old, n = 3). Blood samples (heparin plasma) were collected from a forearm vein 0.25, 0.5, 1, 2, 4, 8, and 24 h after administration. The plasma samples were deproteinized with acetonitrile. After centrifugation, the obtained supernatant was diluted with internal standard solution (0.01 mol/L ammonium formate/acetonitrile = 4/1) and centrifuged again. The compound concentration in the supernatant was measured by LC/MS/MS.

The mass spectrometer was equipped with an electrospray ion source and operated in positive ion mode. The HPLC conditions were as follows: column, L-column ODS (2.1 mm × 150 mm); mobile phase, 0.01 mol/L ammonium formate (containing 0.2% formic acid)/acetonitrile = 6/4; flow rate, 0.2 mL/min; column temperature, 40 °C.

In Vivo Efficacy in Cynomolgus Monkeys. Compound **16b** (1 or 3 mg/kg) suspended in 0.5% methylcellulose containing 1.2% citric acid, or 0.5% methylcellulose containing 1.2% citric acid alone, was orally administered to fasted adult castrated cynomolgus monkeys (6−11 years old, n = 3). Blood samples (heparin plasma) were collected from a femoral vein without anesthesia at 0, 1, 2, 4, 8, 24, and 48 h after administration. LH concentrations in the plasma were measured by bioassays using mouse testicular cells.[11a]

Molecular Modeling Studies. Molecular modeling was carried out using the Insight II software package (Accelrys, San Diego, CA) unless otherwise stated. A homology model of the human GnRH receptor was constructed using the crystal structure of bovine rhodopsin (PDB code 1F88) as a template. The initial binding mode of **1** was modeled by referring to the binding mode of a similar compound as previously reported,[11a] and the complex structure between **1** and the human GnRH receptor was subjected to energy minimization.

The urea part of **1** seems to participate in the hydrogen bonding interactions with the side chain of Asn102 (transmembrane 2, TM2) and the main chain amides of Gln106 and Trp107 (extracellular loop 1, ECL1) as depicted in Figure 2. All potential hydrogen bonding functionalities of the urea moiety are utilized for intermolecular hydrogen bonding. Since Asn102 of TM2 was reported as the binding site for the C-terminal amide of LHRH based on mutagenesis of the human receptor,[24] the urea part of **1** might be a mimic of the C-terminal part of GnRH. The carbonyl group at the opposite position of the difluorobenzyl moiety in the six-membered ring is located within hydrogen bonding distance of the Ser194 side chain of ECL2. Considering the importance of the carbonyl group at this site, hydrogen bonding interaction with Ser194 might be critical for the tight binding of **1** with the human LHRH receptor.

■ ASSOCIATED CONTENT

Supporting Information. Elemental analysis data and HPLC results for compounds **6a**−**h**, **15a**−**j**, and **16a**,**b**; enzyme and radioligand binding assays. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +81-6-6300-6283. Fax: +81-6-6300-6312. E-mail: Miwa_Kazuhiro@takeda.co.jp.

■ ACKNOWLEDGMENT

We thank Dr. Keiji Kamiyama, Dr. Akihiro Tasaka, Dr. Nobuhiro Suzuki, and Tatsuya Watanabe for encouragement and helpful discussions throughout this work. We also acknowledge Tomohiro Kaku and Dr. Kenichi Miyata for supporting the synthetic work, and Dr. Shiro Takekawa, Dr. Masataka Harada, Yumiko Akinaga, Tomoko Kaisho, Mari Asada, Tomoko Urushibara, Koichi Iida, Shinichi Niwa, Dr. Yukihiro Ikeda, Dr. Takayasu Ito, and Junzo Takahashi for supporting the biochemical and pharmacological experiments.

■ ABBREVIATIONS USED

GnRH, gonadotropin-releasing hormone; LHRH, luteinizing hormone-releasing hormone; FBS, fetal bovine serum; CYP, cytochrome P450; GPCR, G-protein-coupled receptor; AA, arachidonic acid; CHO, Chinese hamster ovary; LH, luteinizing hormone; FSH, follicle-stimulating hormone; SAR, structure−activity relationship; NBS, *N*-bromosuccinimide; AIBN, 2,2′-azobis(isobutyronitrile); CDI, 1,1′-carbonyldiimidazole; DEPC, diethyl phosphorocyanidate

■ REFERENCES

(1) (a) Schally, A. V.; Arimura, A.; Kastin, A. J.; Matsuo, H.; Baba, Y.; Redding, T. W.; Nair, R. M. G.; Debeljuk, L.; White, W. F. Gonadotropin-Releasing Hormone: One Polypeptide Regulates Secretion of Luteinizing and Follicle-Stimulating Hormones. *Science* **1971**, *173*, 1036–1038. (b) Matsuo, H.; Baba, Y.; Nair, R. M. G.; Arimura, A.; Schally, A. V. Structure of the Porcine LH- and FSH-Releasing Hormone. I. The Proposed Amino Acid Sequence. *Biochem. Biophys. Res. Commun.* **1971**, *43*, 1334–1339.

(2) (a) Sealfon, S. C.; Weinstein, H.; Millar, R. P. Molecular Mechanisms of Ligand Interaction with the Gonadotropin-Releasing Hormone Receptor. *Endocr. Rev.* **1997**, *18*, 180–205. (b) Millar, R. P.; Lu, Z.-L.; Pawson, A. J.; Flanagan, C. A.; Morgan, K.; Maudsley, S. R. Gonadotropin-Releasing Hormone Receptors. *Endocr. Rev.* **2004**, *25*, 235–275.

(3) (a) Kaiser, U. B.; Conn, P. M.; Chin, W. W. Studies of Gonadotropin-Releasing Hormone (GnRH) Action Using GnRH Receptor-Expressing Pituitary Cell Lines. *Endocr. Rev.* **1997**, *18*, 46–70. (b) Cheng, K. W.; Leung, P. C. K. The Expression, Regulation and Signal Transduction Pathways of the Mammalian Gonadotropin-Releasing Hormone Receptor. *Can. J. Physiol. Pharmacol.* **2000**, *78*, 1029–1052.

(4) Fujino, M.; Fukuda, T.; Shinagawa, S.; Kobayashi, S.; Yamazaki, I.; Nakamura, R.; Seely, J. H.; White, W. F.; Rippel, R. H. Synthetic Analogs of Luteinizing Hormone Releasing Hormone (LH-RH) Substituted in Position 6 and 10. *Biochem. Biophys. Res. Commun.* **1974**, *60*, 406–413.

(5) (a) Belchetz, P. E.; Plant, T. M.; Nakai, Y.; Keogh, E. J.; Knobil, E. Hypophysial Responses to Continuous and Intermittent Delivery of Hypopthalamic Gonadotropin-Releasing Hormone. *Science* **1978**, *202*, 631–633. (b) Conn, P. M.; Crowley, W. F., Jr. Gonadotropin-Releasing Hormone and Its Analogues. *N. Engl. J. Med.* **1991**, *324*, 93–103.

(6) Filicori, M.; Flamigni, C. GnRH Agonists and Antagonists: Current Clinical Status. *Drugs* **1988**, *35*, 63–82.

(7) (a) Karten, M. J.; Rivier, J. E. Gonadotropin-Releasing Hormone Analog Design. Structure−Function Studies toward the Development of Agonists and Antagonists: Rationale and Perspective. *Endocr. Rev.* **1986**, *7*, 44–66. (b) Dutta, A. S. Luteinizing Hormone-Releasing Hormone (LHRH) Agonists. *Drugs Future* **1988**, *13*, 43–57.

(8) (a) Blithe, D. L. Applications for GnRH Antagonists. *Trends Endocrinol. Metab.* **2001**, *12*, 238–240. (b) Huirne, J. A. F.; Lambalk, C. B. Gonadotropin-Releasing-Hormone-Receptor Antagonists. *Lancet* **2001**, *358*, 1793–1803. (c) Nagaraja, N. V.; Pechstein, B.; Erb, K.; Klipping, C.; Hermann, R.; Locher, M.; Derendorf, H. Pharmacokinetic/Pharmacodynamic Modeling of Luteinizing Hormone (LH) Suppression and LH Surge Delay by Cetrorelix after Single and Multiple Doses in Healthy Premenopausal Women. *J. Clin. Pharmacol.* **2003**, *43*, 243–251. (d) Schultze-Mosgau, A.; Griesinger, G.; Altgassen, C.; Otte, S.; von; Hornung, D.; Diedrich, K. New Developments in the Use of Peptide Gonadotropin-Releasing Hormone Antagonists versus Agonists. *Expert Opin. Invest. Drugs* **2005**, *14*, 1085–1097.

(9) (a) Zhu, Y.-F.; Chen, C. Recent Advances in Small Molecule Gonadotropin-Releasing Hormone Receptor Antagonists. *Expert Opin. Ther. Pat.* **2004**, *14*, 187–199. (b) Sarma, P. K. S.; Tiwari, A.; Kondaskar, A.; Cliffe, I. A. Peptidomimetic GnRH Receptor Antagonists for the Treatment of Reproductive and Proliferative Diseases. *Expert Opin. Ther. Pat.* **2006**, *16*, 733–751. (c) Msaouel, P.; Diamanti, E.; Tzanela, M.; Koutsilieris, M. Luteinising Hormone-Releasing Hormone Antagonists in Prostate Cancer Therapy. *Expert Opin. Emerging Drugs* **2007**, *12*, 285–299.

(10) Zhu, Y.-F.; Chen, C.; Struthers, R. S. Nonpeptide Gonadotropin Releasing Hormone Antagonists. *Annu. Rep. Med. Chem.* **2004**, *39*, 99–110.

(11) (a) Cho, N.; Harada, M.; Imaeda, T.; Imada, T.; Matsumoto, H.; Hayase, Y.; Sasaki, S.; Furuya, S.; Suzuki, N.; Okubo, S.; Ogi, K.; Endo, S.; Onda, H.; Fujino, M. Discovery of a Novel, Potent, and Orally Active Nonpeptide Antagonist of the Human Luteinizing Hormone-Releasing Hormone (LHRH) Receptor. *J. Med. Chem.* **1998**, *41*, 4190–4195. (b) Sasaki, S.; Cho, N.; Nara, Y.; Harada, M.; Endo, S.; Suzuki, N.; Furuya, S.; Fujino, M. Discovery of a Thieno[2,3-*d*] pyrimidine-2,4-dione Bearing a *p*-Methoxyureidophenyl Moiety at the 6-Posision: A Highly Potent and Orally Bioavailable Non-Peptide Antagonist for the Human Luteinizing Hormone-Releasing Hormone Receptor. *J. Med. Chem.* **2003**, *46*, 113–124. (c) Imada, T.; Cho, N.; Imaeda, T.; Hayase, Y.; Sasaki, S.; Kasai, S.; Harada, M.; Matsumoto, H.; Endo, S.; Suzuki, N.; Fuyura, S. Design, Synthesis, and Structure−Activity Relationships of Thieno[2,3-*b*]pyridin-4-one Derivatives as a Novel Class of Potent, Orally Active, Non-Peptide Luteinizing Hormone-Releasing Hormone Receptor Antagonists. *J. Med. Chem.* **2006**, *49*, 3809–3825. (d) Sasaki, S.; Imaeda, T.; Hayase, Y.; Shimizu, Y.; Kasai, S.; Cho, N.; Harada, M.; Suzuki, N.; Furuya, S.; Fujino, M. A New Class of Potent Nonpeptide Luteinizing Hormone-Releasing Hormone (LHRH) Antagonists: Design and Synthesis of 2-Phenylimidazo[1,2-*a*]-pyrimidin-5-ones. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2073–2077.

(12) (a) Zhu, Y.-F.; Struthers, R. S.; Connors, P. J., Jr.; Gao, Y.; Gross, T. D.; Saunders, J.; Wilcoxen, K.; Reinhart, G. J.; Ling, N.; Chen, C. Initial Structure−Activity Relationship Studies of a Novel Series of Pyrrolo[1,2-*a*]pyrimid-7-ones as GnRH Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 399–402. (b) Zhu, Y.-F.; Wilcoxen, K.; Saunders, J.; Guo, Z.; Gao, Y.; Connors, P. J., Jr.; Gross, T. D.; Tucci, F. C.; Struthers, R. S.; Reinhart, G. J.; Xie, Q.; Chen, C. A Novel Synthesis of 2-Arylpyrrolo[1,2-*a*]pyrimid-7-ones and Their Structure−Activity Relationships as Potent GnRH Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 403–406. (c) Wilcoxen, K. M.; Zhu, Y.-F.; Connors, P. J., Jr.; Saunders, J.; Gross, T. D.; Gao, Y.; Reinhart, G. J.; Struthers, R. S.; Chen, C. Synthesis and Initial Structure-Activity Relationships of a Novel Series of Imidazolo[1,2-*a*]pyrimid-5-ones as Potent GnRH Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2179–2183. (d) Gross, T. D.; Zhu, Y.-F.; Saunders, J.; Wilcoxen, K. M.; Gao, Y.; Connors, P. J., Jr.; Guo, Z.; Struthers, R. S.; Reinhart, G. J.; Chen, C. Design, Synthesis and Structure−Activity Relationships of Novel Imidazolo[1,2-*a*]pyrimid-5-ones as Potent GnRH Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2185–2187. (e) Tucci, F. C.; Zhu, Y.-F.; Guo, Z.; Gross, T. D.; Connors, P. J., Jr.; Struthers, R. S.; Reinhart, G. J.; Wang, X.; Saunders, J.; Chen, C. A Novel Synthesis of 7-Aryl-8-fluoro-pyrrolo[1,2-*a*]pyrimid-4-ones as Potent Stable GnRH Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3491–3495. (f) Zhu, Y.-F.; Guo, Z.; Gross, T. D.; Gao, Y.; Connors, P. J., Jr.; Struthers, R. S.; Xie, Q.; Tucci, F. C.; Reinhart, G. J.; Wu, D.; Saunders, J.; Chen, C. Design and Structure−Activity Relationships of 2-Alkyl-3-aminomethyl-6-(3-methoxyphenyl)-7-methyl-8-(2-fluorobenzyl)imidazolo[1,2-*a*]pyrimid-5-ones as Potent GnRH Receptor Antagonists. *J. Med. Chem.* **2003**, *46*, 1769–1772. (g) Zhu, Y.-F.; Gross, T. D.; Guo, Z.; Connors, P. J., Jr.; Gao, Y.; Tucci, F. C.; Struthers, R. S.; Reinhart, G. J.; Saunders, J.; Chen, T. K.; Bonneville, A. L. K.; Chen, C. Identification of 1-Arylmethyl-3-(2-aminoethyl)-5-aryluracil as Novel Gonadotropin-Releasing Hormone Receptor Antagonists. *J. Med. Chem.* **2003**, *46*, 2023–2026. (h) Guo, Z.; Zhu, Y.-F.; Tucci, F. C.; Gao, Y.; Struthers, R. S.; Saunders, J.; Gross, T. D.; Xie, Q.; Reinhart, G. J.; Chen, C. Synthesis and Structure−Activity Relationships of 1-Arylmethyl-3-(2-aminopropyl)-5-aryl-6-methyluracils as Potent GnRH Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3311–3315. (i) Tucci, F. C.; Zhu, Y.-F.; Guo, Z.; Gross, T. D.; Connors, P. J., Jr.; Struthers, R. S.; Reinhart, G. J.; Saunders, J.; Chen, C. Synthesis and Structure−Activity Relationships of 1-Arylmethyl-3-(1-methyl-2-amino)ethyl-5-aryl-6-methyluracils as Antagonists of the Human GnRH Receptor. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3317–3322. (j) Guo, Z.; Chen, Y.; Wu, D.; Zhu, Y.-F.; Struthers, R. S.; Saunders, J.; Xie, Q.; Chen, C. Synthesis and Structure−Activity Relationships of Thieno-[2,3-*d*]pyrimidine-2,4-dione Derivatives as Potent GnRH Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3617–3622. (k) Guo, Z.; Zhu, Y.-F.; Gross, T. D.; Tucci, F. C.; Gao, Y.; Moorjani, M.; Connors, P. J., Jr.; Rowbottom, M. W.; Chen, Y.; Struthers, R. S.; Xie, Q.; Saunders, J.; Reinhart, G.; Chen, T. K.; Bonneville, A. L. K.; Chen, C. Synthesis and Structure−Activity Relationships of 1-Arylmethyl-5-aryl-6-methyluracils as Potent Gonadotropin-Releasing Hormone Receptor Antagonists. *J. Med. Chem.* **2004**, *47*, 1259–1271. (l) Tucci, F. C.; Zhu, Y.-F.; Guo, Z.; Gross, T. D.; Connors, P. J., Jr.; Gao, Y.; Rowbottom, M. W.; Struthers, R. S.; Reinhart, G. J.; Xie, Q.; Chen, T. K.; Bozigian, H.; Bonneville, A. L. K.; Fisher, A.; Jin, L.; Saunders, J.; Chen, C. 3-(2-Aminoalkyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-6-methyluracils as Orally Bioavailable Antagonists of the Human Gonadotropin Releasing Hormone Receptor. *J. Med. Chem.* **2004**, *47*, 3483–3486. (m) Rowbottom, M. W.; Tucci, F. C.; Zhu, Y.-F.; Guo, Z.; Gross, T. D.; Reinhart, G. J.; Xie, Q.; Struthers, R. S.; Saunders, J.; Chen, C. Synthesis and structure−activity relationships of (*R*)-1-alkyl-3-[2-(2-amino)phenethyl]-5-(2-fluorophenyl)-6-methyluracils as human GnRH receptor antagonists. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2269–2274. (n) Rowbottom, M. W.; Tucci, F. C.; Connors, P. J., Jr.; Gross, T. D.; Zhu, Y.-F.; Guo, Z.; Moorjani, M.; Acevedo, O.; Carter, L.; Sullivan, S. K.; Xie, Q.; Fisher, A.; Struthers, R. S.; Saunders, J.; Chen, C. Synthesis and Structure−Activity Relationships of Uracil Derived Human GnRH Receptor Antagonists: (*R*)-3-[2-(2-Amino)phenethyl]-1-(2,6-difluorobenzyl)-6-methyluracils

Containing a Substituted Thiophene or Thiazole at C-5. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4967–4973. (o) Tucci, F. C.; Zhu, Y.-F.; Struthers, R. S.; Guo, Z.; Gross, T. D.; Rowbottom, M. W.; Acevedo, O.; Gao, Y.; Saunders, J.; Xie, Q.; Reinhart, G. J.; Liu, X.-J.; Ling, N.; Bonneville, A. K. L.; Chen, T.; Bozigian, H.; Chen, C. 3-[(2*R*)-Amino-2-phenylethyl]-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-6-methylpyrimidin-2,4-dione (NBI 42902) as a Potent and Orally Active Antagonist of the Human Gonadotropin-Releasing Hormone Receptor. Design, Synthesis, and in Vitro and in Vivo Characterization. *J. Med. Chem.* **2005**, *48*, 1169–1178. (p) Guo, Z.; Wu, D.; Zhu, Y.-F.; Tucci, F. C.; Pontillo, J.; Saunders, J.; Xie, Q.; Struthers, R. S.; Chen, C. A Convenient One-Pot Synthesis of Asymmetric 1,3,5-Triazine-2,4,6-triones and Its Application towards a Novel Class of Gonadotropin-Releasing Hormone Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 693–698. (q) Pontillo, J; Chen, C. Efficient Synthesis of Bicyclic Oxazolino- And Thiazolino[3,2-*c*]pyrimidine-5,7-diones and Its Application to the Synthesis of GnRH Antagonists. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1407–1411. (r) Guo, Z; Chen, Y.; Huang, C. Q.; Gross, T. D.; Pontillo, J.; Rowbottom, M. W.; Saunders, J.; Struthers, S.; Tucci, F. C.; Xie, Q.; Wade, W.; Zhu, Y.-F.; Wu, D.; Chen, C. Uracils as Potent Antagonists of the Human Gonadotropin-Releasing Hormone Receptor without Atropisomers. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2519–2522. (s) Guo, Z.; Wu, D.; Zhu, Y.-F.; Tucci, F. C.; Regan, C. F.; Rowbottom, M. W.; Struthers, R. S.; Xie, Q.; Reijmers, S; Sullivan, S. K.; Sai, Y.; Chen, C. Structure−Activity Relationships of 1,3,5-Triazine-2,4,6-triones as Human Gonadotropin-Releasing Hormone Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3685–3690. (t) Pontillo, J.; Guo, Z.; Wu, D.; Struthers, R. S.; Chen, C. Synthesis of Aryl-1,2,4-triazine-3,5-diones as Antagonists of the Gonadotropin-Releasing Hormone Receptor. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4363–4366. (u) Chen, M.; Guo, Z.; Lanier, M. C.; Zhao, L.; Betz, S. F.; Huang, C. Q.; Loweth, C. J.; Ashweek, N. J.; Liu, X.-J.; Struthers, R. S.; Bradbury, M. J.; Behan, J. W.; Wen, J.; O'Brien, Z.; Saunders, J.; Zhu, Y.-F. Identification of 2-(4,5,6,7-Tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridin-3-yl)-ethylamine Derivatives As Novel GnRH Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3845–3850. (v) Lanier, M. C.; Feher, M.; Ashweek, N. J.; Loweth, C. J.; Rueter, J. K.; Slee, D. H.; Williams, J. P.; Zhu, Y.-F.; Sullivan, S. K.; Brown, M. S. Selection, Synthesis, and Structure−Activity Relationship of Tetrahydropyrido[4,3-*d*]pyrimidine-2,4-diones as Human GnRH Receptor Antagonists. *Bioorg. Med. Chem.* **2007**, *15*, 5590–5603. (w) Betz, S. F.; Zhu, Y.-F.; Chen, C.; Struthers, R. S. Non-Peptide Gonadotropin-Releasing Hormone Receptor Antagonists. *J. Med. Chem.* **2008**, *51*, 3331–3348. (x) Chen, C.; Chen, Y.; Pontillo, J.; Guo, Z.; Huang, C. Q.; Wu, D.; Madan, A.; Chen, T.; Wen, J.; Xie, Q.; Tucci, F. C.; Rowbottom, M.; Zhu, Y.-F.; Wade, W.; Saunders., J; Bozigian, H.; Struthers, R. S. Potent and Orally Bioavailable Zwitterion GnRH Antagonists with Low CYP3A4 Inhibitory Activity. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3301–3305. (y) Zhao, L.; Guo, Z.; Chen, Y.; Hu, T.; Wu, D.; Zhu, Y.-F.; Rowbottom, M.; Gross, T. D.; Tucci, F. C.; Struthers, R. S.; Xie, Q.; Chen, C. 5-Aryluracils as Potent GnRH Antagonists: Characterization of Atropisomers. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3344–3349. (z) Regan, C. F.; Guo, Z.; Chen, Y.; Huang, C. Q.; Chen, M.; Jiang, W.; Rueter, J. K.; Coon, T.; Chen, C.; Saunders, J.; Brown, M. S.; Betz, S. F.; Struthers, R. S.; Yang, C.; Wen, J.; Madan, A.; Zhu, Y.-F. Zwitterionic Uracil Derivatives as Potent GnRH Receptor Antagonists with Improved Pharmaceutical Properties. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4503–4507.

(13) Chen, C.; Wu, D.; Guo, Z.; Xie, Q.; Reinhart, G. J.; Madan, A.; Wen, J.; Chen, T.; Huang, C. Q.; Chen, M.; Chen, Y.; Tucci, F. C.; Rowbottom, M.; Pontillo, J.; Zhu, Y.-F.; Wade, W.; Saunders, J.; Bozigian, H.; Struthers, R. S. Discovery of Sodium *R*-(+)-4-{2-[5-(2-Fluoro-3-methoxyphenyl)-3-(2-fluoro-6-[trifluoromethyl]benzyl)-4-methyl-2,6-dioxo-3,6-dihydro-2*H*-pyrimidin-1-yl]-1-phenylethylamino}butyrate (Elagolix), a Potent and Orally Available Nonpeptide Antagonist of the Human Gonadotropin-Releasing Hormone Receptor. *J. Med. Chem.* **2008**, *51*, 7478–7485.

(14) (a) DeVita, R. J.; Hollings, D. D.; Goulet, M. T.; Wyvratt, M. J.; Fisher, M. H.; Lo, J.-L; Yang, Y. T.; Cheng, K.; Smith, R. G. Identification and Initial Structure−Activity Relationships of a Novel Non-Peptide Quinolone GnRH Receptor Antagonist. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2615–2620. (b) DeVita, R. J.; Goulet, M. T.; Wyvratt, M. J.; Fisher, M. H.; Lo, J.-L; Yang, Y. T.; Cheng, K.; Smith, R. G. Investigation of the 4-*O*-Alkylamine Substituent of Non-Peptide Quinolone GnRH Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2621–2624. (c) Walsh, T. F.; Toupence, R. B.; Young, J. R.; Huang, S. X.; Ujjainwalla, F.; DeVita, R. J.; Goulet, M. T.; Wyvratt, M. J., Jr.; Fisher, M. H.; Lo, J.-L; Ren, N.; Yudkovitz, J. B.; Yang, Y. T.; Cheng, K.; Smith, R. G. Potent Antagonists of Gonadotropin Releasing Hormone Receptors Derived from Quinolone-6-Carboxamides. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 443–447. (d) Young, J. R.; Huang, S. X.; Chen, I.; Walsh, T. F.; DeVita, R. J.; Wyvratt, M. J., Jr.; Goulet, M. T.; Ren, N.; Lo, J.; Yang, Y. T.; Yudkovitz, J. B.; Cheng, K.; Smith, R. G. Quinolones as Gonadotropin Releasing Hormone (GnRH) Antagonists: Simultaneous Optimization of the C(3)-Aryl and C(6)-Substituents. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1723–1727. (e) DeVita, R. J.; Walsh, T. F.; Young, J. R.; Jiang, J.; Ujjainwalla, F.; Toupence, R. B.; Parikh, M.; Huang, S. X.; Fair, J. A.; Goulet, M. T.; Wyvratt, M. J.; Lo, J.-L.; Ren, N.; Yudkovitz, J. B.; Yang, Y. T.; Cheng, K.; Cui, J.; Mount, G.; Rohrer, S. P.; Schaeffer, J. M.; Rhodes, L.; Drisko, J. E.; McGowan, E.; MacIntyre, D. E.; Vincent, S.; Carlin, J. R.; Cameron, J.; Smith, R. G. A Potent, Nonpeptidyl 1*H*-Quinolone Antagonist for the Gonadotropin-Releasing Hormone Receptor. *J. Med. Chem.* **2001**, *44*, 917–922. (f) Chu, L.; Hutchins, J. E.; Weber, A. E.; Lo, J.-L; Yang, Y.-T.; Cheng, K.; Smith, R. G.; Fisher, M. H.; Wyvratt, M. J.; Goulet, M. T. Initial Structure−Activity Relationship of a Novel Class of Nonpeptidyl GnRH Receptor Antagonists: 2-Arylindoles. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 509–513. (g) Chu, L.; Lo, J.-L; Yang, Y.-T.; Cheng, K.; Smith, R. G.; Fisher, M. H.; Wyvratt, M. J.; Goulet, M. T. SAR Studies of Novel 5-Substituted 2-Arylindoles as Nonpeptidyl GnRH Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 515–517. (h) Lin, P.; Marino, D.; Lo, J.-L; Yang, Y. T.; Cheng, K.; Smith, R. G.; Fisher, M. H.; Wyvratt, M. J.; Goulet, M. T. 2-(3,5-Dimethylphenyl)tryptamine Derivatives That Bind to the GnRH Receptor. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1073–1076. (i) Lin, P.; Parikh, M.; Lo, J.-L; Yang, Y. T.; Cheng, K.; Smith, R. G.; Fisher, M. H.; Wyvratt, M. J.; Goulet, M. T. Heterocyclic Derivatives of 2-(3,5-Dimethylphenyl)tryptamine as GnRH Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1077–1080. (j) Ashton, W. T.; Sisco, R. M.; Yang, Y. T.; Lo, J.-L; Yudkovitz, J. B.; Cheng, K.; Goulet, M. T. Substituted Indole-5-carboxamides and -acetamides as Potent Nonpeptide GnRH Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1723–1726. (k) Ashton, W. T.; Sisco, R. M.; Yang, Y. T.; Lo, J.-L; Yudkovitz, J. B.; Gibbons, P. H.; Mount, G. R.; Ren, R. N.; Butler, B. S.; Cheng, K.; Goulet, M. T. Potent Nonpeptide GnRH Receptor Antagonists Derived from Substituted Indole-5-carboxamides and -acetamides Bearing a Pyridine Side-Chain Terminus. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1727–1731. (l) Ashton, W. T.; Sisco, R. M.; Kieczykowski, G. R.; Yang, Y. T.; Yudkovitz, J. B.; Cui, J.; Mount, G. R.; Ren, R. N.; Wu, T.-J.; Shen, X.; Lyons, K. A.; Mao, A.-H.; Carlin, J. R.; Karanam, B. V.; Vincent, S. H.; Cheng, K.; Goulet, M. T. Orally Bioavailable, Indole-Based Nonpeptide GnRH Receptor Antagonists with High Potency and Functional Activity. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2597–2602. (m) Young, J. R.; Huang, S. X.; Walsh, T. F.; Wyvratt, M. J., Jr.; Yang, Y. T.; Yudkovitz, J. B.; Cui, J.; Mount, G. R.; Ren, R. N.; Wu, T.-J.; Shen, X.; Lyons, K. A.; Mao, A.-H.; Carlin, J. R.; Karanam, B. V.; Vincent, S. H.; Cheng, K.; Goulet, M. T. 2-Arylindoles as Gonadotropin Releasing Hormone (GnRH) Antagonists: Optimization of the Tryptamine Side Chain. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 827–832. (n) Simeone, J. P.; Bugianesi, R. L.; Ponpipom, M. M.; Yang, Y. T.; Lo, J.-L.; Yudkovitz, J. B.; Cui, J.; Mount, G. R.; Ren, R. N.; Creighton, M.; Mao, A.-H.; Vincent, S. H.; Cheng, K.; Goulet, M. T. Modification of the Pyridine Moiety of Non-Peptidyl Indole GnRH Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3329–3332. (o) Jiang, J.; DeVita, R. J.; Goulet, M. T.; Wyvratt, M. J.; Lo, J.-L.; Ren, N.; Yudkovitz, J. B.; Cui, J.; Yang, Y. T.; Cheng, K.; Rohrer, S. P. Syntheses and Structure−Activity Relationship Studies of Piperidine-Substituted Quinolones as Nonpeptide Gonadotropin Releasing Hormone Antagonists. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1795–1798. (p) DeVita, R. J.; Parikh, M.; Jiang, J.; Fair, J. A.; Young, J. R.; Walsh, T. F.; Goulet, M. T.; Lo, J.-L.;

Ren, N.; Yudkovitz, J. B.; Cui, J.; Yang, Y. T.; Cheng, K.; Rohrer, S. P; Wyvratt, M. J. Identification of Neutral 4-*O*-Alkyl Quinolone Nonpeptide GnRH Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5599–5603.

(15) (a) Luthin, D. R.; Hong, Y.; Pathak, V. P.; Paderes, G.; Nared-Hood, K. D.; Castro, M. A.; Vazir, H.; Li, H.; Tompkins, E.; Christie, L.; May, J. M.; Anderson, M. B. The Discovery of Novel Small Molecule Non-Peptide Gonadotropin Releasing Hormone (GnRH) Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3467–3470. (b) Luthin, D. R.; Hong, Y.; Tompkins, E.; Anderes, K. L.; Paderes, G.; Kraynov, E. A.; Castro, M. A.; Nared-Hood, K. D.; Castillo, R.; Gregory, M.; Vazir, H.; May, J. M.; Anderson, M. B. Characterization of Mono- and Diaminopyrimidine Derivatives as Novel, Nonpeptide Gonadotropin Releasing Hormone (GnRH) Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3635–3639. (c) Anderes, K. L.; Luthin, D. R.; Castillo, R.; Kraynov, E. A.; Castro, M.; Nared-Hood, K.; Gregory, M. L.; Pathak, V. P.; Christie, L. C.; Paderes, G.; Vazir, H.; Ye, Q.; Anderson, M. B; May, J. M. Biological Characterization of a Novel, Orally Active Small Molecule Gonadotropin-Releasing Hormone (GnRH) Antagonist Using Castrated and Intact Rats. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 688–695. (d) Li, H.; Anderes, K. L.; Kraynov, E. A.; Luthin, D. R.; Do, Q.-Q.; Hong, Y.; Tompkins, E.; Sun, E. T.; Rajapakse, R.; Pathak, V. P.; Christie, L. C.; Vazir, H.; Castillo, R.; Gregory, M. L.; Castro, M.; Nared-Hood, K.; Paderes, G.; Anderson, M. B. Discovery of a Novel, Orally Active, Small Molecule Gonadotropin-Releasing Hormone (GnRH) Receptor Antagonist. *J. Med. Chem.* **2006**, *49*, 3362–3367.

(16) (a) Hashimoto, K.; Tatsuta, M.; Kataoka, M.; Yasoshima, K.; Shogase, Y.; Shimazaki, M.; Yura, T.; Li, Y.; Yamamoto, N.; Gupta, J. B.; Urbahns, K. Benzimidazole Derivatives as Novel Nonpeptide Luteinizing Hormone-Releasing Hormone (LHRH) Antagonists. Part 1: Benzimidazole-5-sulfonamides. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 799–803. (b) Li, Y.; Kataoka, M.; Tatsuta, M.; Yasoshima, K.; Yura, T.; Urbahns, K; Kiba, A.; Yamamoto, N.; Gupta, J. B.; Hashimoto, K. Benzimidazole Derivatives As Novel Nonpeptide Luteinizing Hormone-Releasing Hormone (LHRH) Antagonists. Part 2: Benzimidazole-5-sulfonamides. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 805–807. (c) Tatsuta, M.; Kataoka, M.; Yasoshima, K.; Sakakibara, S.; Shogase, Y.; Shimazaki, M.; Yura, T.; Li, Y.; Yamamoto, N.; Gupta, J.; Urbahns, K. Benzimidazoles as Non-Peptide Luteinizing Hormone-Releasing Hormone (LHRH) Antagonists. Part 3: Discovery of 1-(1*H*-benzimidazol-5-yl)-3-*tert*-butylurea Derivatives. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2265–2269.

(17) (a) Pelletier, J. C.; Chengalvala, M.; Cottom, J.; Feingold, I.; Garrick, L.; Green, D.; Hauze, D.; Huselton, C.; Jetter, J.; Kao, W.; Kopf, G. S.; Lundquist, J. T., IV; Mann, C.; Mehlmann, J.; Rogers, J.; Shanno, L.; Wrobel, J. 2-Phenyl-4-piperazinylbenzimidazoles: Orally Active Inhibitors of the Gonadotropin Releasing Hormone (GnRH) Receptor. *Bioorg. Med. Chem.* **2008**, *16*, 6617–6640. (b) Green, D. M.; Goljer, I.; Andraka, D. S.; Chengalvala, M.; Shanno, L.; Hurlburt, W.; Pelletier, J. C. Parallel Synthesis of 2-Aryl-4-aminobenzimidazoles and Their Evaluation as Gonadotropin Releasing Hormone Antagonists. *J. Comb. Chem.* **2009**, *11*, 117–125. (c) Pelletier, J. C.; Chengalvala, M. V.; Cottom, J. E.; Feingold, I. B.; Green, D. M.; Hauze, D. B.; Huselton, C. A.; Jetter, J. W.; Kopf, G. S.; Lundquist, J. T., IV; Magolda, R. L.; Mann, C. W.; Mehlmann, J. F.; Rogers, J. F.; Shanno, L. K.; Adams, W. R.; Tio, C. O.; Wrobel, J. E. Discovery of 6-({4-[2-(4-*tert*-Butylphenyl)-1*H*-benzimidazol-4-yl]piperazin-1-yl}methyl)quinoxaline (WAY-207024): An Orally Active Antagonist of the Gonadotropin Releasing Hormone Receptor (GnRH-R). *J. Med. Chem.* **2009**, *52*, 2148–2152. (d) Hauze, D. B.; Chengalvala, M. V.; Cottom, J. E.; Feingold, I. B.; Garrick, L.; Green, D. M.; Huselton, C.; Kao, W.; Kees, K.; Lundquist, J. T., IV; Mann, C. W.; Mehlmann, J. F.; Rogers, J. F.; Shanno, L.; Wrobel, J.; Pelletier, J. C. Small Molecule Antagonists of the Gonadotropin-Releasing Hormone (GnRH) Receptor: Structure−Activity Relationships of Small Heterocyclic Groups Appended to the 2-Phenyl-4-piperazinyl-benzimidazole Template. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1986–1990. (e) Vera, M. D.; Lundquist, J. T., IV; Chengalvala, M. V.; Cottom, J. E.; Feingold, I. B.; Garrick, L. M.; Green, D. M.; Hauze, D. B.; Mann, C. W.; Mehlmann, J. F.; Rogers, J. F.; Shanno, L.; Wrobel, J. E.; Pelletier, J. C. Synthesis and Biological Evaluation of Piperazinyl Heterocyclic Antagonists of the Gonadotropin Releasing Hormone (GnRH) Receptor. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2512–2515.

(18) Arnould, J. C.; Delouvrié, B.; Boutron, P.; Dossetter, A. G.; Foote, K. M.; Hamon, A.; Hancox, U.; Harris, C. S.; Hutton, M.; Lamorlette, M.; Matusiak, Z. Synthesis and Structure−Activity Relationships of Thieno-[2,3-*b*]pyrroles as Antagonists of the GnRH Receptor. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6448–6454.

(19) (a) Randolph, J. T.; Waid, P.; Nichols, C.; Sauer, D.; Haviv, F.; Diaz, G.; Bammert, G.; Besecke, L. M.; Segreti, J. A.; Mohning, K. M.; Bush, E. N.; Wegner, C. D.; Greer, J. Nonpeptide Luteinizing Hormone-Releasing Hormone Antagonists Derived from Erythromycin A: Design, Synthesis, and Biological Activity of Cladinose Replacement Analogues. *J. Med. Chem.* **2004**, *47*, 1085–1097. (b) Randolph, J. T.; Sauer, D. R.; Haviv, F.; Nilius, A. M.; Greer, J. Elimination of Antibacterial Activities of Non-Peptide Luteinizing Hormone-Releasing Hormone (LHRH) Antagonists Derived from Erythromycin A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1599–1602.

(20) Hara, T.; Araki, H.; Kusaka, M.; Harada, M.; Cho, N.; Suzuki, N.; Furuya, S.; Fujino, M. Suppression of a Pituitary−Ovarian Axis by Chronic Oral Administration of a Novel Nonpeptide Gonadotropin-Releasing Hormone Antagonist, TAK-013, in Cynomolgus Monkeys. *J. Clin. Endocrinol. Metab.* **2003**, *88*, 1697–1704.

(21) Betz, S. F.; Lio, F. M.; Gao, Y.; Reinhart, G. J.; Guo, Z.; Mesleh, M. F.; Zhu, Y.-F.; Struthers, R. S. Determination of the Binding Mode of Thienopyrimidinedione Antagonists to the Human Gonadotropin Releasing Hormone Receptor Using Structure−Activity Relationships, Site-Directed Mutagenesis, and Homology Modeling. *J. Med. Chem.* **2006**, *49*, 6170–6176.

(22) Yamagami, C.; Ogura, T.; Takao, N. Hydrophobicity Parameters Determined by Reversed-Phase Liquid Chromatography I. Relationship between Capacity Factors and Octanol−Water Partition Coefficients for Monosubstituted Pyrazines and the Related Pyridines. *J. Chromatogr.* **1990**, *514*, 123–136.

(23) Masuda, Y.; Sugo, T.; Kikuchi, T.; Kawata, A.; Satoh, M.; Fujisawa, Y.; Itoh, Y.; Wakimasu, M.; Ohtaki, T. Receptor Binding and Antagonist Properties of a Novel Endothelin Receptor Antagonist, TAK-044 {Cyclo[D-α-aspartyl-3-[(4-phenylpiperazin-1-yl)carbonyl]-L-alanyl-L-α-aspartyl-D-2-(2-thienyl)-glycyl-L-leucyl-D-tryptophyl]disodium Salt}, in Human Endothelin$_A$ and Endothelin$_B$ Receptors. *J. Pharmacol. Exp. Ther.* **1996**, *279*, 675–685.

(24) Davidson, J. S.; McArdle, C. A.; Davies, P.; Elario, R.; Flanagan, C. A.; Millar, R. P. Asn^{102} of the Gonadotropin-Releasing Hormone Receptor Is a Critical Determinant of Potency for Agonists Containing C-terminal Glycinamide. *J. Biol. Chem.* **1996**, *271*, 15510–15514.

(25) Koradi, R.; Billeter, M.; Wüthrich, K. MOLMOL: A Program for Display and Analysis of Macromolecular Structures. *J. Mol. Graphics* **1996**, *14*, 51–55.