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

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Design, synthesis, and structure−activity relationships of thieno[2,3-*b*]pyridin-4-one-based non-peptide luteinizing hormone-releasing hormone (LHRH) receptor antagonists are described. Starting with the thienopyridin-4-one derivative **26d** (T-98475) an optimization study was performed, which resulted in the identification of a highly potent and orally bioavailable LHRH receptor antagonist, 3-(*N*-benzyl-*N*-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-2-[4-(1-hydroxy-1-cyclopropanecarboxamido)phenyl]-5-isobutyryl-4-oxothieno[2,3-*b*]pyridine (**33c**). Compound **33c** displayed subnanomolar in vitro activities for the human receptor and its oral administration caused effective suppression of the plasma LH levels in castrated male cynomolgus monkeys. Furthermore, SAR studies revealed that a hydroxyalkylamido moiety on the 2-phenyl ring is virtually equivalent to an alkylureido moiety, at least in this series of compounds.

Introduction

Luteinizing hormone-releasing hormone (LHRH), also known as gonadotropin-releasing hormone (GnRH), is a hypothalamic decapeptide hormone that controls the reproductive axis—gonadal function and ovarian cycles.[1] It is synthesized and released from the hypothalamus in a pulsatile manner and interacts with the specific G-protein coupled receptor (GPCR)[2] on the pituitary gonadotrope cells, where it triggers the biosynthesis and secretion of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH).[3] These gonadotropins, in turn, stimulate gonadal steroidogenesis and gametogenesis in testes and ovaries. Continuous stimulation of the LHRH receptor by potent LHRH agonists ultimately causes down-regulation and receptor desensitization, resulting in the blockade of reproductive hormonal signaling—"biochemical castration".[4] Through this peculiar mechanism, several peptidic LHRH agonists, represented by leuprorelin ([D-Leu^6,Pro^9-NHEt]LHRH),[5] paved the way for useful therapeutic application in the treatment of endocrine-based diseases, such as prostate cancer, breast cancer, endometriosis, uterine leiomyoma, and precocious puberty.[6,7] However, it is well recognized that these LHRH agonists occasionally evoke an initial gonadal hormone surge due to overstimulation of the receptor, known as the "flare effect",[4] leading to symptom exacerbation.

Currently, several peptidic LHRH antagonists have been developed and there is compelling evidence indicating their advantage. Clinical studies have demonstrated that they attenuate steroid hormone levels from onset and thus alleviate disease symptoms without the concomitant flare effect.[8] One of the major drawbacks of these peptidic antagonists, however, is poor oral bioavailability, and they are usually administered by daily subcutaneous injection, intranasal spray, or suitable depot form. In contrast, non-peptide LHRH antagonists offer the potential for oral administration, and therefore, could provide desirable therapeutic agents for clinical applications in the above conditions. These observations have fueled the recent surge of intensive efforts for developing orally active non-peptide LHRH antagonists.[9] Until recently, several research groups have engaged in this project and a plethora of small molecule LHRH antagonists have been reported.[10−17]

Lead Generation and Drug Design

In our search for a non-peptide LHRH antagonist, we first focused on the type II β-turn involving residues 5−8 (Tyr-Gly-Leu-Arg) of LHRH, which was considered to be the dominant structure for its binding to the receptor.[7] The underlying premise of our strategy is that the key structural element, the β-turn structure of LHRH or leuprorelin, could be replaced by a bicyclic "scaffold", and introduction of important functional moieties for receptor binding on to the scaffold would provide a non-peptide LHRH receptor ligand (Chart 1). To explore a lead molecule, "directed screening" of our chemical library was conducted. Screening of compounds selected for their similarity to antagonists of other GPCRs led to the identification of thieno-[2,3-*b*]pyridin-4-one **4a** as a weak LHRH receptor binder. Comparing the structure of the lead compound **4a** with the β-turn portion of LHRH and leuprorelin, we hypothesized that the 4-methoxyphenyl, 2-methoxybenzyl, and ethyl ester groups would correspond to the side chains of the Tyr^5, D-Leu^6, and Leu^7 residues of leuprorelin, respectively.[10] From this point of view, compound **4a** is lacking in a basic moiety corresponding to the Arg^8 residue. We thus attempted to introduce an appropriate amino functionality into **4a** and found that introduction of a basic amino moiety into the 3-position significantly increased the receptor binding affinity. Encouraged by this success, we started a medicinal chemistry program to develop thienopyridin-4-one-based non-peptide LHRH antagonists. Fol-

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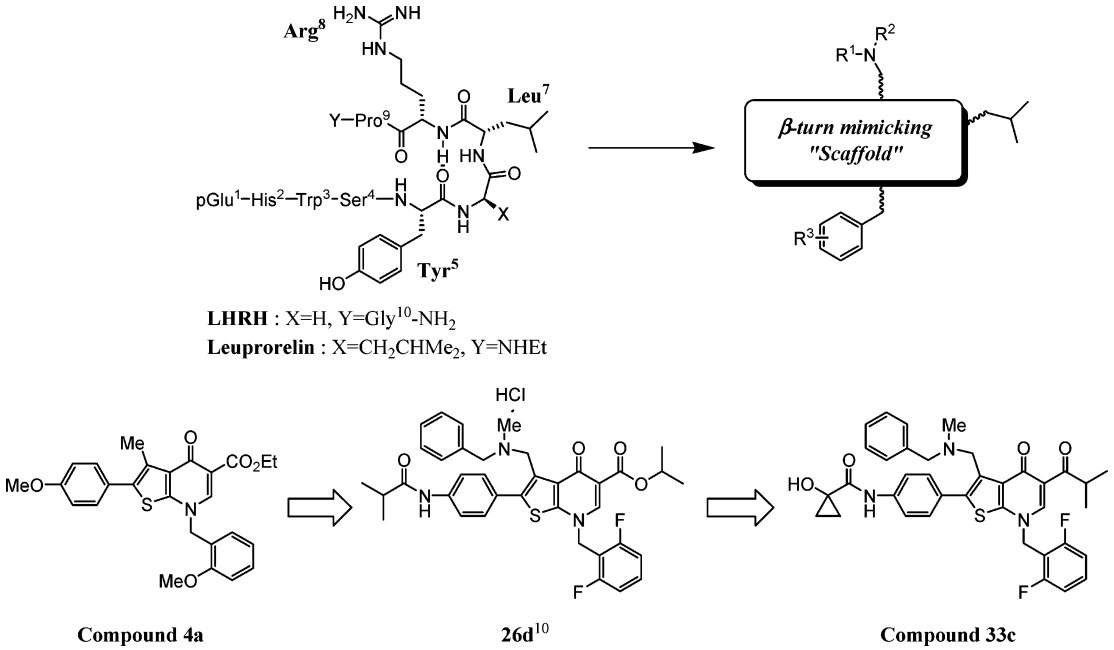
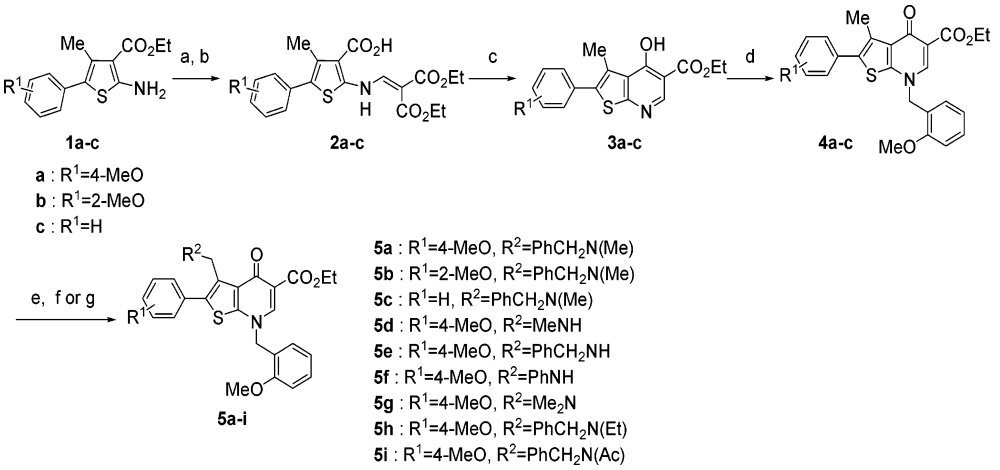
Chart 1



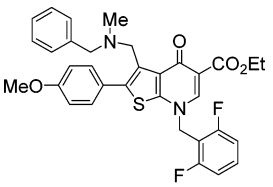
Scheme 1[a]



[a] Reagents: (a) diethyl ethoxymethylenemalonate; (b) KOH−EtOH, dioxane; (c) PPE; (d) 2-methoxybenzyl chloride, NaH, DMF; (e) NBS, AIBN, CCl_4; (f) R^2H (amine), $^iPr_2NEt$, DMF; (g) *N*-benzylacetamide, NaH, DMF.

lowing this line, we discovered the first potent and orally active non-peptide LHRH receptor antagonist, **26d** (T-98475), and we reported the initial structure−activity relationship (SAR) of this class.[10] However, the in vivo antagonism of **26d** when administered orally was not as effective as we had expected, which was considered to be due to its inadequate oral absorption and/or insufficient in vitro antagonism. In search for a more potent LHRH antagonist with improved in vivo efficacy, the following two strategies were employed: (i) further optimization of each substituent of **26d** and (ii) replacement of the thienopyridin-4-one scaffold with other heterocyclic surrogates. The latter strategy of investigating novel classes of LHRH antagonists resulted in the identification of thieno[2,3-*d*]pyrimidine-2,4-diones[11] and imidazo[1,2-*a*]pyrimidin-5-ones.[12]

In this paper, we wish to report the design, synthesis, and SARs of thieno[2,3-*b*]pyridin-4-one derivatives and their biological activity. The subsequent optimization study culminated in the identification of a more potent and orally bioavailable non-peptide LHRH receptor antagonist, a thienopyridin-4-one bearing a 4-(1-hydroxy-1-cyclopropanecarboxamido)phenyl group at the 2-position (**33c**). We also describe how molecular modeling studies suggested a good correlation between compound **9j** and the β-turn portion of LHRH and how this helped the design of the para-substituent of the 2-phenyl ring.

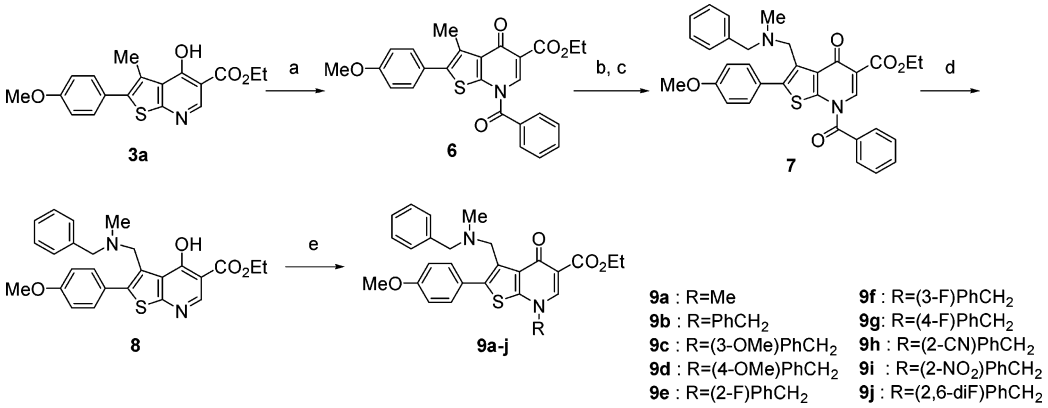


Compound 9j

Chemistry

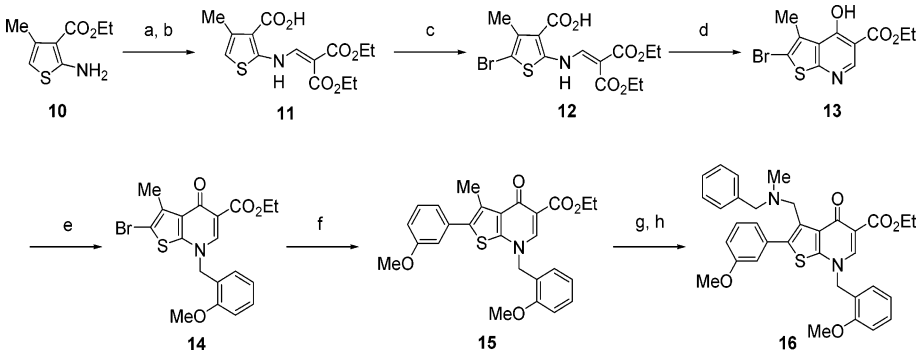
The thieno[2,3-*b*]pyridin-4-one derivatives were generally synthesized according to the previously reported procedure[10] shown in Scheme 1. Reaction of the multifunctionalized aminothiophenes **1a**−**c**,[18] prepared from the corresponding phenylacetones using Gewald's methodology,[19] with diethyl

Scheme 2[a]



[a] Reagents: (a) PhCOCl, pyridine; (b) NBS, AIBN, CCl_4; (c) *N*-benzylmethylamine, $^iPr_2NEt$, DMF; (d) NaOEt, EtOH, CH_2Cl_2; (e) R−X, NaH, DMF.

Scheme 3[a]



[a] Reagents: (a) diethyl ethoxymethylenemalonate; (b) KOH−EtOH, dioxane; (c) Br_2, pyridine, $CHCl_3$; (d) PPE; (e) 2-methoxybenzyl chloride, K_2CO_3, KI, DMF; (f) 3-methoxyphenylboronic acid, $Pd(PPh_3)_4$, 2 M Na_2CO_3, DME; (g) NBS, AIBN, CCl_4; (h) *N*-benzylmethylamine, $^iPr_2NEt$, DMF.

ethoxymethylenemalonate, followed by selective alkaline hydrolysis of the 3-ethyl ester, gave the thiophene-3-carboxylic acids **2a**−**c**. Subsequently, intramolecular cyclization of **2a**−**c** in polyphosphate ester (PPE) afforded the thieno[2,3-*b*]pyridines **3a**−**c**. Benzylation of **3a**−**c** predominantly occurred at the 7-position to give the *N*-benzyl analogues **4a**−**c** contaminated with a small amount of the *O*-benzyl byproducts, which were easily removed by recrystallization. Radical bromination of **4a**−**c** with *N*-bromosuccinimide (NBS) proceeded selectively at the 3-methyl group, and the intermediary bromomethyl compounds were derivatized by reaction with a variety of amines or *N*-benzylacetamide under basic conditions to provide the corresponding amines **5a**−**h** and amide **5i**.

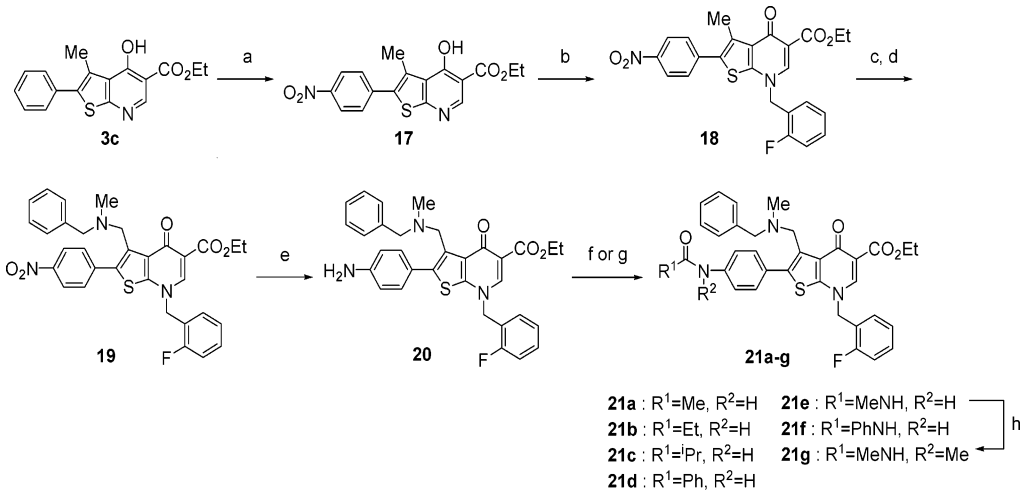
Using the general procedure shown in Scheme 1, it would be cumbersome to prepare various 7-substituted thienopyridin-4-ones. Hence, we developed the convenient synthetic route depicted in Scheme 2. Acylation of the thienopyridine **3a** selectively took place at the 7-position, in a manner similar to that described above for benzylation to give the *N*-benzoyl analogue **6**. The benzoyl group of **6** was sufficiently stable in the bromination step, and the resulting 3-bromomethyl compound was treated with 1 equiv of *N*-benzylmethylamine to generate the tertiary amine **7**. Deprotection of the benzoyl group of **7** with sodium ethoxide cleanly gave the requisite thienopyridine **8**, which was derivatized with various alkyl halides to afford the target compounds **9a**−**j**.

To develop an efficient route for preparing substituted 2-phenyl analogues, the Suzuki aryl coupling reaction was employed as a key step and the preparation of the 2-(3-methoxyphenyl) derivative **16** is outlined in Scheme 3. The thiophene-3-carboxylic acid **11**, readily obtained from the commercially available 2-aminothiophene **10**, was converted to the 5-bromothiophene-3-carboxylic acid **12** by ionic bromination. Ring closure of **12** with PPE, followed by benzylation, produced the 2-bromothienopyridin-4-one **14**. Compound **14** was then subjected to the Suzuki coupling reaction with 3-methoxyphenylboronic acid in the presence of a zerovalent palladium catalyst to furnish the coupling product **15**, which was converted to the desired compound **16** by bromination and subsequent incorporation of an *N*-benzylmethylamino moiety.

The 7-(2-fluorobenzyl) derivatives **21a**−**g** bearing 4-amido or 4-ureido moieties on the 2-phenyl ring were prepared by the route shown in Scheme 4. Nitration of the thienopyridine **3c** gave rise to a mixture of the mono- and dinitrated products, presumably due to its electron-rich properties. The mixture was successfully separated by silica gel column chromatography to afford the desired 4-nitrophenyl derivative **17** in moderate yield. After benzylation of **17**, an *N*-benzylmethylamino moiety was introduced into the 3-methyl group of **18** by the usual two-step protocol to yield the nitrophenyl compound **19**. Reduction of **19** with iron powder−hydrochloric acid provided the corresponding aniline **20**, which in turn was converted to the target amides **21a**−**d** and ureas **21e,f** by reaction with appropriate acyl chlorides, acid anhydrides, or isocyanates. In addition, methylation of the methylurea **21e** occurred with complete regioselectivity toward the aniline nitrogen to furnish compound **21g**. The structure of **21g** was confirmed by the different chemical shifts of the two methyl groups of the urea in its ^1H NMR spectrum (see the Experimental Section).

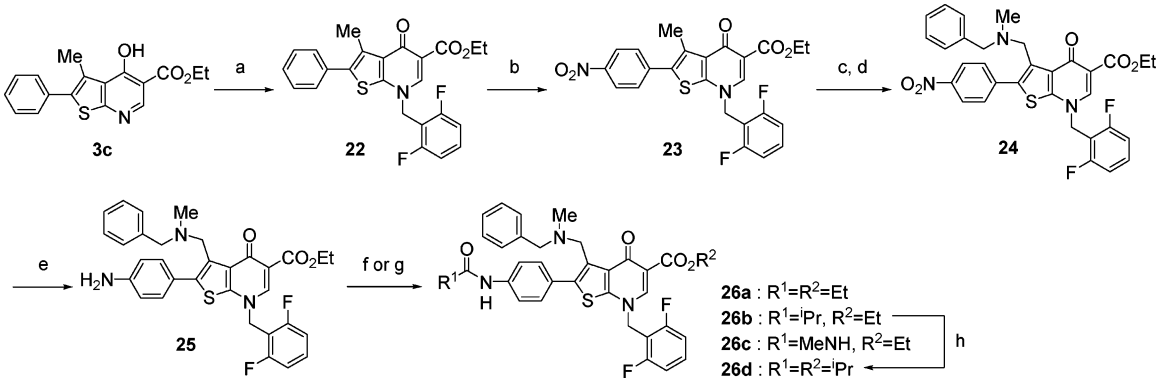
The synthetic route to the 2-(4-amidophenyl) and 2-(4-ureidophenyl) derivatives **26a**−**d** bearing a 2,6-difluorobenzyl group at the 7-position is presented in Scheme 5. Since handling

Scheme 4[a]



[a] Reagents: (a) $NaNO_3$, concd H_2SO_4; (b) 2-fluorobenzyl chloride, K_2CO_3, KI, DMF; (c) NBS, AIBN, CCl_4; (d) *N*-benzylmethylamine, $^iPr_2NEt$, DMF; (e) Fe, concd HCl, EtOH; (f) R^1COCl or $(R^1CO)_2O$, Et_3N, CH_2Cl_2; (g) MeNCO or PhNCO, pyridine, THF; (h) MeI, K_2CO_3, DMF.

Scheme 5[a]



[a] Reagents: (a) 2,6-difluorobenzyl chloride, K_2CO_3, KI, DMF; (b) $NaNO_3$, concd H_2SO_4; (c) NBS, AIBN, CCl_4; (d) *N*-benzylmethylamine, $^iPr_2NEt$, DMF; (e) Fe, concd HCl, EtOH; (f) EtOCl or ^{i}PrCOCl, Et_3N, CH_2Cl_2; (g) MeNCO, pyridine, THF; (h) $Ti(O^iPr)_4$, ^{i}PrOH.

of the 4-nitrophenyl derivative **17** proved to be tedious due to its low solubility, an alternative route was developed. To circumvent the intermediate **17**, benzylation was conducted prior to nitration. After difluorobenzylation of **3c**, nitration of **22** successfully proceeded at the para-position of the 2-phenyl ring to generate the 4-nitrophenyl compound **23** in good yield. This may be attributed to the relative electron-deficiency of the thienopyridin-4-one nucleus and the 2,6-difluorophenyl ring. Subsequently, conversion to the aniline **25** from **23** was carried out in a manner similar to that described above for the preparation of the 7-(2-fluorobenzyl) derivative **20** shown in Scheme 4. The aniline **25** was then reacted with acid chlorides or methyl isocyanate to afford the amides **26a,b** and methylurea **26c**. Finally, transesterification of **26b** with titanium(IV) isopropoxide−2-propanol[20] produced the isopropyl ester **26d**.

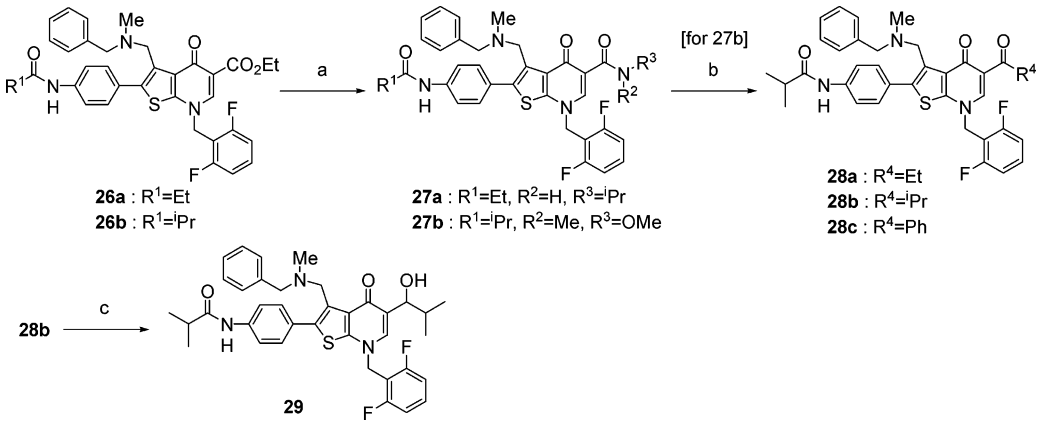
Chemical modification of the 5-ester group was performed by the route depicted in Scheme 6. Facile one-step amidation of the esters **26a,b** with the appropriate amine−aluminum complex using trimethylaluminum[21] worked well to provide the diamides **27a,b**. The Weinreb amide **27b** was subsequently reacted with appropriate Grignard reagents to give the corresponding ketones **28a**−**c**. In addition, the isopropyl ketone **28b** was reduced by sodium borohydride to yield the secondary alcohol **29**.

The synthesis of the ketone derivatives **32a**,**b** and **33a**−**c** bearing various hydrogen-bonding functional groups at the para-position of the 2-phenyl ring was conducted by the procedure shown in Scheme 7. For this purpose, an efficient route that involved initial ketone formation prior to amide or urea derivatization was carried out. Protection of the primary amine of **25** with a trifluoroacetyl group, followed by exposure to *N*,*O*-dimethylhydroxylamine−aluminum complex afforded the Weinreb amide **30** in excellent yield. Compound **30** served as a versatile precursor and was converted to the aniline **31** by treatment with isopropylmagnesium chloride and subsequent removal of the trifluoroacetyl group. The preparation of the target amide and urea derivatives was accomplished as follows: reaction of **31** with methyl isocyanate to provide the methylurea **32a**, condensation of **31** with Boc-glycine using (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP reagent) and subsequent deprotection with trifluoroacetic acid to give the 2-aminoacetamide **32b**, condensation of **31** with methoxymethoxyacetic acid using PyBOP reagent and successive deprotection to furnish the 2-hydroxyacetamide **33a**, and acylation of **31** with appropriate acetoxyacyl chlorides and subsequent removal of the acetyl group to afford the hydroxyalkylamides **33b,c**.

Results and Discussion

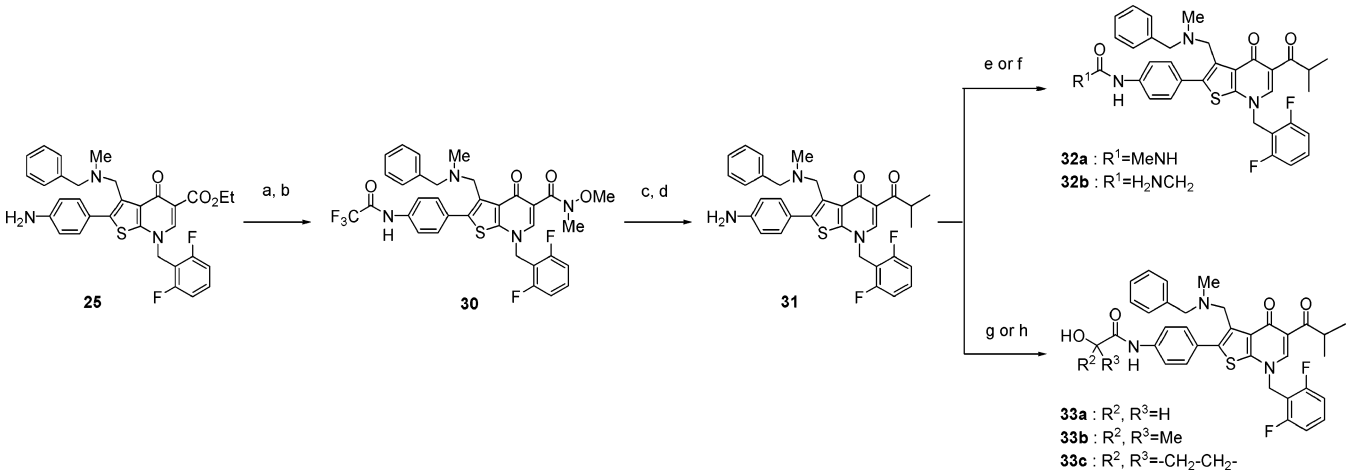
In Vitro Receptor Binding Studies. The SAR studies of thienopyridin-4-one derivatives were undertaken on the basis of data from an in vitro binding assay using cloned Chinese

Scheme 6[a]



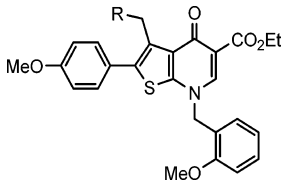
[a] Reagents: (a) R^2NHR^3, Me_3Al, $^iPr_2NEt$ (if necessary), CH_2Cl_2; (b) R^4MgX, THF or dioxane; (c) $NaBH_4$, MeOH.

Scheme 7[a]



[a] Reagents: (a) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2; (b) *N*,*O*-dimethylhydroxylamine hydrochloride, $^iPr_2NEt$, Me_3Al, CH_2Cl_2; (c) ^{i}PrMgCl, dioxane; (d) 2 N NaOH, MeOH; (e) MeNCO, pyridine, THF; (f) (1) Boc-Gly-OH, PyBOP, $^iPr_2NEt$, CH_2Cl_2, (2) TFA; (g) (1) $MeOCH_2OCH_2CO_2H$, PyBOP, $^iPr_2NEt$, CH_2Cl_2, (2) TFA; (h) (1) $AcOC(R^2R^3)COCl$, Et_3N, CH_2Cl_2, (2) 5 N NaOH, EtOH.

Table 1. Physicochemical Data and Binding Affinities of Compounds **4a**, **5a**, and **5d**−**i**



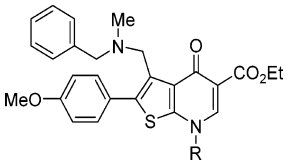
compd	R	mp (°C)	recryst solvent	formula	IC_{50}[a] (μM)
4a	H	165−167	CH_2Cl_2−Et_2O	$C_{26}H_{25}NO_5S$·0.5H_2O	10
5d	MeNH	118−120	Et_2O	$C_{27}H_{28}N_2O_5S$·HBr·0.5H_2O	2
5e	$PhCH_2NH$	118−119	EtOAc−Et_2O	$C_{33}H_{32}N_2O_5S$·HCl·0.9H_2O	2
5f	PhNH	173−174	Et_2O−hexane	$C_{32}H_{30}N_2O_5S$·0.2H_2O	42%[b]
5g	Me_2N	117−119	Et_2O	$C_{28}H_{30}N_2O_5S$·HBr·1.75H_2O	0.5
5a	$PhCH_2N(Me)$	147−152	EtOAc−Et_2O	$C_{34}H_{34}N_2O_5S$·HCl·1.8H_2O	0.07
5h	$PhCH_2N(Et)$	87−91	acetone−Et_2O	$C_{35}H_{36}N_2O_5S$·$(CO_2H)_2$·1.5H_2O	0.3
5i	$PhCH_2N(Ac)$	77−82	EtOAc−$^iPr_2O$	$C_{35}H_{34}N_2O_6S$·0.5EtOAc	7

[a] The binding affinity is reported as the IC_{50} value, which is the antagonist concentration required to inhibit the specific binding of [^{125}I]leuprorelin to LHRH receptor by 50%. Chinese hamster ovary (CHO) cells expressing human LHRH receptors were used as the source for LHRH receptors. All data are expressed as means of two or three determinations. [b] Percent inhibition at 20 μM.

hamster ovary (CHO) cells expressing the human LHRH receptor and [^{125}I]leuprorelin as a ligand.

First, thienopyridin-4-ones possessing various amino functionalities at the 3-position were investigated, and the results and physicochemical data are shown in Table 1. Introduction of a basic amino moiety, important for receptor binding, into the 3-methyl group generally induced a significant increase in affinity.[10] In fact, the methylamino derivative **5d** displayed 5-fold higher affinity than the initial lead **4a**. The benzylamine (**5e**) was equipotent with the methylamine (**5d**), whereas the aniline (**5f**) showed marked decrease in binding affinity. In contrast, incorporation of secondary amino groups led to a boost

Table 2. Physicochemical Data and Binding Affinities of Compounds **5a**, **8**, and **9a**−**j**



compd	R	mp (°C)	recryst solvent	formula	IC_{50}[a] (μM)
8	H	175−177	EtOAc	$C_{26}H_{26}N_2O_4S$	>20
9a	Me	217−220	EtOAc−^{i}Pr$_2$O	$C_{27}H_{28}N_2O_4S$·HCl·2.5H_2O	5
9b	$PhCH_2$	203−208	EtOAc−^{i}Pr$_2$O	$C_{33}H_{32}N_2O_4S$·HCl·0.2H_2O	0.2
5a	(2-OMe)$PhCH_2$	147−152	EtOAc−Et_2O	$C_{34}H_{34}N_2O_5S$·HCl·1.8H_2O	0.07
9c	(3-OMe)$PhCH_2$	109−113	Et_2O	$C_{34}H_{34}N_2O_5S$·HCl·H_2O·0.2Et_2O	0.4
9d	(4-OMe)$PhCH_2$	200−204	EtOAc−^{i}Pr$_2$O	$C_{34}H_{34}N_2O_5S$·HCl·0.95H_2O	0.3
9e	(2-F)$PhCH_2$	203−207	EtOAc−^{i}Pr$_2$O	$C_{33}H_{31}N_2O_4SF$·HCl·0.6H_2O	0.03
9f	(3-F)$PhCH_2$	221−224	EtOAc−^{i}Pr$_2$O	$C_{33}H_{31}N_2O_4SF$·HCl·0.5H_2O	0.3
9g	(4-F)$PhCH_2$	227−230	EtOAc−^{i}Pr$_2$O	$C_{33}H_{31}N_2O_4SF$·HCl·0.5H_2O	0.4
9h	(2-CN)$PhCH_2$	196−198	EtOAc−^{i}Pr$_2$O	$C_{34}H_{31}N_3O_4S$·HCl·0.5H_2O	0.1
9i	(2-NO_2)$PhCH_2$	214−216	EtOAc−^{i}Pr$_2$O	$C_{33}H_{31}N_3O_6S$·HCl·0.5H_2O	0.1
9j	(2,6-F_2)$PhCH_2$	189−192	EtOAc−^{i}Pr$_2$O	$C_{33}H_{30}N_2O_4SF_2$·HCl·1.5H_2O	0.006

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

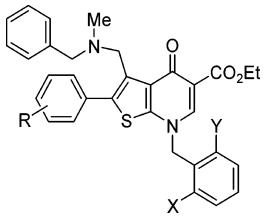
in activity. The dimethylamino derivative **5g** was 4-fold more potent than **5d,e**. Surprisingly, when one methyl group of **5g** was replaced with a benzyl group, the *N*-benzylmethylamino derivative **5a** showed excellent affinity with a half-maximal inhibition concentration (IC_{50}) of 70 nM, which was ca. 140-fold higher than that of **4a**. In addition, substitution of an ethyl (**5h**) or acetyl (**5i**) moiety for the methyl group of **5a** diminished the affinity. Accordingly, the *N*-benzyl-*N*-methylaminomethyl group was selected as the 3-substituent for subsequent investigation.

Second, effects of the 7-substituent on receptor binding affinity were examined for compounds bearing the *N*-benzyl-*N*-methylaminomethyl group at the 3-position, and the results and physicochemical data are presented in Table 2. The nonsubstituted analogue **8** showed greatly reduced activity, but the methyl derivative **9a** was found to exhibit significant affinity, although it was ca. 70-fold less potent than the 2-methoxybenzyl derivative **5a**. Thus, substituted benzyl derivatives were next examined. Deletion (**9b**) or transposition (**9c,d**) of the 2-methoxy group of **5a** caused a deleterious effect on binding affinity. Replacement of the methoxy group with a fluorine atom (**9e**) led to about a 2-fold increase in activity compared to **5a**. As in the case of the methoxybenzyl compounds, transposition (**9f,g**) of the fluorine atom of **9e** resulted in more than 10-fold decrease in activity, which indicates that ortho-substitution is preferable to meta- or para-substitution of the 7-benzyl moiety. With respect to the ortho-substituent, fluoro (**9e**) was more potent than methoxy (**5a**), cyano (**9h**), and nitro (**9i**). It should be noted that addition of another fluorine atom into the ortho-position of **9e** produced a further increase in activity, and the affinity of the 2,6-difluorobenzyl analogue **9j** reached a nanomolar level. The result suggested that 2-fluoro- or 2,6-difluorobenzyl groups are favorable 7-substituents and the binding site around the 7-substituent is mainly hydrophobic.

After obtaining the successful results above, we began to probe for effects of the substituent on the 2-phenyl ring, and the results and physicochemical data are shown in Table 3. Initially, a methoxy group on the 2-phenyl ring was investigated for compounds bearing the 2-methoxybenzyl moiety at the 7-position. Omission (**5c**) as well as transposition (**5b**, **16**) of the 4-methoxy group resulted in a 3−10-fold decrease in affinity compared with **5a**, which clearly indicated that para-substitution is preferable to ortho- or meta-substitution and led us to further examine the para-substituent. As discussed below in molecular modeling studies, superimposition of compound **9j** on LHRH suggested that the para-substituent on the 2-phenyl ring could correspond to the C-terminal region of LHRH. Since the C-terminal amide of LHRH has been reported to interact with the hydrogen-bonding amino acid residue (Asn102 of the second transmembrane helix) of the receptor,[22] introduction of hydrogen-bonding functional groups, such as an amide or urea, were investigated at this position. In the 7-(2-fluorobenzyl) series, the amino compound **20** showed almost equal affinity to the methoxy compound **9e**; however, acylation of the amino group of **20** elicited a notable enhancement in activity, as we had anticipated. Indeed, the amide derivatives **21a**−**d** exhibited subnanomolar to nanomolar affinities and the activities increased in the order of acetamide **21a** < benzamide **21d** < isobutyrylamide **21c** < propionylamide **21b**. Introduction of a methylureido group led to a further enhancement in activity and the methylurea **21e** displayed extremely high affinity with an IC_{50} value of 70 pM. Conversely, incorporation of sterically demanding phenylurea (**21f**) and N-methylation of the aniline nitrogen (**21g**) caused more than 30-fold decrease in activity compared with **21e**. Furthermore, additional inclusion of a fluorine atom into the ortho-position of the 7-benzyl group of **21b**,**c** (**26a**,**b**) increased the activity by more than 3-fold in the amide series. On the other hand, additional inclusion of a fluorine atom produced a minimal effect on the affinity in the urea series, and the difluorobenzyl derivative **26c** possessed almost comparable affinity to the monofluorobenzyl counterpart **21e**. With respect to the para-substituent on the 2-phenyl ring, it can be seen that both the hydrogen-donating NH and terminal small alkyl groups are crucial for high-affinity binding and that a steric constraint may exist around the recognition site interacting with this part of the molecule.

Table 4 shows the effects of the 5-substituent on binding affinity, with compounds bearing the 4-propionyl- or 4-isobutyrylamidophenyl groups at the 2-position. Since it is widely acknowledged that an ester group is metabolically labile in vivo, a more stable 5-substituent, such as an amide, ketone, or alcohol, was explored. Although the amide **27a** was less potent than the

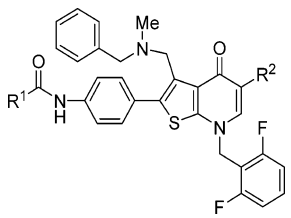
Table 3. Physicochemical Data and Binding Affinities of Compounds **5a**−**c**, **9e**, **16**, **20**, **21a**−**g**, and **26a**−**c**



compd	R	X	Y	mp (°C)	recryst solvent	formula	IC_{50}[a] (nM)
5c	H	MeO	H	117−120	EtOAc−Et_2O	$C_{33}H_{32}N_2O_4S$·HCl·1.1H_2O	200
5b	2-MeO			119−122	EtOAc−Et_2O	$C_{34}H_{34}N_2O_5S$··HCl·1.5H_2O	700
16	3-MeO			115−120	EtOAc−Et_2O	$C_{34}H_{34}N_2O_5S$·HCl·1.5H_2O	500
5a	4-MeO			147−152	EtOAc−Et_2O	$C_{34}H_{34}N_2O_5S$··HCl·1.8H_2O	70
9e	4-MeO	F	H	203−207	EtOAc−iPr_2O	$C_{33}H_{31}N_2O4S$·F·HCl·0.6H_2O	30
20	4-NH_2			158−160	$CHCl_3$−Et_2O	$C_{32}H_{30}N_3O_3S$·F·0.5H_2O	40
21a	4-MeCONH			118−120	EtOH	$C_{34}H_{32}N_3O_4S$·F·H_2O	2
21b	4-EtCONH			221−223	EtOH−Et_2O	$C_{35}H_{34}N_3O_4S$·F·3.0H_2O	0.4
21c	4-^{i}PrCONH			188−192	EtOH−Et_2O	$C_{36}H_{36}N_3O_4S$·F·2.0H_2O	0.6
21d	4-PhCONH			141−143	EtOH−Et_2O	$C_{39}H_{34}N_3O_4S$·F·2.5H_2O	1
21e	4-MeNHCONH			216−220	EtOH−EtOAc	$C_{34}H_{33}N_4O_4SF$·2.0H_2O	0.07
21f	4-PhNHCONH			205−207	EtOH−EtOAc	$C_{39}H_{35}N_4O_4SF$·2.5H_2O	30
21g	4-MeNHCON(Me)			>300	EtOH−EtOAc	$C_{35}H_{35}N_4O_4SF$·H_2O	2
26a	4-EtCONH	F	F	100−102	EtOAc−hexane	$C_{35}H_{33}N_3O_4SF_2$·0.75H_2O	0.1
26b	4-^{i}PrCONH			185−186	EtOH−Et_2O	$C_{36}H_{35}N_3O_4SF_2$·HCl·1.5H_2O	0.2
26c	4-MeNHCONH			214−215	EtOH−EtOAc	$C_{34}H_{32}N_4O_4SF_2$	0.06

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

Table 4. Physicochemical Data and Binding Affinities of Compounds **26a**,**b**,**d**, **27a**, **28a**−**c**, and **29**



compd	R^1	R^2	mp (°C)	recryst solvent	formula	IC_{50}[a] (nM)
26a	Et	CO_2Et	100−102	EtOAc−hexane	$C_{35}H_{33}N_3O_4SF_2$·0.75H_2O	0.1
26b	^{i}Pr	CO_2Et	185−186	EtOH−Et_2O	$C_{36}H_{35}N_3O_4SF_2$·HCl·1.5H_2O	0.2
26d	^{i}Pr	$CO_2{}^i$Pr	168−170	EtOH−Et_2O	$C_{37}H_{37}N_3O_4SF_2$·HCl·0.5H_2O	0.2
27a	Et	CONH^{i}Pr	144−146	EtOAc−hexane	$C_{36}H_{36}N_4O_3SF_2$·3.5H_2O	8
28a	^{i}Pr	COEt	250−256	EtOH−EtOAc	$C_{36}H_{35}N_3O_3SF_2$·HCl·0.5H_2O	0.4
28b	^{i}Pr	CO^{i}Pr	192−197	EtOH−EtOAc	$C_{37}H_{37}N_3O_3SF_2$·HCl·0.5H_2O	0.2
28c	^{i}Pr	COPh	198−200	EtOAc	$C_{40}H_{35}N_3O_3SF_2$·HCl·0.5H_2O	0.1
29	^{i}Pr	CH(OH)^{i}Pr	232−234	EtOAc−Et_2O	$C_{37}H_{39}N_3O_3SF_2$·HCl·2.0H_2O	0.4

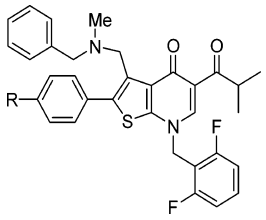
[a] The binding affinity is reported as the IC_{50} value, which is the antagonist concentration required to inhibit the specific binding of [^{125}I]leuprorelin to LHRH receptor by 50%. Chinese hamster ovary (CHO) cells expressing human LHRH receptors were used as the source for LHRH receptors. All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

esters **26a,d**, the ketones **28a**−**c** and alcohol **29** were well-tolerated and showed low subnanomolar affinities (IC_{50} < 0.5 nM).

It is well-known that highly lipophilic compounds tend to be targets of metabolic enzymes and bind to proteins nonspecifically. Considering physicochemical properties, the phenyl ketone **28c** (log *D* value of 7.29) is more lipophilic than the isopropyl ketone **28b** (log *D* value of 6.33) and the affinity of **28b** was acceptable in comparison with that of **28c**. Moreover, introduction of urea or urea-mimicking moieties into the para-position of the 2-phenyl ring might fill the gap as for the mono- and difluorobenzyl derivatives shown in Table 3. Hence, the isopropyl ketone was chosen as the 5-substituent for further investigation.

Effects of the para-substituent of the 2-phenyl group on binding affinity were reinvestigated with compounds bearing the isopropyl ketone at the 5-position, and the results and physicochemical data are presented in Table 5. The methylurea **32a** exhibited excellent binding affinity with an IC_{50} value of 0.1 nM, but it was slightly less potent than the ethyl ester counterpart **26c** (see Table 3). It is widely recognized that ureas tend to show poor oral absorption, resulting from relatively low solubility and/or poor membrane permeability due to their strong hydrogen-bonding ability. We thus set out to explore substituents that could have a similar function to urea and envisioned that incorporation of a hydrogen-donating group to mimic the terminal NH of urea (e.g. a hydroxy or amino group), at the α-carbon of the amido moiety would provide a urea-mimicking substituent with potent in vitro activity and improved oral absorption. Incorporation of an amino group into the amide (**32b**) reduced the activity, whereas incorporation of a hydroxy group into the amide did not essentially affect the affinity. The

Table 5. Physicochemical Data and Binding Affinities of Compounds **28b**, **32a**,**b**, and **33a**−**c**



compd	R	mp (°C)	recryst solvent	formula	IC_{50}[a] (nM)
28b	^{i}PrCONH	192−197	EtOH−EtOAc	$C_{37}H_{37}N_3O_3SF_2$·HCl·0.5H_2O	0.2
32a	MeNHCONH	222−226	$CHCl_3$−EtOAc	$C_{35}H_{34}N_4O_3SF_2$·HCl·0.5H_2O·0.2$CHCl_3$	0.1
32b	H_2NCH_2CONH	197−199	EtOH	$C_{35}H_{34}N_4O_3SF_2$·2.0HCl·1.5H_2O	9
33a	$HOCH_2CONH$	197−199	EtOH	$C_{35}H_{33}N_3O_4SF_2$·HCl·0.5H_2O	0.2
33b	$HOC(Me)_2CONH$	178−180	$CHCl_3$−Et_2O	$C_{37}H_{37}N_3O_4SF_2$·0.5H_2O	0.2
33c	(1-OH)^{c}PrCONH	184−185	EtOH	$C_{37}H_{35}N_3O_4SF_2$	0.1

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

Table 6. Species Specificities for Binding to LHRH Receptors and Inhibitory Effects on LHRH-Stimulated Arachidonic Acid (AA) Release of Compounds **26d**, **32a**, and **33c**

	IC_{50}[c] (nM)				
	binding affinity[a]			inhibition of AA release[b]	
compd	human[d]	monkey[d]	rat[e]	human	monkey
LHRH	10	6[f]	7	nd[g]	nd[g]
leuprorelin	0.3	0.8[f]	0.5	nd[g]	nd[g]
26d	0.2	2 (4[f])	60	0.6	nd[g]
32a	0.1	0.2	200	0.07	nd[g]
33c	0.1	0.9	150	0.1	15

[a] The binding affinity is reported as the IC_{50} value, which is the antagonist concentration required to inhibit the specific binding of [^{125}I]leuprorelin to LHRH receptor by 50%. [b] Inhibition of LHRH-stimulated arachidonic acid (AA) release from CHO cells expressing human or monkey LHRH receptors was measured to evaluate the functional LHRH antagonism of the test compounds. The IC_{50} value is the antagonist concentration required to inhibit the LHRH-stimulated AA release from CHO cells by 50%. [c] All data are expressed as means of two or three determinations. [d] Chinese hamster ovary (CHO) cells expressing human or monkey LHRH receptors were used as the source for LHRH receptors. [e] Rat anterior pituitaries were used as the source for LHRH receptors (see ref 10). [f] Monkey anterior pituitaries were used as the source for LHRH receptors (see ref 10). [g] nd = not determined.

hydroxyalkylamides **33a**−**c** proved to be well-tolerated and displayed comparable, or marginally increased, activities to the isobutyrylamide **28b**. It should be noted that the 1-hydroxycyclopropanecarboxamide **33c** displayed equal affinity to the methylurea **32a** and was the most potent among the hydroxyalkylamide series with an IC_{50} value of 0.1 nM. Therefore, compound **33c** was selected for further in vitro and in vivo evaluation.

Species Specificities and In Vitro Functional Antagonism. The 1-hydroxycyclopropanecarboxamide **33c** as well as the isobutyrylamide **26d** and methylurea **32a** were evaluated for their binding affinities to LHRH receptors of particular species and for in vitro functional antagonism, i.e., inhibitory potencies of LHRH-stimulated arachidonic acid release from CHO cells expressing the human and monkey receptors. The results are summarized in Table 6. Compounds **26d**, **32a**, and **33c** had more than 50-fold higher affinities than LHRH for the human receptor. In addition, these three compounds proved to be more potent than the superagonist leuprorelin for the human receptor. It can be seen from Table 6 that there are remarkable species specificities in binding affinities similar to those observed for the previously reported **26d**.[10] Compounds **32a** and **33c** displayed 2−2000-fold selectivity for the human receptor over the monkey and rat receptors. Considering **26d** as a "benchmark", incorporation of the additional hydrogen-bonding functionalities into the amide moiety (**32a**, **33c**) significantly increased the affinities for the human and monkey receptors; however, it induced no improvement in affinity for the rat receptor. Thus, it was considered reasonable that further evaluations of these compounds should be undertaken using monkey as well as human receptors.

As for in vitro functional antagonism, compounds **32a** and **33c** had no agonistic effects and potently antagonized LHRH function on CHO cells expressing the human and monkey receptors. Moreover, it is noteworthy that the 1-hydroxycyclopropanecarboxamide **33c** showed almost equal activity to the methylurea **32a**, while the isobutyrylamide **26d** was ca. 9-fold less potent than **32a** for the human receptor. Comparing the binding affinities and in vitro functional antagonism of these compounds for the human and monkey receptors, compound **33c** was essentially equipotent with **32a**. This suggests that the incorporated hydroxy group of **33c** might function equivalently to the terminal NH of **32a**. Similar to binding affinity, species specificities in functional antagonism were again observed. The in vitro functional antagonism of the human receptor exceeded that of the monkey receptor by 150-fold.

In Vivo Studies. Finally, the in vivo antagonism of the 1-hydroxycyclopropanecarboxamide **33c** was evaluated for the suppression of plasma LH concentrations in castrated male cynomolgus monkeys, and the results are shown in Table 7. As we had expected, oral administration of a 30 mg/kg dose of compound **33c** exhibited almost complete suppression of the circulating LH levels in monkeys. In addition, exposure to **33c**, even at a 10 mg/kg dose (po), caused effective and sustained suppression. The suppressive effect of **33c** at both 10 and 30 mg/kg doses (po) was unambiguously more potent than that of **26d** at a 60 mg/kg dose (po), and compound **33c** displayed longer duration of action than **26d**.[10] Furthermore, the in vivo antagonism of **33c** was virtually equipotent to that of the thienopyrimidine-2,4-dione TAK-013 at the same dosages.[11] In these experiments the maximum plasma concentrations of **33c** were 330 nM (reached 8 h after administration) and 56 nM (reached 4 h after administration) at 30 and 10 mg/kg doses, respectively, which indicates that compound **33c** showed good oral absorption in monkeys. As a consequence, compound **33c** potently antagonized the elevated LHRH function at the receptor level that was elicited by castration in male cynomolgus

Table 7. Time Course of Plasma LH Concentrations in Castrated Male Cynomolgus Monkeys after Oral Administration of Compound **33c** at 10 and 30 mg/kg Doses

	LH concentration[a] (mU/mL)					
	at 0 h	at 2 h	at 4 h	at 8 h	at 24 h	at 48 h
vehicle	0.62 ± 0.14	0.69 ± 0.10	0.67 ± 0.14	0.62 ± 0.06	0.65 ± 0.12	0.64 ± 0.09
dose = 10 mg/kg	0.48 ± 0.04	0.38 ± 0.07	0.27 ± 0.03	0.13 ± 0.03	0.11 ± 0.03	0.56 ± 0.07
dose = 30 mg/kg	0.56 ± 0.08	0.45 ± 0.05	0.31 ± 0.05	0.18 ± 0.06	0.07 ± 0.03	0.62 ± 0.07

[a] Cynomolgus monkeys (male, 4−9 years old) were castrated more than 6 months prior to the examination. Compound **33c** (10 or 30 mg/kg, 3 mL/kg, $n = 3$ for each group) suspended in 0.5% methylcellulose containing 1.2% citric acid, or 0.5% methylcellulose containing 1.2% citric acid alone (3 mL/kg, $n = 3$), was administered orally. Values shown are the mean ± SEM.

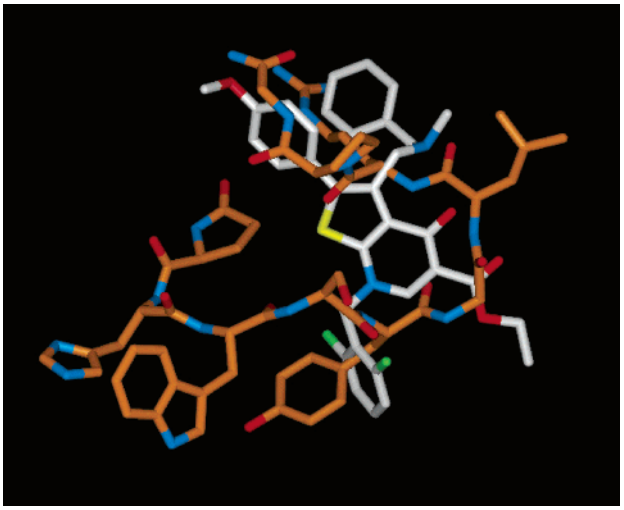


Figure 1. Superimposition of the Putative Active Conformation of LHRH and Compound **9j**: Carbon atoms for LHRH and compound **9j** are colored orange and white, respectively. Nitrogen, oxygen, sulfur, and fluorine atoms are colored cyan, red, yellow, and light green, respectively.

monkeys, and the antagonism lasted for more than 24 h at both 30 and 10 mg/kg doses (po). These results clearly demonstrate that compound **33c** is a potent and orally active LHRH antagonist. The excellent in vivo effectiveness of **33c** results from its potent in vitro activities and/or improved oral absorption.

Molecular Modeling Studies. To rationalize the underlying premise of our strategy, superimposition of compound **9j** on LHRH was investigated. The putative active conformation of LHRH was constructed on the basis of the reported dihedral angles as described by Momany.[23] Initially, we hypothesized that the 4-methoxyphenyl and ethyl ester groups of the lead compound **4a** would correspond to the side chains of the Tyr^5 and Leu^7 residues of LHRH, respectively. Moreover, we speculated that the 2-methoxybenzyl group of **4a** might mimic the side chain of the D-Leu^6 residue of leuprorelin.[10] The SAR study revealed that incorporation of an amino moiety into the 3-methyl group of **4a** led to a significant increase in activity, as shown in Table 1. Hence, we revised the superimposition of a thienopyridin-4-one derivative on LHRH, in accordance with this result. The overlay of the three-dimensional structure of compound **9j** and LHRH is illustrated in Figure 1. In the new model, good fits were obtained as a whole and certain specific points of match become apparent. Namely, the thienopyridine scaffold is superimposed on the type II β-turn portion and the difluorobenzyl and *N*-benzyl-*N*-methylaminomethyl moieties fit well with the Tyr^5 and Arg^8 residues, respectively. In addition, the ethyl ester group extends toward the Gly^6 residue, which corresponds to the D-Leu^6 residue of leuprorelin. It should be noted that a good overlay between the 4-methoxyphenyl group and the Gly^{10}-NH_2 residue is observed. This indicates that the para-substituent of the 2-phenyl ring of **9j** could correspond to the C-terminal amide portion of LHRH. Since Asn102 of the second transmembrane helix was reported as the binding site for the C-terminal amide of LHRH, based on the mutagenesis of the human receptor,[22] compounds bearing a hydrogen-bonding functional group at this position would be expected to interact strongly with the receptor and provide a more potent antagonist. In fact, incorporation of an amido or ureido moiety into the para-position of the 2-phenyl ring successfully increased the activity, as shown in Table 3. The molecular modeling studies suggested that compound **9j** could share a similar receptor binding site with LHRH, especially in the type II β-turn portion and C-terminal region. A recent study has revealed that the binding affinity of a thieno[2,3-*d*]pyrimidine-based antagonist to the human LHRH receptor was affected by mutation of Ser118, Asp302, and His306.[24] Considering that His306 of the seventh transmembrane helix is located adjacent to Asn102 of the second transmembrane helix, these data support our revised hypothesis as well.

Conclusion

We have carried out a medicinal chemistry program to develop thienopyridin-4-one-based non-peptide LHRH receptor antagonists starting from the lead compound **4a** identified by directed screening. According to the strategy of introducing important functional groups for receptor binding into a bicyclic "scaffold", we first attempted to introduce a basic amino moiety into **4a** that would correspond to the Arg^8 residue of LHRH. Gratifyingly, introduction of a basic moiety into the 3-methyl group gained a dramatic increase in binding affinity to provide the *N*-benzyl-*N*-methylaminomethyl derivative **5a**. The encouraging results led us to start a further optimization study of each substituent surrounding the thienopyridin-4-one scaffold. It was revealed that the 2-fluorobenzyl or 2,6-difluorobenzyl moieties were favorable 7-substituents. On the basis of molecular modeling studies, the 4-amido- or 4-ureidophenyl derivatives were designed, and introduction of these hydrogen-bonding moieties into the 2-position caused a remarkable increase in activity. With respect to the 5-substituent, the ketone was found to have comparable affinity to the corresponding ester. Finally, intensive efforts were made toward searching for a urea-mimicking substituent at the para-position of the 2-phenyl group, to obtain potent in vitro activity and improved oral absorption. Compounds with a hydroxy or amino group at the α-carbon of the amido moiety, e.g. hydroxyalkylamides, were designed, prepared, and evaluated. Consequently, we have succeeded in the identification of a highly potent and orally active non-peptide LHRH antagonist, the 1-hydroxycyclopropanecarboxamide derivative **33c**. Compound **33c** exhibited high binding affinity and potent in vitro antagonism for the human receptor, with IC_{50} values of 0.1 nM for both. Effective and sustained suppression of the plasma LH levels was accomplished in castrated male cynomolgus monkeys, with sufficient duration of action (more than 24 h) after oral administration of **33c** at 10 and 30 mg/kg

doses. The in vivo efficacy of **33c** after oral dosing was unequivocally more potent than that of the previously reported **26d** and almost equipotent to that of the thieno[2,3-*d*]pyrimidine-2,4-dione TAK-013. The SAR data obtained here was taken into consideration during the course of optimizing the thienopyrimidine-2,4-dione[11] and imidazopyrimidin-5-one[12] series. Furthermore, these results indicated that the hydroxyalkylamides are virtually equivalent to alkylureas with regard to their hydrogen-bonding ability, at least in the context of in vitro activity.

The medicinal chemistry and biological studies disclosed here demonstrate that the thieno[2,3-*b*]pyridin-4-one nucleus is an excellent scaffold and the thienopyridin-4-one derivatives constitute the first class of potent non-peptide human LHRH receptor antagonists.

Experimental Section

Chemistry. General Procedures. All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. The proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on either a Varian Gemini-200 (200 MHz) or a JEOL JNM-LA300 (300 MHz) spectrometer. Chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard. All *J* values are given in hertz. The infrared (IR) spectra were measured on a JACSO FTIR spectrometer. FAB mass spectra were recorded on a JEOL JMS-HX110. Elemental analyses were within ±0.4% of theoretical values and were determined at Takeda Analytical Research Laboratories, Osaka, Japan. Reagents and solvents were obtained from commercial sources and used without further purification. Flash chromatography was performed with Merck silica gel 60 (art. 9385; 230−400 mesh), and reaction progress was determined by thin-layer chromatography (TLC) analysis on silica gel 60 F_{254} plates (Merck). Visualization was done with UV light (254 nm) or iodine. Yields are of purified compounds and were not optimized.

Ethyl *N*-[3-Carboxy-5-(4-methoxyphenyl)-4-methyl-2-thienyl]-aminomethylenemalonate (2a). A mixture of **1a**[18] (10 g, 34.3 mmol) and ethyl ethoxymethylenemalonate (7.45 g, 34.5 mmol) was stirred at 120 °C for 2 h. After being cooled, the mixture was triturated with Et_2O. The yellow precipitate was collected by filtration, washed with ice-cold Et_2O, and dried to give the corresponding triester compound (14.2 g, 90%) as a yellow crystalline powder. Mp: 122−123 °C. ^{1}H NMR ($CDCl_3$): δ 1.32 (3H, t, J = 7.1 Hz), 1.38 (3H, t, J = 7.2 Hz), 1.41 (3H, t, J = 7.2 Hz), 2.34 (3H, s), 3.85 (3H, s), 4.25 (2H, q, J = 7.1 Hz), 4.38 (2H, q, J = 7.2 Hz), 4.45 (2H, q, J = 7.2 Hz), 6.95 (2H, d, J = 8.8 Hz), 7.31 (2H, d, J = 8.8 Hz), 8.22 (1H, d, J = 13.4 Hz), 12.74 (1H, d, J = 13.1 Hz); IR (KBr): 2984, 1720, 1707, 1688, 1653, 1599, 1518, 1499 cm^{-1}.

A solution of KOH (5.0 g, 75.7 mmol) in EtOH (30 mL) was added to a solution of the triester compound (7.0 g, 15.2 mmol) in dioxane (60 mL) at 70 °C. After being stirred at 70 °C for 10 min, the mixture was stirred at room temperature for a further 30 min and acidified with 2 N HCl (40 mL). The resulting suspension was concentrated in vacuo (removal of dioxane and EtOH). The precipitate was collected by filtration and dried to afford **2a** (6.1 g, 93%) as a yellow crystalline powder. Mp: 184−187 °C. ^{1}H NMR (DMSO-d_6): δ 1.24 (3H, t, J = 7.1 Hz), 1.28 (3H, t, J = 7.2 Hz), 2.30 (3H, s), 3.80 (3H, s), 4.15 (2H, q, J = 7.1 Hz), 4.24 (2H, q, J = 7.2 Hz), 7.03 (2H, d, J = 8.7 Hz), 7.37 (2H, d, J = 8.7 Hz), 8.08 (1H, d, J = 13.6 Hz), 12.41 (1H, d, J = 13.6 Hz). IR (KBr): 3422, 2980, 1719, 1653, 1607, 1551, 1512 cm^{-1}.

Ethyl *N*-[3-Carboxy-5-(2-methoxyphenyl)-4-methyl-2-thienyl]-aminomethylenemalonate (2b). Compound **2b** was prepared in 88% yield from **1b** (two steps) by a procedure similar to that described for **2a**. Mp: 167−169 °C (from Et_2O−hexane). ^{1}H NMR (DMSO-d_6): δ 1.23 (3H, t, J = 7.1 Hz), 1.28 (3H, t, J = 7.1 Hz), 2.13 (3H, s), 3.80 (3H, s), 4.14 (2H, q, J = 7.1 Hz), 4.24 (2H, q, J = 7.1 Hz), 6.99−7.16 (2H, m), 7.26−7.30 (1H, m), 7.38−7.47 (1H, m), 8.06 (1H, d, J = 13.8 Hz), 12.40 (1H, d, J = 13.8 Hz).

Ethyl *N*-[3-Carboxy-4-methyl-5-phenyl-2-thienyl]aminomethylenemalonate (2c). Compound **2c** was prepared in 90% yield from **1c** (two steps) by a procedure similar to that described for **2a**. Mp: 187−190 °C (from EtOH−H_2O). ^{1}H NMR (DMSO-d_6): δ 1.24 (3H, t, J = 7.2 Hz), 1.28 (3H, t, J = 7.2 Hz), 2.34 (3H, s), 4.15 (2H, q, J = 7.2 Hz), 4.24 (2H, q, J = 7.2 Hz), 7.39−7.54 (5H, m), 8.09 (1H, d, J = 13.3 Hz), 12.42 (1H, d, J = 13.3 Hz).

Ethyl 4-Hydroxy-2-(4-methoxyphenyl)-3-methylthieno[2,3-*b*]-pyridine-5-carboxylate (3a). Compound **2a** (6.0 g, 13.8 mmol) was added portionwise to polyphosphate ester (PPE; 60 g) at 120 °C. The mixture was stirred at 120 °C for 0.5 h. After being cooled, the reaction mixture was poured into ice−water and extracted with $CHCl_3$. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography (EtOAc−hexane, 1:4). The crystalline product was recrystallized from EtOH to provide **3a** (3.18 g, 67%) as orange crystals. Mp: 162−163 °C. ^{1}H NMR ($CDCl_3$): δ 1.47 (3H, t, J = 7.1 Hz), 2.63 (3H, s), 3.87 (3H, s), 4.49 (2H, q, J = 7.1 Hz), 6.99 (2H, d, J = 8.8 Hz), 7.44 (2H, d, J = 8.8 Hz), 8.84 (1H, s), 12.11 (1H, s). IR (KBr): 3434, 2992, 1692, 1601, 1582, 1535, 1504 cm^{-1}. FAB-MS *m/z*: 344 (M + H). Anal. ($C_{18}H_{17}NO_4S$) C, H, N, S.

Ethyl 4-Hydroxy-2-(2-methoxyphenyl)-3-methylthieno[2,3-*b*]-pyridine-5-carboxylate (3b). Compound **3b** was prepared in 73% yield from **2b** by a procedure similar to that described for **3a**, as an oil. ^{1}H NMR ($CDCl_3$): δ 1.47 (3H, t, J = 7.1 Hz), 2.48 (3H, s), 3.85 (3H, s), 4.49 (2H, q, J = 7.1 Hz), 6.99−7.08 (2H, m), 7.32−7.45 (2H, m), 8.84 (1H, s), 12.10 (1H, s).

Ethyl 4-Hydroxy-3-methy-2-phenylthieno[2,3-*b*]pyridine-5-carboxylate (3c). Compound **3c** was prepared in 60% yield from **2c** by a procedure similar to that described for **3a**. Mp: 155−157 °C (from EtOH). ^{1}H NMR ($CDCl_3$): δ 1.47 (3H, t, J = 7.1 Hz), 2.66 (3H, s), 4.49 (2H, q, J = 7.1 Hz), 7.39−7.55 (5H, m), 8.86 (1 H, s), 12.14 (1H, s). Anal. ($C_{17}H_{15}NO_3S$) C, H, N, S.

Ethyl 4,7-Dihydro-7-(2-methoxybenzyl)-2-(4-methoxyphenyl)-3-methyl-4-oxothieno[2,3-*b*]pyridine-5-carboxylate (4a). Sodium hydride (60% in oil; 0.123 g, 3.08 mmol) was added to a mixture of **3a** (1.0 g, 2.91 mmol) in DMF (25 mL) at 0 °C, and the mixture was then stirred at room temperature for 30 min. Subsequently, 2-methoxybenzyl chloride (0.91 g, 5.81 mmol) was added to the reaction mixture, and stirring was continued at room temperature for 23 h and at 70 °C for a further 2 h. After evaporation of the solvent in vacuo, aqueous NH_4Cl was added to the residue and the resulting mixture was extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). The solution was concentrated in vacuo, and the residue was purified by flash column chromatography (EtOAc−hexane, 4:1, EtOAc, then EtOAc−MeOH, 9:1) to give **4a** (0.95 g, 70%) as a pale-yellow amorphous powder. Crystallization from CH_2Cl_2−Et_2O afforded yellow columns. Mp: 165−167 °C. ^{1}H NMR ($CDCl_3$): δ 1.41 (3H, t, J = 7.1 Hz), 2.65 (3H, s), 3.85 (3H, s), 3.86 (3H, s), 4.39 (2H, q, J = 7.1 Hz), 5.16 (2H, s), 6.92−7.00 (4H, m), 7.21−7.41 (4H, m), 8.41 (1H, s). IR (KBr): 2980, 1727, 1684, 1621, 1609, 1589, 1497 cm^{-1}. Anal. ($C_{26}H_{25}NO_5S\cdot0.5H_2O$) C, H, N.

Ethyl 4,7-Dihydro-7-(2-methoxybenzyl)-2-(2-methoxyphenyl)-3-methyl-4-oxothieno[2,3-*b*]pyridine-5-carboxylate (4b). Compound **4b** was prepared in 57% yield from **3b** by a procedure similar to that described for **4a**. Mp: 194−196 °C (from EtOAc). ^{1}H NMR ($CDCl_3$): δ 1.40 (3H, t, J = 7.1 Hz), 2.51 (3H, s), 3.81 (3H, s), 3.86 (3H, s), 4.39 (2H, q, J = 7.1 Hz), 5.15 (2H, s), 6.91−7.04 (4H, m), 7.23−7.42 (4H, m), 8.41 (1H, s). Anal. ($C_{26}H_{25}NO_5S\cdot0.1H_2O$) C, H, N.

Ethyl 4,7-Dihydro-7-(2-methoxybenzyl)-3-methyl-4-oxo-2-phenylthieno[2,3-*b*]pyridine-5-carboxylate (4c). Compound **4c** was prepared in 91% yield from **3c** by a procedure similar to that described for **4a**, as an amorphous powder. ^{1}H NMR ($CDCl_3$): δ 1.41 (3H, t, J = 7.1 Hz), 2.69 (3H, s), 3.87 (3H, s), 4.40 (2H, q, J = 7.1 Hz), 5.17 (2H, s), 6.92−7.00 (2H, m), 7.22−7.44 (7H, m), 8.43 (1H, s). Anal. ($C_{25}H_{23}NO_4S\cdot0.4H_2O$) C, H, N.

Ethyl 3-(*N*-Benzyl-*N*-methylaminomethyl)-4,7-dihydro-7-(2-methoxybenzyl)-2-(4-methoxyphenyl)-4-oxothieno[2,3-*b*]pyridine-5-carboxylate Hydrochloride (5a). A mixture of **4a** (0.35 g, 0.76 mmol), *N*-bromosuccinimide (NBS; 0.135 g, 0.76 mmol), and 2,2′-azobis(isobutyronitrile) (AIBN; 13 mg, 0.079 mmol) in CCl_4 (5 mL) was heated under reflux for 2 h. After being cooled, an insoluble material was removed by filtration and the residue was washed with a small portion of $CHCl_3$. The filtrate was diluted with $CHCl_3$ and washed with brine. The aqueous phase was re-extracted with $CHCl_3$. The extract was washed with brine and dried ($MgSO_4$). After removal of the solvent in vacuo, the residue was purified by flash column chromatography ($CHCl_3$−MeOH, 99:1) to provide the corresponding bromomethyl compound (0.37 g, 90%) as a pale-yellow solid. Recrystallization from EtOAc gave colorless needles. Mp: 200−201 °C. ^{1}H NMR ($CDCl_3$): δ 1.40 (3H, t, J = 7.1 Hz), 3.86 (6H, s), 4.40 (2H, q, J = 7.1 Hz), 5.05 (2H, s), 5.16 (2H, s), 6.92−7.04 (4H, m), 7.23−7.28 (1H, m), 7.34−7.43 (1H, m), 7.57 (2H, d, J = 8.9 Hz), 8.46 (1H, s). IR (KBr): 2980, 1725, 1607, 1588, 1497 cm^{-1}.

N-Benzylmethylamine (1.05 mL, 8.14 mmol) was added to a mixture of the bromomethyl compound (4.0 g, 7.37 mmol) and *N*,*N*-diisopropylethylamine (1.54 mL, 8.84 mmol) in DMF (70 mL). After being stirred at room temperature for 1 h, the mixture was concentrated in vacuo and saturated $NaHCO_3$ was added to the residue. The resulting mixture was extracted with EtOAc, and the extract was washed with brine and dried ($MgSO_4$). The solution was concentrated in vacuo, and the residue was purified by flash column chromatography (CH_2Cl_2−MeOH, 99:1 to 4:1) to afford the free amine of **5a** (3.30 g, 77%) as a white amorphous powder. ^{1}H NMR (free amine in $CDCl_3$): δ 1.39 (3H, t, J = 7.1 Hz), 2.19 (3H, brs), 3.73 (2H, brs), 3.86 (3H, s), 3.87 (3H, s), 4.20 (2H, s), 4.39 (2H, q, J = 7.1 Hz), 5.18 (2H, s), 6.93−7.01 (4H, m), 7.10−7.32 (5H, m), 7.37 (1H, t, J = 3.9 Hz), 7.74 (2H, d, J = 4.4 Hz), 8.41 (1H, s). The free amine was treated with 1 M ethereal HCl to afford **5a** as a white crystalline powder (from EtOAc−Et_2O). Mp: 147−152 °C. IR (KBr): 1719, 1605, 1502, 1253 cm^{-1}. FAB-MS *m*/*z*: 583 (M + H). Anal. ($C_{34}H_{34}N_2O_5S$·HCl·1.8H_2O) C, H, N.

Compounds **5b**−**h** were prepared by a procedure similar to that described for **5a**, and the physicochemical data are shown in Table 1 (**5d**−**h**) and Table 3 (**5b**,**c**).

Ethyl 3-(*N*-Acetyl-*N*-benzylaminomethyl)-4,7-dihydro-7-(2-methoxybenzyl)-2-(4-methoxyphenyl)-4-oxothieno[2,3-*b*]pyridine-5-carboxylate (5i). Sodium hydride (60% in oil; 27 mg, 0.675 mmol) was added to a solution of *N*-benzylacetamide (99 mg, 0.66 mmol) in DMF (3 mL) at 0 °C under a nitrogen atmosphere. After being stirred at 0 °C for 30 min, a solution of the intermediary bromomethyl compound (0.30 g, 0.55 mmol) obtained in the preparation of **5a** in DMF (8 mL) was added to the mixture. Then, the resulting mixture was stirred at room temperature for 4 h. After removal of the solvent in vacuo, aqueous NH_4Cl was added to the residue and the resulting mixture was extracted with EtOAc. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography ($CHCl_3$−MeOH, 99:1 to 97:3) to provide **5i** (56 mg, 17%) as a pale-yellow oil. Crystallization from EtOAc−^{i}Pr_2O afforded pale-yellow crystals. Mp: 77−82 °C. ^{1}H NMR ($CHCl_3$): δ 1.38, 1.41 (3H, each t, J = 7.1 Hz), 1.64, 2.16 (3H, each s), 3.84 (3H, s), 3.86, 3.88 (3H, each s), 4.27 (2H, s), 4.37, 4.40 (2H, each q, J = 7.1 Hz), 5.05, 5.37 (2H, each s), 5.11, 5.13 (2H, each s), 6.81−7.02 (8H, m), 7.13−7.21 (3H, m), 7.30−7.45 (2H, m), 8.38, 8.40 (1H, each s). Anal. ($C_{35}H_{34}N_2O_6S$·0.5EtOAc) C, H, N.

Ethyl 7-Benzoyl-4,7-dihydro-2-(4-methoxyphenyl)-3-methyl-4-oxothieno[2,3-*b*]pyridine-5-carboxylate (6). Benzoyl chloride (1.78 mL, 15.3 mmol) was added dropwise to a mixture of **3a** (5.0 g, 14.6 mmol) in pyridine (100 mL) at 0 °C. After being stirred at room temperature for 2.5 h, the reaction was quenched by adding EtOH (1 mL). After evaporation of the solvent in vacuo, water was added to the residue and the resulting mixture was extracted with CH_2Cl_2. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography (CH_2Cl_2−MeOH, 95:5) to give a white amorphous powder. Crystallization from EtOAc afforded **6** (6.41 g, 98%) as white crystals. Mp: 110−112 °C. ^{1}H NMR ($CDCl_3$): δ 1.14 (3H, t, J = 7.7 Hz), 2.42 (3H, s), 3.85 (3H, s), 4.26 (2H, q, J = 7.2 Hz), 6.98 (2H, d, J = 6.7 Hz), 7.40 (2H, d, J = 8.9 Hz), 7.57 (2H, t, J = 7.6 Hz), 7.70 (1H, t, J = 5.9 Hz), 8.27 (2H, d, J = 7.0 Hz), 9.14 (1H, s). IR (KBr): 2972, 1717, 1607, 1580, 1522, 1502, 1454 cm^{-1}. Anal. ($C_{25}H_{21}NO_5S$) C, H, N.

Ethyl 3-(*N*-Benzyl-*N*-methylaminomethyl)-4-hydroxy-2-(4-methoxyphenyl)thieno[2,3-*b*]pyridine-5-carboxylate (8). A mixture of **6** (6.39 g, 14.3 mmol), NBS (2.67 g, 15.0 mmol), and AIBN (0.47 g, 2.86 mmol) in CCl_4 (100 mL) was heated under reflux for 1 h. After being cooled, the mixture was poured into water and extracted with $CHCl_3$. The extract was washed with brine and dried ($MgSO_4$). The solution was concentrated in vacuo to provide the corresponding bromomethyl compound as a crystalline solid, which was dissolved in DMF (100 mL). Then, *N*,*N*-diisopropylethylamine (2.52 mL, 14.7 mmol) and *N*-benzylmethylamine (1.9 mL, 14.7 mmol) were added successively to the DMF solution. After being stirred at room temperature for 40 min, the mixture was concentrated in vacuo. Water was added to the residue and the resulting mixture was extracted with CH_2Cl_2. The extract was washed with brine and dried ($MgSO_4$). Evaporation of the solvent in vacuo afforded the crude **7** as a brown oil, which was dissolved in CH_2Cl_2 (100 mL) and EtOH (50 mL). A solution of sodium ethoxide (0.69 g, 10.1 mmol) in EtOH (50 mL) was added to the solution at 0 °C. After being stirred at room temperature for 4 h, the reaction mixture was neutralized with AcOH. After removal of the solvent in vacuo, water was added to the residue and the resulting mixture was extracted with $CHCl_3$. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography (CH_2Cl_2−MeOH, 95:5) to provide a white amorphous powder. Crystallization from EtOAc gave **8** (8.72 g, 61% from **6**) as white crystals. Mp: 175−177 °C. ^{1}H NMR ($CDCl_3$): δ 1.45 (3H, t, J = 7.2 Hz), 2.35 (3H, s), 3.75 (2H, s), 3.89 (3H, s), 3.92 (2H, s), 4.44 (2H, q, J = 7.2 Hz), 7.01 (2H, d, J = 6.7 Hz), 7.21−7.37 (7H, m), 8.87 (1H, s). IR (KBr): 3424, 3000, 1686, 1607, 1504 cm^{-1}. Anal. ($C_{26}H_{26}N_2O_4S$) C, H, N.

Ethyl 3-(*N*-Benzyl-*N*-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-2-(4-methoxyphenyl)-4-oxothieno[2,3-*b*]pyridine-5-carboxylate Hydrochloride (9j). Compound **9j** was prepared in 66% yield (free amine) from **8** by a procedure similar to that described for **4a**. Mp: 189−192 °C (from EtOAc−^{i}Pr_2O, HCl salt). ^{1}H NMR (free amine in $CDCl_3$): δ 1.42 (3H, t, J = 6.9 Hz), 2.91 (3H, s), 3.88 (3H, s), 4.37−4.70 (6H, m), 5.45 (2H, s), 6.95−7.07 (4H, m), 7.28−7.32 (3H, m), 7.42−7.52 (8H, m), 8.64 (1H, m). IR (KBr): 3574, 1725, 1605, 1504, 1473, 1259, 1180, 1035 cm^{-1}. FAB-MS *m*/*z*: 589 (M + H). Anal. ($C_{33}H_{30}N_2O_4SF_2$·HCl·1.5H_2O) C, H, N.

Compounds **9a**−**i** were prepared by a procedure similar to that described for **4a**, and the physicochemical data are shown in Table 2.

Ethyl *N*-[3-Carboxy-4-methyl-2-thienyl]aminomethylenemalonate (11). Compound **11** was prepared in 86% yield from **10** (two steps) by a procedure similar to that described for **2a**. Mp: 187−189 °C (from EtOH−H_2O). ^{1}H NMR (DMSO-d_6): δ 1.24 (3H, t, J = 7.1 Hz), 1.27 (3H, t, J = 7.1 Hz), 2.30 (3H, s), 4.14 (2H, q, J = 7.1 Hz), 4.23 (2H, q, J = 7.1 Hz), 6.68 (1H, s), 8.08 (1H, d, J = 13.4 Hz), 12.54 (1H, d, J = 13.4 Hz).

Ethyl *N*-(5-Bromo-3-carboxy-4-methyl-2-thienyl)aminomethylenemalonate (12). A solution of bromine (5.8 mL, 0.113 mol) in $CHCl_3$ (50 mL) was added dropwise to a mixture of **11** (30.3 g, 92.6 mmol) and pyridine (38 mL, 0.47 mol) in $CHCl_3$ (200 mL). After being stirred at room temperature for 40 min, the mixture was concentrated in vacuo and the residue was treated with dilute HCl. The precipitate was collected by filtration, washed with water and a small portion of Et_2O, and dried to afford **12** (34.1 g, 91%) as a brown crystalline powder, which was used in the following reaction without further purification. Mp: 182−185 °C (dec). ^{1}H NMR (DMSO-d_6): δ 1.25 (3H, t, J = 7.1 Hz), 1.26 (3H, t, J = 7.1 Hz), 2.27 (3H, s), 4.15 (2H, q, J = 7.1 Hz), 4.23 (2H, q, J = 7.1 Hz), 7.93 (1H, d, J = 13.4 Hz), 12.38 (1H, d, J = 13.2 Hz). IR

(KBr): 3120, 2984, 1688, 1634, 1574, 1547, 1499 cm^{-1}. FAB-MS *m/z*: 406 (M + H; ^{79}Br), 408 (M + H; ^{81}Br).

Ethyl 2-Bromo-4-hydroxy-3-methythieno[2,3-*b*]pyridine-5-carboxylate (13). Compound **13** was prepared in 61% yield from **12** by a procedure similar to that described for **3a**. Mp: 214−216 °C (from EtOH). ^1H NMR ($CDCl_3$−CD_3OD): δ 1.47 (3H, t, J = 7.1 Hz), 2.60 (3H, s), 4.50 (2H, q, J = 7.1 Hz), 8.82 (1H, s). Anal. ($C_{11}H_{10}NO_3SBr$) C, H, N, S.

Ethyl 2-Bromo-4,7-dihydro-7-(2-methoxybenzyl)-3-methyl-4-oxothieno[2,3-*b*]pyridine-5-carboxylate (14) 2-Methoxybenzyl chloride (1.60 g, 10.2 mmol) was added to a mixture of **13** (2.15 g, 6.80 mmol), K_2CO_3 (1.41 g, 10.2 mmol), and KI (0.56 g, 3.37 mmol) in DMF (25 mL). The resulting mixture was stirred at room temperature for 30 min and at 50 °C for a further 2 h. After being cooled, the mixture was concentrated in vacuo. Water was added to the residue and the resulting mixture was extracted with EtOAc−THF (4:1). The extract was washed with brine and dried ($MgSO_4$). After evaporation of the solvent in vacuo, the residue was purified by flash column chromatography ($CHCl_3$−MeOH, 99:1 to 98:2) to give **14** (2.70 g, 91%) as a pale-yellow solid. Recrystallization from EtOAc−Et_2O afforded white crystals. Mp: 161−163 °C. ^1H NMR ($CDCl_3$): δ 1.40 (3H, t, J = 7.1 Hz), 2.60 (3H, s), 3.85 (3H, s), 4.38 (2H, q, J = 7.1 Hz), 5.09 (2H, s), 6.92−7.02 (2H, m), 7.19−7.24 (1H, m), 7.35−7.44 (1H, m), 8.39 (1H, s). IR (KBr): 2926, 1727, 1690, 1609, 1495, 1439 cm^{-1}. FAB-MS *m/z*: 436 (M + H; ^{79}Br), 438 (M + H; ^{81}Br). Anal. ($C_{19}H_{18}NO_4SBr$) C, H, N.

Ethyl 4,7-Dihydro-7-(2-methoxybenzyl)-2-(3-methoxyphenyl)-3-methyl-4-oxothieno[2,3-*b*]pyridine-5-carboxylate (15). Tetrakis-(triphenylphosphine)palladium(0) (0.163 g, 0.141 mmol) was added to a mixture of **14** (0.615 g, 1.41 mmol), 3-methoxyphenylboronic acid (0.535 g, 2.11 mmol), and 2 M Na_2CO_3 (3.53 mL, 7.06 mmol) in 1,2-dimethoxyethane (30 mL) under an argon atmosphere. The resulting mixture was refluxed with stirring under an argon atmosphere for 2 h. After being cooled, the mixture was diluted with EtOAc and an insoluble material was removed by filtration through Celite. The filtrate was diluted with water and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). After evaporation of the solvent in vacuo, the residue was purified by flash column chromatography (EtOAc−hexane 7:3 to 9:1, EtOAc, and then EtOAc−MeOH 99:5 to 9:1) to afford **15** (0.446 g, 68%) as a white amorphous powder. ^1H NMR ($CDCl_3$): δ 1.41 (3H, t, J = 7.1 Hz), 2.69 (3H, s), 3.84 (3H, s), 3.87 (3H, s), 4.39 (2H, q, J = 7.1 Hz), 5.16 (2H, s), 6.87−7.02 (5H, m), 7.22−7.42 (3H, m), 8.42 (1H, s). IR (KBr): 3440, 2938, 1727, 1688, 1607, 1493, 1465 cm^{-1}. Anal. ($C_{26}H_{25}NO_5S\cdot0.5H_2O$) C, H, N.

Ethyl 3-(*N*-Benzyl-*N*-methylaminomethyl)-4,7-dihydro-7-(2-methoxybenzyl)-2-(3-methoxyphenyl)-4-oxothieno[2,3-*b*]pyridine-5-carboxylate Hydrochloride (16). Compound **16** was prepared in 65% yield from **15** (two steps) by a procedure similar to that described for **5a**. Mp: 115−120 °C (from EtOAc−Et_2O, HCl salt). ^1H NMR (free amine in $CDCl_3$): δ 1.39 (3H, t, J = 7.1 Hz), 2.16 (3H, brs), 3.69 (2H, brs), 3.80 (3H, s), 3.87 (3H, s), 4.23 (2H, s), 4.39 (2H, q, J = 7.1 Hz), 5.18 (2H, s), 6.90−7.00 (3H, m), 7.12−7.42 (9H, m), 7.68 (1H, brs), 8.40 (1H, s). Anal. ($C_{34}H_{34}N_2O_5S\cdot HCl\cdot1.5H_2O$) C, H, N.

Ethyl 4-Hydroxy-3-methyl-2-(4-nitrophenyl)thieno[2,3-*b*]pyridine-5-carboxylate (17). A solution of sodium nitrate (1.87 g, 22.0 mmol) in concentrated H_2SO_4 (2 mL) was added dropwise to a solution of **3c** (6.27 g, 20.0 mmol) in concentrated H_2SO_4 (20 mL) at 0 °C. After being stirred at 0 °C for 30 min, the mixture was poured into ice−water and extracted with $CHCl_3$. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography (EtOAc−hexane, 1:4) to give **17** (4.20 g, 55%) as a yellow solid. Recrystallization from EtOAc−hexane provided yellow crystals. Mp: 260−261 °C. ^1H NMR ($CDCl_3$): δ 1.49 (3H, t, J = 7.2 Hz), 2.70 (3H, s), 4.51 (2H, q, J = 7.2 Hz), 7.71 (2H, d, J = 8.9 Hz), 8.34 (2H, d, J = 8.9 Hz), 8.89 (1H, s). IR (KBr): 3002, 1692, 1605, 1514, 1350, 1290 cm^{-1}. FAB-MS *m/z*: 359 (M + H). Anal. ($C_{17}H_{14}N_2O_5S$) C, H, N, S.

Ethyl 4,7-Dihydro-7-(2-fluorobenzyl)-3-methyl-2-(4-nitrophenyl)-4-oxothieno[2,3-*b*]pyridine-5-carboxylate (18). Compound **18** was prepared in 60% yield from **17** by a procedure similar to that described for **14**. Mp: 184−186 °C (from EtOH). ^1H NMR ($CDCl_3$): δ 1.41 (3H, t, J = 7.1 Hz), 2.74 (3H, s), 4.41 (2H, q, J = 7.1 Hz), 5.26 (2H, s), 7.12−7.28 (3H, m), 7.35−7.48 (1H, m), 7.58 (2H, d, J = 8.6 Hz), 8.28 (2H, d, J = 8.6 Hz), 8.39 (1H, s). Anal. ($C_{24}H_{19}N_2O_5SF$) C, H, N.

Ethyl 3-(*N*-Benzyl-*N*-methylaminomethyl)-4,7-dihydro-7-(2-fluorobenzyl)-2-(4-nitrophenyl)-4-oxothieno[2,3-*b*]pyridine-5-carboxylate Hydrochloride (19). Compound **19** was prepared in 41% yield from **18** (two steps) by a procedure similar to that described for **5a**. Mp: 140−144 °C (from CH_2Cl_2−Et_2O, HCl salt). ^1H NMR (free amine in $CDCl_3$): δ 1.40 (3H, t, J = 7.1 Hz), 2.20 (3H, s), 3.67 (2H, s), 4.22 (2H, s), 4.41 (2H, q, J = 7.1 Hz), 5.27 (2H, s), 7.10−7.28 (8H, m), 8.07 (2H, d, J = 8.9 Hz), 8.07 (2H, d, J = 8.9 Hz), 8.36 (1H, m). Anal. ($C_{32}H_{28}N_3O_5SF\cdot HCl\cdot0.85H_2O\cdot0.1CH_2Cl_2$) C, H, N.

Ethyl 2-(4-Aminophenyl)-3-(*N*-benzyl-*N*-methylaminomethyl)-4,7-dihydro-7-(2-fluorobenzyl)-4-oxothieno[2,3-*b*]pyridine-5-carboxylate (20). Concentrated HCl (10 mL) was added dropwise to a mixture of **19** (3.29 g, 5.0 mmol) and iron powder (1.12 g, 20.0 mmol) in EtOH (10 mL) at 0 °C. After being stirred at 0 °C for 1 h, the mixture was poured into ice−water and treated with 5 N NaOH. The resulting suspension was diluted with $CHCl_3$ and an insoluble material was removed by filtration through Celite. The aqueous phase was separated and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with brine and dried ($MgSO_4$). After removal of the solvent in vacuo, the residue was purified by flash column chromatography ($CHCl_3$−MeOH, 9:1) to give **20** (2.18 g, 79%) as a yellow solid. Recrystallization from $CHCl_3$−Et_2O afforded a pale-yellow crystalline powder. Mp: 158−160 °C. ^1H NMR ($CDCl_3$): δ 1.35 (3H, t, J = 7.2 Hz), 2.04 (3H, s), 3.65 (2H, s), 4.17 (2H, s), 4.35 (2H, q, J = 7.2 Hz), 5.47 (2H, s), 6.74 (2H, d, J = 8.4 Hz), 7.07−7.28 (7H, m), 7.30−7.50 (4H, m), 8.64 (1H, s). IR (KBr): 3330, 1723, 1607, 1495, 1446, 1377, 1311 cm^{-1}. FAB-MS *m/z*: 556 (M + H). Anal. ($C_{32}H_{30}N_3O_3SF\cdot0.5H_2O$) C, H, N.

Ethyl 3-(*N*-Benzyl-*N*-methylaminomethyl)-4,7-dihydro-7-(2-fluorobenzyl)-4-oxo-2-(4-propionylamidophenyl)thieno[2,3-*b*]-pyridine-5-carboxylate (21b). A solution of propionic anhydride (0.26 mL, 2.0 mmol) in CH_2Cl_2 (1 mL) was added dropwise to a solution of **20** (0.11 g, 0.20 mmol) and triethylamine (0.56 mL, 4.0 mmol) in CH_2Cl_2 (2 mL) at 0 °C. After being stirred at room temperature for 6 h, the mixture was poured into ice−water and extracted with $CHCl_3$. The extract was washed with brine and dried ($MgSO_4$). The solution was concentrated in vacuo, and the residue was purified by flash column chromatography ($CHCl_3$−MeOH, 9:1) to afford **21b** (0.12 g, quant.) as a pale-yellow solid. Recrystallization from EtOH−Et_2O gave white needles. Mp: 221−223 °C. ^1H NMR ($CDCl_3$): δ 1.22 (3H, t, J = 7.5 Hz), 1.41 (3H, t, J = 7.0 Hz), 2.44 (2H, q, 7.5 Hz), 2.56 (3H, s), 4.16 (2H, s), 4.40 (2H, q, J = 7.0 Hz), 4.58 (2H, s), 5.61 (2H, s), 7.20−7.40 (7H, m), 7.40−7.55 (4H, m), 7.75 (2H, d, J = 8.7 Hz), 8.89 (1H, s). IR (KBr): 2970, 1692, 1605, 1502, 1446, 1385, 1321, 1270, 1183 cm^{-1}. FAB-MS *m/z*: 612 (M + H). Anal. ($C_{35}H_{34}N_3O_4SF\cdot3.0H_2O$) C, H, N.

Compounds **21a**,**c**,**d** were prepared by a procedure similar to that described for **21b**, and the physicochemical data are shown in Table 3.

Ethyl 3-(*N*-Benzyl-*N*-methylaminomethyl)-4,7-dihydro-7-(2-fluorobenzyl)-2-[4-(3-methylureido)phenyl]-4-oxothieno[2,3-*b*]-pyridine-5-carboxylate (21e). Methyl isocyanate (0.32 mL, 5.4 mmol) was added dropwise to a solution of **20** (0.57 g, 1.0 mmol) and pyridine (1 mL) in THF (10 mL) at 0 °C. After stirring at room temperature for 10 h, the mixture was concentrated in vacuo. Water was added to the residue and the resulting mixture was extracted with CH_2Cl_2. The extract was washed with brine and dried ($MgSO_4$). After evaporation of the solvent in vacuo, the residue was purified by flash column chromatography ($CHCl_3$−MeOH, 95:5) to give **21e** (0.46 g, 73%) as a pale-yellow amorphous powder. Crystallzation from EtOH−EtOAc provided a pale-yellow powder. Mp: 216−220 °C. ^1H NMR ($CDCl_3$): δ 1.44 (3H, t, J = 7.3 Hz),

2.25 (3H, s), 2.84 (3H, s), 4.35 (2H, s), 4.43 (2H, q, J = 7.2 Hz), 4.90 (2H, s), 5.61 (2H, s), 7.25−7.65 (13H, m), 8.85 (1H, s). Anal. ($C_{34}H_{33}N_4O_4SF\cdot 2.0H_2O$) C, H, N.

Compounds **21f** were prepared by a procedure similar to that described for **21e**, and the physicochemical data are shown in Table 3.

Ethyl 3-(*N*-Benzyl-*N*-methylaminomethyl)-4,7-dihydro-2-[4-(1,3-dimethylureido)phenyl]-7-(2-fluorobenzyl)-4-oxothieno[2,3-*b*]pyridine-5-carboxylate (21g). Iodomethane (14.2 mg, 0.10 mmol) was added dropwise to a mixture of **21e** (30.6 mg, 0.05 mmol) and K_2CO_3 (6.90 mg, 0.05 mmol) in DMF (1 mL) at 0 °C. After being stirred at room temperature for 14 h, the mixture was poured into ice−water and extracted with $CHCl_3$. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography ($CHCl_3$−MeOH, 9:1) to give **21g** (10 mg, 32%) as a pale-yellow solid. Recrystallization from EtOH−EtOAc afforded a pale-yellow crystalline powder. Mp: >300 °C. ^{1}H NMR (CD_3OD): δ 1.29 (3H, s), 1.41 (3H, t, J = 7.2 Hz), 2.70 (3H, s), 2.80 (3H, s), 3.62 (2H, s), 4.43 (2H, q, J = 7.2 Hz), 4.52 (2H, s), 5.70 (2H, s), 7.17−7.36 (4H, m), 7.39−7.55 (9H, m), 8.96 (1H, s). IR (KBr): 3394, 1690, 1603, 1543, 1499, 1386, 1319 cm^{-1}. FAB-MS m/z: 627 (M + H). Anal. ($C_{35}H_{35}N_4O_4SF\cdot H_2O$) C, H, N.

Ethyl 7-(2,6-Difluorobenzyl)-4,7-dihydro-3-methyl-4-oxo-2-phenylthieno[2,3-*b*]pyridine-5-carboxylate (22). Compound **22** was prepared in 90% yield from **3c** by a procedure similar to that described for **14**. Mp: 171−173 °C (from EtOAc−Et_2O). ^{1}H NMR ($CDCl_3$): δ 1.41 (3H, t, J = 6.9 Hz), 2.68 (3H, s), 4.40 (2H, q, J = 6.9 Hz), 5.26 (2H, s), 7.00 (2H, t, J = 10.8 Hz), 7.34−7.48 (6H, m), 8.39 (1H, s). IR (KBr): 1680, 1624, 1497, 1466 cm^{-1}. FAB-MS m/z: 440 (M + H). Anal. ($C_{24}H_{19}NO_3SF_2$) C, H, N.

Ethyl 7-(2,6-Difluorobenzyl)-4,7-dihydro-3-methyl-2-(4-nitrophenyl)-4-oxothieno[2,3-*b*]pyridine-5-carboxylate (23). Compound **23** was prepared in 71% yield from **22** by a procedure similar to that described for **17**. Mp: 215−217 °C (from EtOAc−$CHCl_3$−Et_2O). ^{1}H NMR ($CDCl_3$): δ 1.42 (3H, t, J = 7.2 Hz), 2.73 (3H, s), 4.41 (2H, q, J = 7.2 Hz), 5.27 (2H, s), 7.02 (2H, t, J = 8.1 Hz), 7.38−7.49 (1H, m), 7.60 (2H, d, J = 8.7 Hz), 8.30 (2H, d, J = 8.7 Hz), 8.41 (1H, s). IR (KBr): 1688, 1628, 1518, 1493, 1470, 1348 cm^{-1}. FAB-MS m/z: 485 (M + H). Anal. ($C_{24}H_{18}N_2O_5SF_2\cdot 0.5H_2O$) C, H, N.

Ethyl 3-(*N*-Benzyl-*N*-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-2-(4-nitrophenyl)-4-oxothieno[2,3-*b*]pyridine-5-carboxylate (24). Compound **24** was prepared in 72% yield from **23** (two steps) by a procedure similar to that described for **5a**. Mp: 145−147 °C (from CH_2Cl_2−Et_2O, free amine). ^{1}H NMR ($CDCl_3$): δ 1.40 (3H, t, J = 7.2 Hz), 2.19 (3H, s), 3.67 (2H, s), 4.21 (2H, s), 4.41 (2H, q, J = 7.2 Hz), 5.28 (2H, s), 7.03 (2H, t, J = 8.1 Hz), 7.12−7.23 (5H, m), 7.39−7.50 (1H, m), 8.10 (2H, d, J = 9.0 Hz), 8.26 (2H, d, J = 9.0 Hz), 8.39 (1H, s). Anal. ($C_{32}H_{27}N_3O_5SF_2\cdot 0.1H_2O$) C, H, N.

Ethyl 2-(4-Aminophenyl)-3-(*N*-benzyl-*N*-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-4-oxothieno[2,3-*b*]pyridine-5-carboxylate (25). Compound **25** was prepared in 96% yield from **24** by a procedure similar to that described for **20**. Mp: 195−196 °C (from EtOAc). ^{1}H NMR ($CDCl_3$): δ 1.25 (3H, t, J = 7.2 Hz), 2.11 (3H, s), 3.65 (2H, s), 4.14 (2H, s), 4.40 (2H, q, J = 7.2 Hz), 5.25 (2H, s), 6.73 (2H, d, J = 8.7 Hz), 7.00 (2H, t, J = 7.8 Hz), 7.11−7.27 (5H, m), 7.35−7.46 (1H, m), 7.64 (2H, d, J = 8.7 Hz), 8.33 (1H, s). IR (KBr): 3352, 1682, 1607, 1495, 1473 cm^{-1}. FAB-MS m/z: 574 (M + H). Anal. ($C_{32}H_{29}N_3O_3SF_2\cdot 0.25H_2O$) C, H, N.

Ethyl 3-(*N*-Benzyl-*N*-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-4-oxo-2-(4-propionylamidophenyl)thieno[2,3-*b*]pyridine-5-carboxylate (26a). Compound **26a** was prepared quantitatively from **25** by a procedure similar to that described for **21b**. Mp: 100−102 °C (from EtOAc−hexane, free amine). ^{1}H NMR ($CDCl_3$): δ 0.88 (3H, t, J = 6.6 Hz), 1.37 (3H, t, J = 7.2 Hz), 2.11 (3H, s), 2.42 (2H, q, J = 6.6 Hz), 3.64 (2H, s), 4.37 (2H, q, J = 7.2 Hz), 4.14 (2H, s), 5.26 (2H, s), 6.97−7.75 (12H, m), 8.00 (1H, s), 8.36 (1H, s). Anal. ($C_{35}H_{33}N_3O_4SF_2\cdot 0.75H_2O$) C, H, N.

Ethyl 3-(*N*-Benzyl-*N*-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-2-(4-isobutyrylamidophenyl)-4-oxothieno[2,3-*b*]pyridine-5-carboxylate Hydrochloride (26b). The free amine of compound **26b** was prepared quantitatively from **25** by a procedure similar to that described for **21b**, and compound **26b** was obtained from the free amine in the usual manner. Mp: 185−186 °C (from EtOH−Et_2O, HCl salt). ^{1}H NMR (free amine in $CDCl_3$): δ 1.28 (6H, d, J = 6.8 Hz), 1.39 (3H, t, J = 7.1 Hz), 2.12 (3H, s), 2.44 (2H, q, J = 7.2 Hz), 2.50−2.60 (1H, m), 3.65 (2H, s), 4.16 (2H, s), 4.40 (2H, q, J = 7.1 Hz), 5.26 (2H, s), 7.01 (2H, t, J = 8.1 Hz), 7.10−7.30 (5H, m), 7.35−7.47 (5H, m), 7.62 (2H, d, J = 8.5 Hz), 7.81 (2H, d, J = 8.5 Hz), 8.35 (1H, s). Anal. ($C_{36}H_{35}N_3O_4SF_2\cdot HCl\cdot 1.5H_2O$) C, H, N.

Ethyl 3-(*N*-Benzyl-*N*-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-2-[4-(3-methylureido)phenyl]-4-oxothieno[2,3-*b*]pyridine-5-carboxylate (26c). Compound **26c** was prepared in 79% yield from **25** by a procedure similar to that described for **21e**. Mp: 214−215 °C (from EtOH−EtOAc, free amine). ^{1}H NMR ($CDCl_3$): δ 1.37 (3H, t, J = 7.2 Hz), 2.00 (3H, s), 2.85 (3H, d, J = 4.5 Hz), 3.52 (2H, s), 4.07 (2H, s), 4.37 (2H, q, J = 7.2 Hz), 5.25 (2H, s), 5.80 (1H, br), 6.99 (2H, t, J = 8.4 Hz), 7.02−7.12 (5H, m), 7.36−7.47 (1H, m), 7.45 (2H, d, J = 8.7 Hz), 7.64 (2H, d, J = 8.7 Hz), 7.72 (1H, br), 8.38 (1H, s). IR (KBr): 3362, 1607, 1499, 1473, 1317, 1238, 1183 cm^{-1}. FAB-MS m/z: 631 (M + H). Anal. ($C_{34}H_{32}N_4O_4SF_2$) C, H, N.

Isopropyl 3-(*N*-Benzyl-*N*-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-2-(4-isobutyrylamidophenyl)-4-oxothieno-[2,3-*b*]pyridine-5-carboxylate Hydrochloride (26d). Titanium(IV) isopropoxide (0.30 mL, 1.0 mmol) was added dropwise to a solution of **26b** (1.29 g, 2.0 mmol) in 2-propanol (50 mL) at 0 °C. After being stirred at room temperature for 12 h, the reaction mixture was poured into a mixture of $CHCl_3$ and water with vigorous stirring. An insoluble material was removed by filtration through Celite. The filtrate was diluted with brine and extracted with $CHCl_3$. The extract was washed with brine and dried (Na_2SO_4). After removal of the solvent in vacuo, the residue was purified by flash column chromatography ($CHCl_3$−MeOH, 5:1). The crude product was recrystallized from EtOAc to provide the free amine of **26d** (1.08 g, 82%) as a white crystalline powder. ^{1}H NMR (free amine in $CDCl_3$): δ 1.28 (6H, d, J = 6.8 Hz), 1.36 (6H, d, J = 6.3 Hz), 2.10 (3H, s), 2.53−2.61 (1H, m), 3.65 (2H, s), 4.16 (2H, s), 5.19−5.27 (1H, m), 5.23 (2H, s), 7.00 (2H, t, J = 8.1 Hz), 7.10−7.26 (5H, m), 7.34−7.42 (1H, m), 7.63 (2H, d, J = 8.3 Hz), 7.78 (2H, d, J = 8.6 Hz), 8.29 (1H, s). The free amine was treated with 10 M ethanolic HCl to give **26d** as colorless needles (from EtOH−Et_2O). Mp: 168−170 °C. IR (KBr): 3400, 2976, 1690, 1603, 1504, 1473 cm^{-1}. FAB-MS m/z: 658 (M + H). Anal. ($C_{37}H_{37}N_3O_4SF_2\cdot HCl\cdot 0.5H_2O$) C, H, N.

3-(*N*-Benzyl-*N*-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-*N*-isopropyl-4-oxo-2-(4-propionylamidophenyl)-thieno[2,3-*b*]pyridine-5-carboxamide (27a). Compound **27a** was prepared in 56% yield from **26a** by a procedure similar to that described for **27b** (see below). Mp: 144−146 °C (from EtOAc−hexane, free amine). ^{1}H NMR ($CDCl_3$): δ 1.25−1.31 (9H, m), 2.04 (3H, s), 2.43 (2H, q, J = 7.2 Hz), 3.63 (2H, s), 4.13 (2H, s), 4.24−4.30 (1H, m), 5.28 (2H, s), 6.95−7.72 (12H, m), 8.67 (1H, s), 10.20 (2H, d, J = 7.2 Hz). Anal. ($C_{36}H_{36}N_4O_3SF_2\cdot 3.5H_2O$) C, H, N.

3-(*N*-Benzyl-*N*-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-2-(4-isobutyrylamidophenyl)-*N*-methoxy-*N*-methyl-4-oxothieno[2,3-*b*]pyridine-5-carboxamide (27b). Trimethylaluminum (15% solution in hexane; 9.64 mL, 20.0 mmol) was added dropwise to a mixture of *N*,*O*-dimethylhydroxylamine hydrochloride (1.95 g, 20.0 mmol) and *N*,*N*-diisopropylethylamine (3.48 mL, 20.0 mmol) in CH_2Cl_2 (50 mL) at 0 °C. After the mixture was stirred at 0 °C for 1 h, a solution of **26b** (5.14 g, 7.98 mmol) in CH_2Cl_2 (20 mL) was added dropwise to the mixture. Then, the reaction mixture was stirred at 0 °C for a further 1 h. The mixture was poured into ice−water and extracted with $CHCl_3$. The extract was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was crystallized from Et_2O−petroleum ether to afford **27b** (4.54 g, 86%) as a yellow crystalline powder. Mp: 152−154 °C. ^{1}H NMR

($CDCl_3$): δ 1.28 (6H, d, J = 7.0 Hz), 2.13 (3H, s), 2.53−2.62 (1H, m), 3.34 (3H, s), 3.62 (2H, s), 3.73 (3H, s), 4.16 (2H, s), 5.21 (2H, s), 6.99 (2H, t, J = 8.1 Hz), 7.15−7.30 (5H, m), 7.34−7.44 (1H, m), 7.62 (2H, d, J = 8.6 Hz), 7.72 (1H, s), 7.83 (2H, d, J = 8.5 Hz). IR (KBr): 1620, 1497, 1473 cm^{-1}. FAB-MS *m*/*z*: 659 (M + H). Anal. ($C_{36}H_{36}N_4O_4SF_2$) C, H, N.

3-(*N*-Benzyl-*N*-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-2-(4-isobutyrylamidophenyl)-4-oxo-5-propionylthieno[2,3-*b*]pyridine Hydrochloride (28a). Ethylmagnesium chloride (2.0 M solution in THF; 0.92 mL, 1.84 mmol) was added dropwise to a suspension of **27b** (0.30 g, 0.46 mmol) in dry THF (10 mL) at 0 °C under an argon atmosphere. The reaction mixture was stirred at 0 °C under an argon atmosphere for 30 min. Then, the mixture was poured into ice−water and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). After evaporation of the solvent in vacuo, the residue was purified by flash column chromatography (EtOAc−MeOH, 10:1) to afford the free amine of **28a** (0.20 g, 69%) as a pale-yellow amorphous powder. ^{1}H NMR (free amine in $CDCl_3$): δ 1.17 (3H, t, J = 7.1 Hz), 1.29 (6H, d, J = 6.8 Hz), 2.10 (3H, s), 2.51−2.58 (1H, m), 3.29 (2H, q, J = 7.1 Hz), 3.64 (2H, s), 4.16 (2H, s), 5.27 (2H, s), 7.00 (2H, t, J = 8.2 Hz), 7.16−7.26 (5H, m), 7.36−7.46 (1H, m), 7.62 (2H, d, J = 8.5 Hz), 7.78 (2H, d, J = 8.5 Hz), 8.34 (1H, s). The free amine was treated with 1 M ethereal HCl to give **28a** as a pale-yellow crystalline powder (from EtOH−EtOAc). Mp: 250−256 °C. IR (KBr): 3422, 3230, 2976, 1694, 1671, 1628, 1595, 1526, 1502, 1475 cm^{-1}. Anal. ($C_{36}H_{35}N_3O_3SF_2$·HCl·0.5H_2O) C, H, N.

Compounds **28b,c** were prepared by a procedure similar to that described for **28a**, and the physicochemical data are shown in Table 4.

3-(*N*-Benzyl-*N*-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-5-(1-hydroxy-2-methylpropyl)-2-(4-isobutyrylamidophenyl)-4-oxothieno[2,3-*b*]pyridine Hydrochloride (29). Sodium borohydride (0.20 g, 5.4 mmol) was added in one portion to a solution of **28b** (1.0 g, 1.5 mmol) in MeOH (30 mL) at 0 °C. After being stirred at 0 °C for 1 h, the mixture was diluted with saturated $NaHCO_3$ and extracted with CH_2Cl_2. The extract was washed with brine and dried (Na_2SO_4). The solution was concentrated in vacuo, and the residue was purified by flash column chromatography (EtOAc−MeOH, 10:1 to 3:1) to give the free amine of **29** (0.66 g, 68%) as a brown crystalline powder. The free amine was treated with 1 M ethereal HCl to provide **29** as yellow crystals (from EtOAc−Et_2O). Mp: 232−234 °C. ^{1}H NMR (HCl salt in DMSO-d_6): δ 0.76−1.05 (6H, m), 1.12 (6H, d, J = 6.6 Hz), 2.06 (1H, brs), 2.51 (1H, m), 2.68 (3H, s), 4.14−4.79 (4H, m), 5.32 (1H, brs), 5.65 (2H, d, J = 12.6 Hz), 5.78 (2H, d, J = 12.6 Hz), 7.24−7.28 (7H, m), 7.39 (2H, d, J = 8.7 Hz), 7.35−7.45 (1H, m), 7.84 (2H, d, J = 8.7 Hz), 8.28 (1H, s), 10.27 (1H, s). IR (KBr): 3412, 2972, 2362, 1686, 1611, 1522, 1473, 1408 cm^{-1}. FAB-MS *m*/*z*: 644 (M + H). Anal. ($C_{37}H_{39}N_3O_3SF_2$·HCl·2.0H_2O) C, H, N.

3-(*N*-Benzyl-*N*-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-*N*-methoxy-*N*-methyl-4-oxo-2-(4-trifluoroacetamidophenyl)thieno[2,3-*b*]pyridine-5-carboxamide (30). A solution of trifluoroacetic anhydride (0.65 mL, 5.0 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a solution of **25** (1.15 g, 2.0 mmol) and triethylamine (0.56 mL, 4.0 mmol) in CH_2Cl_2 (20 mL) at 0 °C. After being stirred at room temperature for 1 h, the mixture was poured into ice−water and extracted with CH_2Cl_2. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography (EtOAc−MeOH, 9:1) and the purified compound was then triturated with Et_2O to give the corresponding trifluoroacetamide compound (1.31 g, 98%) as a pale-yellow amorphous powder. Mp: 114 °C. ^{1}H NMR ($CDCl_3$): δ 1.36 (3H, t, J = 7.2 Hz), 2.13 (3H, s), 3.64 (2H, s), 4.16 (2H, s), 4.37 (2H, q, J = 7.2 Hz), 5.27 (2H, s), 7.01 (2H, t, J = 8.1 Hz), 7.05−7.25 (5H, m), 7.35−7.48 (1H, m), 7.66 (2H, d, J = 8.7 Hz), 7.92 (2H, t, J = 8.7 Hz), 8.36 (1H, s). FAB-MS *m*/*z*: 670 (M + H).

Compound **30** was then prepared in 94% yield from the above amide compound by a procedure similar to that described for **27b** as a white crystalline powder. Mp: 198 °C (from Et_2O). ^{1}H NMR ($CDCl_3$): δ 2.15 (3H, s), 3.35 (3H, s), 3.62 (2H, s), 3.73 (3H, s), 4.15 (2H, s), 5.20 (2H, s), 7.00 (2H, t, J = 7.8 Hz), 7.10−7.30 (5H, m), 7.38−7.48 (1H, m), 7.64 (2H, d, J = 8.4 Hz), 7.71 (1H, s), 7.90 (2H, t, J = 8.4 Hz). IR (KBr): 3029, 2938, 1723, 1622, 1539, 1497, 1471 cm^{-1}. FAB-MS *m*/*z*: 685 (M + H). Anal. ($C_{34}H_{29}N_4O_4SF_5$) C, H, N.

2-(4-Aminophenyl)-3-(*N*-benzyl-*N*-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-5-isobutyryl-4-oxothieno[2,3-*b*]pyridine (31). The precursor of compound **31** [3-(*N*-benzyl-*N*-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-5-isobutyryl-2-(4-trifluoroacetamidophenyl)-4-oxothieno[2,3-*b*]pyridine] was prepared in 75% yield from **30** by a procedure similar to that described for **28a** as a white crystalline powder. Mp: 160 °C (from Et_2O). ^{1}H NMR ($CDCl_3$): δ 1.18 (6H, d, J = 6.6 Hz), 2.12 (3H, s), 3.64 (2H, s), 4.11−4.19 (1H, m), 4.18 (2H, s), 5.27 (2H, s), 7.00 (2H, t, J = 8.4 Hz), 7.10−7.22 (5H, m), 7.36−7.46 (1H, m), 7.66 (2H, d, J = 6.6 Hz), 7.90 (2H, d, J = 6.9 Hz), 8.29 (1H, s). FAB-MS *m*/*z*: 668 (M + H).

NaOH (2 N, 2 mL, 4.0 mmol) was added dropwise to a solution of the above trifluoroacetamide compound (0.67 g, 1.0 mmol) in MeOH (10 mL). After being stirred at 60 °C for 1 h, the reaction mixture was poured into water and extracted with EtOAc. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography (EtOAc−hexane, 1:1) and the purified compound was then crystallized from Et_2O to afford **31** (0.45 g, 79%) as a pale-yellow crystalline powder. Mp: 149 °C. ^{1}H NMR ($CDCl_3$): δ 1.18 (6H, d, J = 6.9 Hz), 2.10 (3H, s), 3.64 (2H, s), 3.83 (2H, s), 4.13−4.23 (1H, m), 4.16 (2H, brs), 5.25 (2H, s), 6.73 (2H, d, J = 9.0 Hz), 6.99 (2H, t, J = 8.1 Hz), 7.10−7.25 (5H, m), 7.25−7.42 (1H, m), 7.59 (2H, d, J = 8.7 Hz), 8.27 (1H, s). IR (KBr): 3445, 3358, 3223, 2971, 2932, 1667, 1595, 1575, 1495, 1472 cm^{-1}. FAB-MS *m*/*z*: 572 (M + H). Anal. ($C_{33}H_{31}N_3O_2SF_2$) C, H, N.

3-(*N*-Benzyl-*N*-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-5-isobutyryl-2-[4-(3-methylureido)phenyl]-4-oxothieno[2,3-*b*]pyridine Hydrochloride (32a). Compound **32a** was prepared quantitatively from **31** by a procedure similar to that described for **21e**. Mp: 222−226 °C (from $CHCl_3$−EtOAc, HCl salt). ^{1}H NMR (free amine in $CDCl_3$): δ 1.19 (6H, d, J = 6.6 Hz), 2.44 (3H, br), 2.76 (3H, d, J = 4.8 Hz), 3.95−4.04 (3H, m), 4.34 (2H, s), 5.34 (2H, s), 6.29 (1H, br), 7.00 (2H, t, J = 8.0 Hz), 7.25 (7H, br), 7.37−7.48 (1H, m), 7.62 (2H, d, J = 8.4 Hz), 8.35 (1H, s), 9.03 (1H, br). Anal. ($C_{35}H_{34}N_4O_3SF_2$·HCl·0.5H_2O·0.2$CHCl_3$) C, H, N.

2-[4-(2-Aminoacetamido)phenyl]-3-(*N*-benzyl-*N*-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-5-isobutyryl-4-oxothieno[2,3-*b*]pyridine Dihydrochloride (32b). PyBOP reagent (0.39 g, 0.75 mmol) was added portionwise to a solution of **31** (0.29 g, 0.50 mmol), *N*-Boc-glycine (0.13 g, 0.75 mmol), and *N*,*N*-diisopropylethylamine (0.13 g, 1.0 mmol) in CH_2Cl_2 (5 mL) at 0 °C. After being stirred at room temperature for 18 h, the reaction mixture was poured into water and extracted with $CHCl_3$. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography ($CHCl_3$−MeOH, 19:1), and the purified compound was recrystallized from EtOH to afford the corresponding *N*-Boc-glycinamide compound (0.21 g, 58%) as a white crystalline powder. Mp: 135−137 °C. ^{1}H NMR ($CDCl_3$): δ 1.18 (6H, d, J = 6.8 Hz), 1.48 (9H, s), 2.09 (3H, s), 3.63 (2H, s), 4.01 (2H, t, J = 5.6 Hz), 4.11−4.21 (1H, m), 4.16 (2H, s), 5.24 (2H, s), 5.66 (1H, t, J = 5.9 Hz), 6.97 (2H, t, J = 8.1 Hz), 7.10−7.21 (5H, m), 7.33−7.43 (1H, m), 7.61 (2H, d, J = 8.5 Hz), 7.74 (2H, d, J = 8.5 Hz), 8.29 (1H, s), 8.82 (1H, brs). FAB-MS *m*/*z*: 729 (M + H).

Trifluoroacetic acid (1 mL) was added to the solution of the above amide compound (0.15 g, 0.2 mmol) in CH_2Cl_2 (1 mL) at 0 °C. After being stirred at room temperature for 18 h, the reaction mixture was concentrated in vacuo, and the residue was dissolved in $CHCl_3$ and washed with saturated $NaHCO_3$. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash column chromatography ($CHCl_3$−MeOH, 9:1),

and the crude product was crystallized from Et_2O to provide the free amine of **32b** (0.12 g, 95%) as a white amorphous powder: ^{1}H NMR (free amine in $CDCl_3$): δ 1.13 (6H, d, J = 6.6 Hz), 2.76 (3H, s), 3.83−3.87 (1H, m), 4.29 (2H, s), 4.46 (2H, s), 4.65 (2H, s), 5.35 (2H, s), 6.94 (2H, t, J = 8.3 Hz), 7.10−7.45 (8H, m), 7.76 (2H, d, J = 8.6 Hz), 8.32 (1H, s), 11.05 (1H, brs). The free amine was treated with 5 M ethanolic HCl to afford **32b** as a white crystalline powder. Mp: 197−199 °C (from EtOH). FAB-MS *m*/*z*: 629 (M + H). IR (KBr): 2936, 1682, 1626, 1593, 1539, 1504, 1471, cm^{-1}. Anal. ($C_{35}H_{34}N_4O_3SF_2$·2.0HCl·1.5H_2O) C, H, N.

3-(*N*-Benzyl-*N*-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-2-[4-(2-hydroxyacetamido)phenyl]-5-isobutyryl-4-oxothieno[2,3-*b*]pyridine Hydrochloride (33a). Compound **33a** was prepared in 98% yield (two steps) from **31** by a procedure similar to that described for **32b**. Mp: 197−199 °C (from EtOH, HCl salt). ^{1}H NMR (free amine in $CDCl_3$): δ 1.18 (6H, d, J = 6.8 Hz), 2.09 (3H, s), 3.61 (2H, s), 4.07−4.19 (1H, m), 4.16 (2H, s), 4.21 (2H, s), 5.24 (2H, s), 6.99 (2H, t, J = 8.3 Hz), 7.10−7.25 (5H, m), 7.32−7.48 (1H, m), 7.63 (2H, d, J = 8.6 Hz), 7.72 (2H, d, J = 8.6 Hz), 8.25 (1H, s), 8.67 (1H, brs). FAB-MS *m*/*z*: 630 (M + H). Anal. ($C_{35}H_{33}N_3O_4SF_2$·HCl·0.5H_2O) C, H, N.

3-(*N*-Benzyl-*N*-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-2-(4-(2-hydroxy-2-methylpropionylamido)phenyl)-5-isobutyryl-4-oxothieno[2,3-*b*]pyridine (33b). Compound **33b** was prepared in 70% yield from **31** (two steps) by a procedure similar to that described for **33c** (see below). Mp: 178−180 °C (from $CHCl_3$−Et_2O). ^{1}H NMR ($CDCl_3$): δ 1.18 (6H, d, J = 6.8 Hz), 1.58 (6H, s), 2.10 (3H, s), 3.08 (1H, brs), 3.64 (2H, s), 4.13−4.21 (1H, m), 4.16 (2H, s), 5.23 (2H, s), 6.99 (2H, t, J = 8.2 Hz), 7.10−7.25 (5H, m), 7.30−7.44 (1H, m), 7.64 (2H, d, J = 8.5 Hz), 7.75 (2H, d, J = 8.5 Hz), 8.25 (1H, s), 8.86 (1H, brs). FAB-MS *m*/*z*: 667 (M + H). Anal. ($C_{37}H_{37}N_3O_4SF_2$·0.5H_2O) C, H, N.

3-(*N*-Benzyl-*N*-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-2-[4-(1-hydroxy-1-cyclopropanecarboxamido)-phenyl]-5-isobutyryl-4-oxothieno[2,3-*b*]pyridine (33c). 1-Acetoxy-1-cyclopropanecarbonyl chloride (3.58 g, 22.0 mmol) was added dropwise to a solution of **31** (11.4 g, 20.0 mmol) and triethylamine (2.43 g, 24.0 mmol) in CH_2Cl_2 (40 mL) at 0 °C. After being stirred at room temperature for 6 h, the reaction mixture was poured into water and extracted with $CHCl_3$. The extract was washed with brine and dried ($MgSO_4$). The solution was concentrated in vacuo, and the residue was purified by flash column chromatography ($CHCl_3$−MeOH, 19:1) to afford the corresponding 1-acetoxy-1-cyclopropanecarboxamide compound (11.2 g, 80%) as a pale-yellow solid. ^{1}H NMR (CD_3OD): δ 1.18 (6H, d, J = 6.8 Hz), 2.11 (3H, s), 3.47 (3H, s), 3.65 (2H, s), 4.10−4.24 (1H, m), 4.18 (2H, s), 4.26 (2H, s), 4,65 (2H, s), 5.27 (2H, s), 7.00 (2H, t, J = 8.3 Hz), 7.10−7.25 (5H, m), 7.33−7.49 (1H, m), 7.67 (2H, d, J = 8.6 Hz), 7.81 (2H, d, J = 8.6 Hz), 8.28 (1H, s), 8.51 (1H, brs). FAB-MS *m*/*z*: 674 (M + H).

NaOH (5 N, 10 mL, 50 mmol) was added to a solution of the above amide compound (5.40 g, 7.74 mmol) in EtOH (40 mL) at 0 °C. After being stirred at 0 °C for 3 h, the mixture was poured into water and extracted with $CHCl_3$. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash column chromatography ($CHCl_3$−MeOH, 9:1) and the purified compound was recrystallized from EtOH to give **33c** (4.53 g, 89%) as a white crystalline powder. Mp: 184−185 °C. ^{1}H NMR ($CDCl_3$): δ 1.18 (6H, d, J = 6.8 Hz), 1.16−1.20 (2H, m), 1.48−1.51 (2H, m), 2.09 (3H, s), 3.64 (2H, s), 3.95 (1H, brs), 4.10−4.20 (1H, m), 4.14 (2H, s), 5.20 (2H, s), 6.99 (2H, t, J = 8.0 Hz), 7.10−7.25 (5H, m), 7.34−7.44 (1H, m), 7.56 (2H, d, J = 8.5 Hz), 7.70 (2H, d, J = 8.5 Hz), 8.21 (1H, s), 8.82 (1H, brs). IR (KBr): 3362, 3065, 2976, 2955, 2872, 1688, 1672, 1590, 1497, 1473 cm^{-1}. FAB-MS *m*/*z*: 656 (M + H). Anal. ($C_{37}H_{35}N_3O_4SF_2$) C, H, N.

In Vitro Binding Assays. Receptor binding assays were carried out as described previously.[10] Briefly, human LHRH receptor cDNA was cloned from a pituitary cDNA library, and CHO cells stably expressing high levels of the recombinant human LHRH receptor were isolated. [^{125}I][Tyr^5]leuprorelin (0.12−0.15 nM) and the membrane fractions of the CHO cells (0.2 mg/mL) were incubated at 25 °C for 60 min in 0.2 mL of assay buffer A [25 mM Tris, 1 mM EDTA, 0.1% bovine serum albumin (BSA), 0.03% NaN_3, 0.25 mM phenylmethanesulfonyl fluoride, 1 μg/mL pepstatin A, 20 μg/mL leupeptin, and 100 μg/mL phosphoramidon, pH 7.5] containing various concentrations of the test compounds. The reaction was terminated by adding 2 mL of ice-cold assay buffer A, and the bound and free ligands were immediately separated by filtration through a poly(ethylenimine)-coated glass microfiber filter (Whatman, GF/F). The filter was washed twice with 2 mL of assay buffer A, and radioactivity was measured using an X-ray counter. Specific binding was determined by subtracting the nonspecific binding, which was measured in the presence of 1 μM unlabeled leuprorelin, from the total binding. The concentration of each test compound that produced 50% inhibition of the specific binding (IC_{50} value) was derived by fitting the data into a pseudo-Hill equation:

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

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

Similarly, the binding experiments to the LHRH receptor of other species were performed as follows. The monkey LHRH receptor cDNA was cloned from a pituitary cDNA library of cynomolgus monkeys, and CHO cells stably expressing high levels of the recombinant monkey LHRH receptor were isolated as described previously.[10] The binding assays were carried out by incubating [^{125}I][Tyr^5]leuprorelin (0.12−0.13 nM) and membranes prepared from the CHO cells (0.25 mg/mL) at 25 °C for 60 min, in the presence or absence of compounds. For the binding experiments to the rat LHRH receptor, [^{125}I][Tyr^5]leuprorelin (0.15 nM) and membranes (0.2 mg/mL) from the anterior pituitary of male Wistar rats were incubated with or without compounds at 4 °C for 90 min.[10]

In Vitro Functional Assays. LHRH-stimulated arachidonic acid release from CHO cells expressing human or monkey LHRH receptors was measured according to the previously reported protocol.[25] 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$_8$]-arachidonic acid (11 kBq/well, NEN Lifescience Products) for 1 day and washed with Dulbecco's modified Eagle's medium (DMEM) supplemented with 20 mM HEPES and 0.2% BSA. The cells were then preincubated with the compounds at 37 °C for 60 min and the reaction was started by addition of LHRH (1 nM). After incubation at 37 °C for 40 min, radioactivity in the medium was measured with a liquid scintillation counter.

In Vivo Efficacy in Cynomolgus Monkeys. Cynomolgus monkeys (male, 4−9 years old) were castrated more than 6 months prior to the examination. The monkeys were trained to sit in a primate-restraining chair during administration of the compounds. Compound **33c** (10 or 30 mg/kg, 3 mL/kg, n = 3 for each group) suspended in 0.5% methylcellulose containing 1.2% citric acid, or 0.5% methylcellulose containing 1.2% citric acid alone (3 mL/kg, n = 3), was administered orally. Blood samples (heparin−plasma) were collected from a femoral vein 0, 2, 4, 8, 24, and 48 h after administration. LH concentrations in the plasma were measured by bioassays using mouse testicular cells.[10]

Molecular Modeling Studies. All calculations were performed on a Silicon Graphics O2 R10000 workstation. Molecular modeling was carried out using the Insight II software package (Accelrys Inc., San Diego, CA). Conformer CC of LHRH as described by Momany[23] was reproduced with the reported dihedral angles and used as the putative active conformation of LHRH.

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Supporting Information Available: Elemental analysis data of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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. Gonadtropin-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) (a) Conn, P. M.; Crowley, W. F., Jr. Gonadotropin-Releasing Hormone And Its Analogues. *N. Engl. J. Med.* **1991**, *324*, 93−103. (b) Goulet, M. T. Gonadotropin Releasing Hormone Antagonists. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed.; Academic Press: New York, 1995; Vol. 30, pp 169−178. (c) Huirne, J. A. F.; Lambalk, C. B. Gonadotropin-Releasing-Hormone-Receptor Antagonists. *Lancet* **2001**, *358*, 1793−1803.

(5) Fujino, M.; Fukuda, T.; Shinagawa, S.; Kobayashi, S.; Yamazaki, I.; Nakayama, 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.

(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) Kardamakis, E.; Tzingounis, V. GnRH Analogues in Reproductive Medicine and Gynaecologic Cancer. In *Biomedical Health Research: Bioactive Peptides in Drug Discovery and Design: Medical Aspects*; Marsoukas, J., Mavromoustakos, T., Eds.; IOS Press: 1999; Vol. 22, pp 275−285. (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.

(9) (a) Zhu, Y.-F.; Chen, C.; Struthers, R. S. Nonpeptide Gonadotropin Releasing Hormone Antagonists. In *Annual Reports in Medicinal Chemistry*; Doherty, A. M., Ed.; Academic Press: New York, 2004; Vol. 39, pp 99−110. (b) Armer, R. E.; Smelt K. H. Non-Peptidic GnRH Receptor Antagonists. *Curr. Med. Chem.* **2004**, *11*, 3017−3028. (c) Zhu, Y.-F.; Chen, C. Recent Advances in Small Molecule GnRH Receptor Antagonists. *Exp. Opin. Ther. Pat.* **2004**, *14*, 187−199.

(10) 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.

(11) 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-Position: A Highly Potent and Orally Bioavailable Non-Peptide Antagonist for the Human Luteinizing Hormone-Releasing Hormone Receptor. *J. Med. Chem.* **2003**, *46*, 113−124.

(12) 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.

(13) (a) De Vita, 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.; 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. (c) Jiang, J.; De Vita, 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. (d) De Vita, 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. (e) 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. (f) 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. (g) 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.

(14) (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) 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. (c) 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. (d) 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. (e) 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. (f) 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. (g) 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. L. K.; Chen, T.; Bozigian, H.; Chen, C. 3-[(2*R*)-Amino-2-phenylethyl]-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-meth-

oxyphenyl)-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.

(15) (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.

(16) (a) 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. (b) 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.

(17) (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.

(18) Cho, N.; Nara, Y.; Harada, M.; Sugo, T.; Masuda, Y.; Abe, A.; Kusumoto, K.; Itoh, Y.; Ohtaki, T.; Watanabe, T.; Furuya, S. Thieno-[2,3-*d*]pyrimidine-3-acetic Acids A New Class of Nonpeptide Endothelin Receptor Antagonists. *Chem. Pharm. Bull.* **1998**, *46*, 1724−1737.

(19) Gewald, K.; Schinke, E.; Böttcher, H. 2-Amino-thiophene aus Methylenaktiven Nitrilen, Carbonylverbindungen und Schwefel. *Chem. Ber.* **1966**, *99*, 94−100

(20) Imwinkelried, R.; Schiess, M.; Seebach, D. Diisopropyl (2*S*,3*S*)-2,3-*O*-isopropylidenetartrate (1,3-Dioxolane-4,5-dicarboxylic Acid, 2,2-Dimethyl-, Bis(1-methylethyl)ester, (4*R*-*trans*)-). *Org. Synth.* **1987**, *65*, 230−235

(21) Basha, A.; Lipton, M.; Weinreb, S. M. A Mild, General Method for Conversion of Esters to Amides. *Tetrahedron Lett.* **1977**, *18*, 4171−4174.

(22) 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.

(23) Momany, F. A. Conformational Energy Analysis of the Molecule, Luteinizing Hormone-Releasing Hormone. 1. Native Decapeptide. *J. Am. Chem. Soc.* **1976**, *98*, 2990−2996.

(24) Betz, S. F.; Reinhart, G. J.; Lio, F. M.; Chen, C.; Struthers, R. S. Overlapping, Nonidentical Binding Sites of Different Classes of Nonpeptide Antagonists for the Human Gonadotropin-Releasing Hormone Receptor. *J. Med. Chem.* **2006**, *49*, 637−647.

(25) 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.

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