3-[(2*R*)-Amino-2-phenylethyl]-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-6-methylpyrimidin-2,4-dione (NBI 42902) as a Potent and Orally Active Antagonist of the Human Gonadotropin-Releasing Hormone Receptor. Design, Synthesis, and in Vitro and in Vivo Characterization

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Further structure−activity relationship studies of a series of substituted uracils at the 1, 3, and 5 positions resulted in the discovery of several potent antagonists of the human gonadotropin-releasing hormone receptor. Uracils bearing a side chain derived from phenylglycinol at the 3-position were shown to be orally bioavailable in monkeys. 3-[(2*R*)-Amino-2-phenylethyl]-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-6-methylpyrimidin-2,4-dione (*R*-**13b**, NBI 42902) displayed subnanomolar binding affinity (K_i = 0.56 nM) and was a potent functional antagonist (IC_{50} = 3.0 nM in Ca^{2+} flux assay) at the human GnRH receptor. It also bound to the monkey GnRH receptor with high affinity (K_i = 3.9 nM). In addition, *R*-**13b** had good plasma exposure in cynomolgus monkeys after oral administration, with a C_{max} of 737 ng/mL and an AUC of 2392 ng/mL·h at a 10 mg/kg dose. Moreover, oral administration of *R*-**13b** to castrated male cynomolgus monkeys resulted in a significant decrease in serum levels of luteinizing hormone. These results demonstrate that compounds from this series of uracils are potent GnRH antagonists with good oral bioavailability and efficacy in nonhuman primates.

Introduction

Gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone-releasing hormone (LH-RH), is a linear decapeptide amide, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, originally isolated and characterized from porcine and ovine hypothalami.[1] GnRH exerts its action at the level of the pituitary by activation of its cell surface receptor, a member of the class A G-protein-coupled receptor superfamily,[2] to stimulate the secretion of the gonadotropins-luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These hormones, in turn, act on the reproductive organs where they participate in the regulation of steroid production, gametogenesis, and ovulation.[3] Several disease conditions, such as endometriosis and prostate cancer, can be treated by suppression of the pituitary−gonadal axis. GnRH peptide superagonists, represented by leuprorelin,[4] which activate and subsequently down-regulate the receptor, are currently used in the treatment of these conditions.[5] Clinical evidence shows that peptidic GnRH antagonists directly lower gonadal sex hormone levels, alleviating disease symptoms without the concomitant flare effect caused by superagonists.[6] An orally bioavailable small molecule will have the advantage of flexible dosing and titration of drug concentrations, which may provide novel clinical management options.

Since the first nonpeptidic antagonist of the human gonadotropin-releasing hormone receptor (hGnRH-R) T-98475 (**1**), which possesses high binding affinity (IC_{50} = 0.2 nM),[7] was reported in 1998, several small molecules from different chemical classes have appeared in the literature.[8] 3-Arylquinolones, represented by compound **2**, have been discovered as potent antagonists of the hGnRH-R (IC_{50} = 0.44 nM). Interestingly, unlike **1** which binds to the rat GnRH receptor (rGnRH-R) with only moderate affinity (IC_{50} = 60 nM), **2** also possesses high binding affinity (IC_{50} = 4 nM) at the rat receptor.[9] Tryptamine compounds such as **3** are reported to be potent GnRH antagonists, and several derivatives from this class have showed oral bioavailability in rats and dogs.[10] Very recently, a potent antagonist of the hGnRH-R, thieno[2,3-*b*]pyrimidin-2,4-dione TAK-013 (**4**), has been reported to be clinically efficacious in humans in suppressing luteinizing hormone when given orally.[11]

Previously, we had reported on a series of pyrazolopyrimidones[12] and imidazolopyrimidones[13] such as **5** and **6** as potent antagonists of the hGnRH-R. In our efforts to optimize small molecules toward oral bioavailability, we focused on reducing the molecular weight of these early compounds. On the basis of the successful optimization toward high potency of imidazolopyrimidone derivatives, we designed and synthesized a series of uracils (pyrimidin-2,4-ones) bearing a 3-(*N*-alkylaminoethyl) group (**7**−**9**, Figure 1).[14] While compounds from this series such as **8** (K_i = 0.5 nM) exhibit high binding affinity,[15] many of them, especially tertiary amines such as **8**, suffer from poor oral bioavailability

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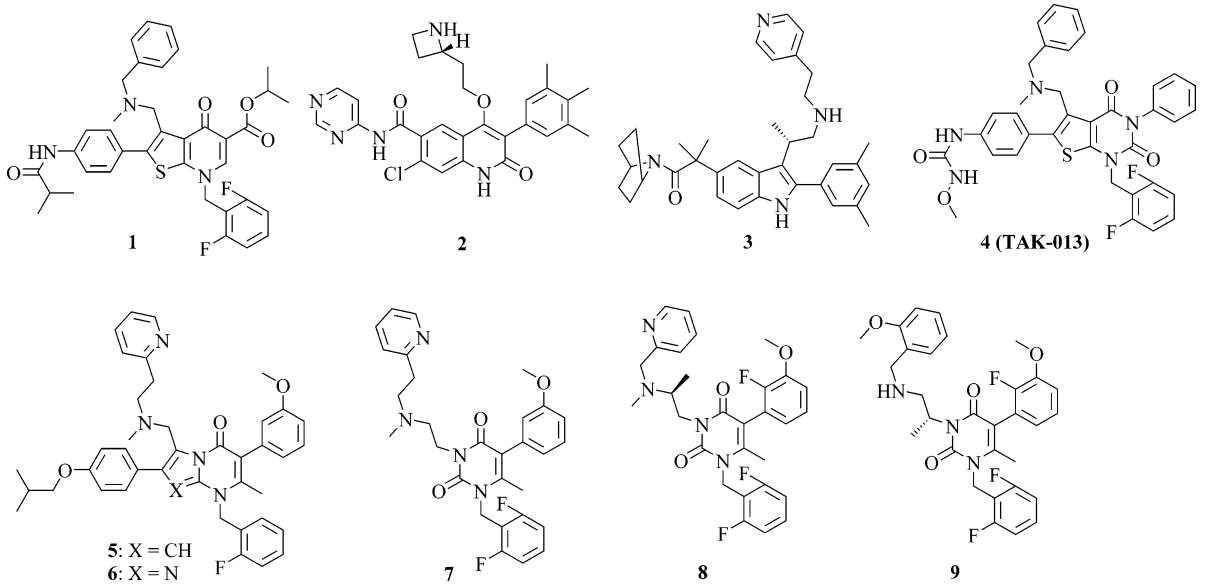


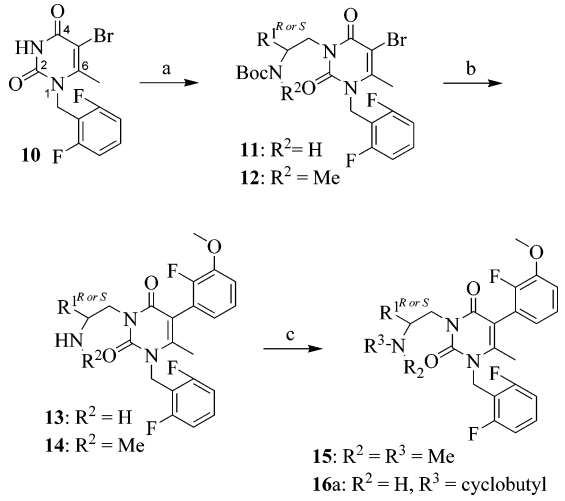
Figure 1. Some small-molecule GnRH antagonists.

because of high clearance. One of the major metabolites from the in vitro liver microsomes assay is identified as the N-dealkylation product, which possesses low binding affinity. Because an *N*-alkyl group such as a benzyl moiety at the basic nitrogen is required for high affinity in **7**−**9**, we designed analogues by moving this small lipophilic group from the nitrogen to the α-carbon of the 2-aminoethyl side chain to eliminate one of the nitrogen−carbon bonds that are easily cleaved through oxidation by enzymes. In a previous communication, we reported on our initial success by using this strategy and synthesized potent compounds with improved metabolic stability.[16] In this paper we describe the details of this structure−activity relationship study on compounds bearing a 2-aminoalkyl side chain at the 3-position of the uracils (**13**−**16**), as well as substitutions at the 5 and 1 positions (**19**, **20**, and **24**).[17] Finally, we report on the pharmacokinetic profiles and oral activity of representative compounds from this series.

Chemistry

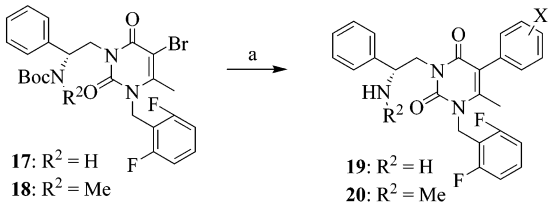
To examine the aminoalkyl side chain at the 3-position of the uracil, **13** and **14** were synthesized according to Scheme 1 starting from 1-(2,6-difluorobenzyl)-5-bromo-6-methyluracil **10**, which was obtained in three steps from readily available chemicals as previously reported.[14] Thus, coupling reactions of **10** with either *R*- or *S*-substituted *N*-Boc-aminoethanols, or their *N*-methyl analogues, under Mitsunobu conditions (DEAD/Ph_3P in THF) gave the corresponding uracils **11** and **12** in 55−70% yields. These were then subjected to a Suzuki coupling reaction with 2-fluoro-3-methoxyphenylboronic acid catalyzed by palladium, followed by TFA treatment, to afford the 5-aryluracils **13** and **14**, respectively, in 35−90% yields. Compounds **13** were N-dimethylated with formaldehyde in the presence of sodium triacetoxyborohydride to give **15** in about 50% yield. Compound **13** could also be monoalkylated with cyclobutanone to give the secondary amine **16a**. To explore the SAR at the 5-position, 5-bromouracils **17** or **18** bearing the *R*-2-aminophenethyl side chain was subjected to Suzuki coupling reactions with various

Scheme 1. Synthesis of 5-(2-Fluoro-3-methoxypheyl)uracils Bearing a 3-Aminoalkyl Side Chain[a]



[a] Reagents and conditions: (a) *R*- or *S*-$R^1CH(NR^2Boc)CH_2OH$, PPh_3, DEAD, THF, room temp, 55−70%; (b) (i) 2-F-3-MeOPh-$B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, benzene, EtOH, DME, reflux; (ii) TFA, CH_2Cl_2, room temp, 35−90%; (c) for **13**, CH_2O or cyclobutanone, $NaBH(OAc)_3$, CH_2Cl_2, room temp, ∼50%.

Scheme 2. Synthesis of Uracils with Various Substituted Phenyl Group at the 5-Position[a]

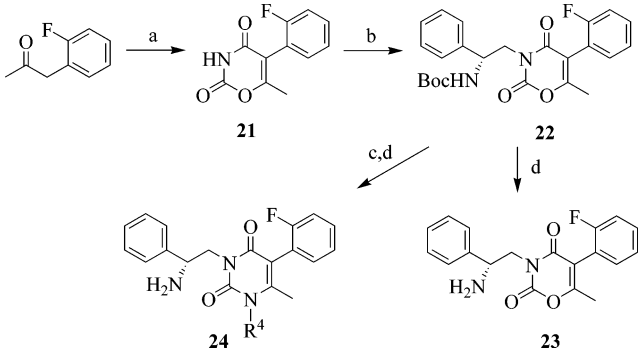


[a] Reagents and conditions: (a) (i) $ArB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, benzene, EtOH, DME, reflux; (ii) TFA, CH_2Cl_2, 30−93%.

substituted phenylboronic acids to afford compounds **19** and **20** after deprotection (Scheme 2).

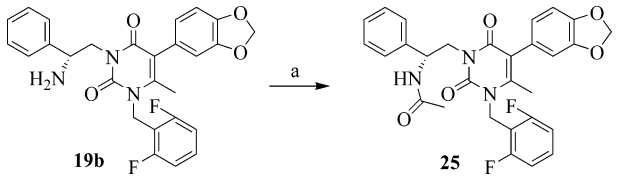
To quickly survey the effect of substitution at the 1-position of the 6-methyluracil, a new synthetic route was developed and is shown in Scheme 3.[17] Thus, reaction of (2-fluorophenyl)acetone with chlorosulfonyl

Scheme 3. Synthesis of Uracils with Various Substituents at the 1-Position[a]



[a] Reagents and conditions: (a) $ClSO_2NCO$, Et_2O, room temp, 33%; (b) (*R*)-PhCH(NHBoc)CH_2OH, PPh_3, DEAD, THF, room temp, 81%; (c) R^4NH_2, heat, 5−60%; (d) TFA, CH_2Cl_2, quantitative.

Scheme 4. Acetylation of Amino Compound **19b**[a]



[a] Reagents and conditions: (a) Ac_2O, Et_3N, CH_2Cl_2, room temp, 93%.

isocyanate, according to the general method previously described by Hassner and co-workers,[18] gave 5-(2-fluorophenyl)-6-methyl-1,3-oxazine-2,4-(*3H*)-dione **21** in 33% yield after separation by crystallization. Alkylation at the 3-position of **21** with *R*-*N*-Boc-2-phenylglycinol, via a Mitsunobu protocol, gave 1,3-oxazine-2,4-dione **22** in 81% yield. Reaction of **22** with various primary amines at 100 °C, followed by *N*-Boc deprotection, gave the desired products **24** in 5−60% isolated yield. Anilines failed to give the corresponding products under these conditions.

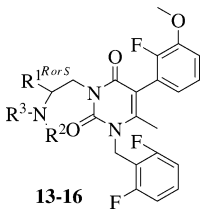
Compound **19b** was acetylated with acetic anhydride in the presence of triethylamine to afford **25** in 93% yield in order to examine the importance of the basic amine in the binding mode of uracil-based GnRH antagonists (Scheme 4).

Pharmacology

The compounds herein synthesized were evaluated for their ability to inhibit [^{125}I-Tyr^5,DLeu^6,NMeLeu^7,Pro^9-NEt]GnRH agonist binding to the cloned human, monkey, and rat GnRH-Rs as previously described,[19] and the data are summarized in Tables 1−3. Selected compounds were tested in the inhibition of GnRH-stimulated calcium flux in RBL cells stably expressing the hGnRH-R to determine functional antagonism. Table 4 summarizes the binding affinities of selected compounds at the human, monkey, and rat GnRH receptors, as well as functional antagonism of these compounds at the human receptor.

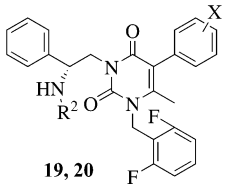
Previously, we reported on the structure−activity relationships of a series of uracils bearing a *N*-alkylaminoethyl side chain at the 3-position as potent antagonists of hGnRH-R.[13c] For example, by incorporation of a methyl group at the β- or α-position of the 3-side-chain in the initial lead **7**, potent antagonists exemplified by **8** and **9** were identified. However,

Table 1. SAR of (*R*)- and (*S*)-3-Aminoalkyluracils **13**−**16** at the hGnRH-R



compd	R^1	R^2	R^3	K_i (nM)
R-**13a**	$PhCH_2$	H	H	66 ± 11
S-**13a**	$PhCH_2$	H	H	16 ± 1.1
R-**13b**	Ph	H	H	0.56 ± 0.03
S-**13b**	Ph	H	H	22 ± 3.1
R-**13c**	i-Bu	H	H	6.4 ± 1.0
S-**13c**	i-Bu	H	H	39 ± 13
R-**13d**	cyclohexyl	H	H	6.5 ± 1.0
S-**13d**	cyclohexyl	H	H	46 ± 12
R-**13e**	i-Pr	H	H	51 ± 18
S-**13e**	i-Pr	H	H	970 ± 226
R-**13f**	Me	H	H	600 ± 87
S-**13f**	Me	H	H	>10000
S-**14a**	$PhCH_2$	Me	H	8.1 ± 0.5
R-**14b**	Ph	Me	H	1.8 ± 0.3
R-**14c**	i-Bu	Me	H	1.2 ± 0.3
S-**15a**	$PhCH_2$	Me	Me	7.9 ± 1.5
R-**15b**	Ph	Me	Me	6.5 ± 0.3
S-**15b**	Ph	Me	Me	15 ± 1.3
S-**15c**	i-Bu	Me	Me	7.5 ± 2.0
R-**15e**	i-Pr	Me	Me	27 ± 11
S-**15e**	i-Pr	Me	Me	1100 ± 460
S-**16a**	Me	H	cyclobutyl	3.6 ± 0.3

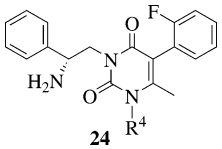
Table 2. SAR of 5-Phenyluracils **19** and **20** at the hGnRH-R



compd	R^2	X	K_i (nM)
R-**13b**	H	2-F, 3-MeO	0.56 ± 0.03
19a	H	3-MeO	2.3 ± 0.1
19b	H	3,4-OCH_2O	3.4 ± 0.3
19c	H	3,4-OCH_2CH_2O	5.1 ± 0.4
19d	H	4-MeS	13 ± 2.3
19e	H	4-PhO	8.5 ± 0.9
19f	H	2-Cl	2.8 ± 0.9
19g	H	2-F	5.6 ± 0.7
19h	H	2-F, 3-Me	3.1 ± 0.2
20a	Me	3-MeO	6.4 ± 0.5
20b	Me	3,4-OCH_2O	5.6 ± 0.7
20c	Me	3,4-OCH_2CH_2O	13 ± 2.5

N-dealkylation by liver enzymes in the in vitro assay generates a 3-(aminopropyl)uracil metabolite, which, without a small *N*-alkyl group, is much less potent in binding. For example, the primary amine *S*-**13f** was inactive, while the corresponding *N*-cyclobutyl analogue *S*-**16a** had a K_i value of 3.6 nM. We anticipated that compounds bearing a 3-side-chain derived from an amino acid, in which a small lipophilic group required for high receptor binding is attached through a carbon−carbon bond, should be metabolically more stable. The successful Mitsunobu reaction of uracil **10** at the 3-position with an alcohol allowed us to quickly examine a series of chiral *N*-Boc-amino alcohols, readily available from commercial sources or from the corresponding

Table 3. SAR of 1-Substitutions of Uracils **24a**−**t** at the hGnRH-R



24

compd	R^4	K_i (nM)
23		>10000
24a	$MeOCH_2CH_2$	1200 ± 210
24b	c-$PrCH_2$	880 ± 83
24c	i-$PrCH_2$	570 ± 69
24d	c-$HxCH_2$	140 ± 24
24e	$PhCH_2CH_2$	880 ± 100
24f	2-$PyCH_2$	64 ± 9
24g	3-$PyCH_2$	980 ± 120
24h	$PhCH_2$	53 ± 4
24i	4-$FC_6H_4CH_2$	63 ± 5
24j	3-$FC_6H_4CH_2$	61 ± 4
24k	2-$FC_6H_4CH_2$	20 ± 2
24l	2-$ClC_6H_4CH_2$	14 ± 4
24m	2-$BrC_6H_4CH_2$	4.6 ± 0.9
24n	2-$MeC_6H_4CH_2$	20 ± 5
24o	2-$MeOPhCH_2$	100 ± 37
24p	2-$CF_3SC_6H_4CH_2$	180 ± 19
24q	2-$CF_3C_6H_4CH_2$	3.1 ± 0.3
24r	2-CF_3-5-$FC_6H_4CH_2$	3.9 ± 0.3
19g	2,6-$F_2C_6H_4CH_2$	5.6 ± 0.7
24s	2-Cl-6-$FC_6H_4CH_2$	0.61 ± 0.1
24t	2-Cl-4-$FC_6H_4CH_2$	21 ± 6

Table 4. Species Selectivity and Antagonistic Activity of Uracils at the GnRH-Rs

	GnRH-R binding K_i (nM)			IC_{50} (nM)[a]
compd	human	monkey	rat	Ca^{2+} flux[b]
R-**13b**	0.56 ± 0.03	3.5 ± 0.3	3000 ± 850	3.0
R-**13d**	6.5 ± 1.0	120 ± 11	3200[c]	6.5
S-**14a**	8.1 ± 0.5	200 ± 17	12000 ± 4700	20
R-**14b**	1.8 ± 0.3	15 ± 9.0		
R-**15b**	6.5 ± 0.3	120 ± 3.7	11000 ± 2300	49
19a	2.3 ± 0.1	45 ± 6.1	7600 ± 1100	5.0
19b	3.4 ± 0.3	42 ± 4.5	9900 ± 2400	6.2
19c	5.1 ± 0.4	74 ± 12	18000 ± 4700	10
20a	6.4 ± 0.3	65 ± 12	7700 ± 1300	34
20b	5.6 ± 0.7	100 ± 7		
24s	0.61 ± 0.1	6.4 ± 1.8	2900 ± 110	2.8

[a] Average of two or more independent determinations. [b] Function at the hGnRH-R. [c] Single determination.

amino acids by a simple reduction. We examined both *R*- and *S*-isomers to determine the chiral selectivity for high affinity to the receptor.

Among the primary amines we examined, the compound derived from *R*-phenylglycinol possessed the best binding affinity (*R*-**13b**, NBI-42902, K_i = 0.56 nM), followed by *R*-leucinol and *R*-cyclohexylglycinol derivatives (*R*-**13c** and *R*-**13d**, K_i = 6.4 and 6.5 nM, respectively). Smaller lipophilic side chains showed further reductions in potency (*R*-**13e** and *R*-**13f**, K_i = 51 and 600 nM, respectively) On the other hand, the larger *R*-phenylalaninol *R*-**13a** only exhibited a K_i value of 66 nM, suggesting that a lipophilic group is important for binding, but there is a size limit for the interaction with the hGnRH-R for the *R*-isomers.

While the *S*-configured compounds **13** generally possessed lower binding affinity than the corresponding *R*-isomers, the *S*-phenylalaninol derivative (*S*-**13a**, K_i = 16 nM) was about 4-fold more potent than the corresponding *R*-isomer, about equivalent to the *S*-phenylglycinol *S*-**13b** (K_i = 22 nM). More importantly, it was dramatically more active than the *S*-alaninol analogue *S*-**13f** (K_i > 10 μM). All these results may suggest that, in addition to a key interaction with the basic amine, the receptor possesses a relatively large pocket lined by lipophilic amino acids to accommodate a group such as a phenyl or cyclohexyl moiety from the *R*-isomeric ligands, or a benzyl moiety from the *S*-isomer.

Interestingly, addition of a small methyl group to the amino nitrogen of the *R*-isomers of these aminoethyl derivatives impacted their binding affinity in an unpredicted fashion. For example, mono- and dimethylation of the phenylglycinol derivative *R*-**13b** reduced its binding affinity about 3- and 11-fold, respectively (*R*-**14b** and *R*-**15b**). On the other hand, methylation of the leucinol derivative *R*-**13c** increased potency over 5-fold (*R*-**14c**, K_i = 1.2 nM), and this was also observed for the *S*-isomers (*S*-**13a**, *S*-**14a**, and *S*-**15a**). One possible explanation for these changes is that the methylation causes the lipophilic side chain to adopt alternative conformations and interact with the receptor in a more or less favorable way. However, alkylation of the much smaller alaninol side chain of *S*-**13f** ($K_i \geq 10$ μM) with the small cyclobutyl group led to a dramatic increase in binding affinity (*S*-**16a**, K_i = 3.6 nM), and this improvement in potency most likely is caused by the additional lipophilic interaction (Table 1).

The SAR of the 5-aromatic group of uracil was also briefly examined with the 3-side-chain kept constant as either (*R*)-2-aminophenethyl or (*R*)-2-(*N*-methyl)aminophenethyl, and the results are shown in Table 2. While the 3-methoxy-, 3,4-methylenedioxy-, and 3,4-ethylenedioxyphenyl derivatives (**19a**−**c**) exhibited good binding affinities (K_i = 2.3−5.1 nM), their N-methylation (**20a**−**c**) resulted in about 2-fold reduction (K_i = 5.6−13 nM). The large and lipophilic 4-phenoxyphenyl derivative (**19e**) possessed a K_i value of 8.5 nM, similar to the 4-methylthiophenyl analogue (**19d**, K_i = 13 nM). The 2-chloro-, 2-fluoro-, and 2-fluoro-3-methylphenyl compounds (**19f**−**h**) had K_i values of 2.8−5.6, and the 2-fluoro-3-methoxyphenyl analogue *R*-**13b** (K_i = 0.56 nM) was significantly more potent than the 3-methoxyphenyl **19a** and 2-fluorophenyl **19g**. The increase in binding affinity of *R*-**13b** over **19a** is most likely a consequence of the enforcement of an orthogonal conformation between the 5-(2-fluoro-3-methoxyphenyl) ring and the uracil core. This phenomenon was observed in the X-ray crystal structure of *R*-**13b** (Figure 2).

A survey of the three-dimensional homology models for the hGnRH-R, built from the human receptor protein by using the X-ray structure of bovine rhodopsin as a template,[2b,20] suggests that two amino acids, Tyr-283 and Tyr-284 in the middle of the transmembrane helix 6, are possible residues to interact with the electron-deficient 1-(2,6-difluorobenzyl) group of *R*-**13b** and its close analogues. A cavity formed by these two residues and Tyr-290 around the Gly-267 could be perfect to host the 2,6-difluorobenzyl group. These two amino acids have been implicated in the binding and function of the peptide GnRH ligand, and thus, either Y283A or Y284A mutant cannot be activated by GnRH.[20] In addition, these residues align with the highly conserved aromatic amino acids of many class A aminergic G-protein-

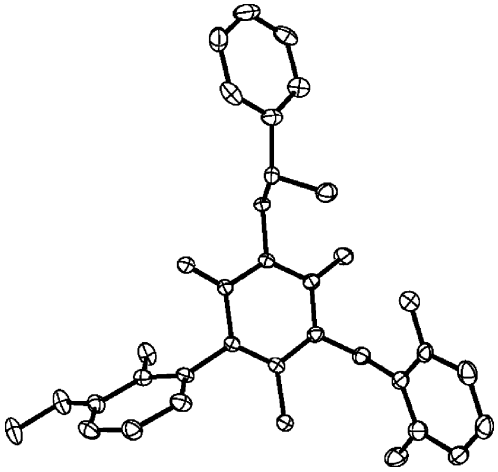


Figure 2. X-ray crystal structure of *R*-**13b** indicates an orthogonal relationship between the 5-(2-fluoro-3-methoxyphenyl) group and the uracil ring.

coupled receptors, which are known to face the putative binding pocket and to be involved in the interaction with small-molecule ligands.[21] We investigated the structure−activity relationships of the substituent at the 1-position to explore this potential interaction further. By use of the chemistry shown in Scheme 3, several analogues were quickly synthesized and tested, and the results are summarized in Table 3. The oxazine **23**, which lacks the 2,6-difluorobenzyl moiety of *R*-**13**, had no measurable binding affinity to the hGnRH-R. Methoxyethyl at this position resulted in a compound with poor affinity (**24a**, K_i = 1.2 μM). Increasing lipophilicity such as isobutyl afforded analogues with moderate binding affinity (i.e., **24c**, K_i = 570 nM), which was improved further in cyclohexylmethyl analogue **24d** (K_i = 140 nM). **24d** was the most potent analogue without an aromatic functionality at this position. In comparison with the phenethyl compound **24e** (K_i = 880 nM), the benzyl analogue **24h** exhibited much better binding affinity (K_i = 53 nM). Interestingly, the 2-pyridylmethyl compound (**24f**, K_i = 64 nM) possessed much higher potency than the 3-pyridyl analogue (**24g**, K_i = 980 nM). These results indicate that an aromatic ring connected by a methylene is favored at this site.

To further explore the SAR at this site and to improve the affinity of 1-benzyluracils as antagonists of the hGnRH-R, we carefully examined a series of substitutions at the benzyl group. Among the monofluorinated analogues, the 2-fluoro derivative (**24k**, K_i = 20 nM) exhibited a binding affinity about 3-fold better than that of the parent **24h**, while 3- or 4-fluorination (**24i** and **24j**) had little effect. For the 2-substituent, it is apparent that the binding affinity increased along with the size. Thus, the K_i values for the 2-fluoro **24k**, 2-methyl **24n**, 2-chloro **24l**, and 2-bromo compound **24m** were 20, 20, 14, and 4.6 nM, respectively. The electron-donating 2-methoxy substitution resulted in a 2-fold reduction in binding (**24o**, K_i = 100 nM), whereas the electron-deficient 2-trifluoromethyl improved binding affinity (**24q**, K_i = 3.1 nM).

These observations prompted us to investigate disubstituted benzyl groups. While a second fluorine at the 6-position of the 2-fluorophenyl ring (**19g**, K_i = 5.6 nM, Table 2) increased binding affinity by about 4-fold, the combination of a strongly electron-withdrawing fluorine and a bulky chlorine at the two ortho positions resulted in compound **24s** with subnanomolar binding affinity (K_i = 0.61 nM). An additional fluorine at the para position of the 1-(2-chlorobenzyl) group of **24l** or at the meta position of the 1-(2-trifluoromethylbenzyl) group of **24q** had no effect on binding affinity (**24t** and **24r**, K_i = 21 and 3.9 nM, respectively).

These results may indicate that a bulky ortho substituent such as chlorine at the benzyl group favors a certain conformation that interacts well with the receptor and could be similar to that observed in the X-ray structure of *R*-**13b**. The SAR results also suggest that the 1-benzyl group interacts with an electron-rich aromatic ring such as tyrosine. Thus, an electron-deficient aromatic ring with a proper dipolar moment would be optimal to have a strong edge-to-face $\sigma-\pi$ interaction[22] or offset face-to-face $\pi-\pi$ stacking with the tyrosine residues.[23]

Acetylation of **19b** at the basic nitrogen afforded the amide **25**, which exhibited almost 500-fold reduction in binding affinity (K_i = 1.5 μM). These data suggest that the basic nitrogen is very important for high binding affinity, possibly through charge−charge attraction. However, since the acetyl group is quite large, it may cause a steric clash with the receptor and consequently a conformational change of the 3-side-chain of **19b**. A hint of this hypothesis is the phenomenon observed from the methylation of **19b**, which led to a modest reduction in binding affinity in **20b**. The role of this basic amine in receptor binding is not very clear.

Characterization of Atropisomeric 5-(2-Fluorophenyl)-6-methyluracils. As seen in the X-ray crystal structure of *R*-**13b**, the 5-(2-fluoro-3-methoxy)phenyl ring lay in an orthogonal orientation to the uracil ring. While this feature contributed to binding affinity at the hGnRH-R, it also created a unique property for these compounds—an interconvertible mixture of atropisomerers. Thus, when examined by NMR, all compounds containing a 5-(2-fluorophenyl) or 5-(2-fluoro-3-methoxyphenyl) group displayed two distinct sets of signals in proton, fluorine, and carbon spectra (see Experimental Section for details). The ratio of the mixture was typically 1:1. This observation was somewhat surprising, since the des-fluoro analogue **19a** did not show evidence of isomers, at least by NMR spectroscopy. The van der Waals radius of a fluorine is comparable to that of hydrogen (1.47 versus 1.20 Å), so it is difficult to infer that steric hindrance plays a major role in the slow rotation around the carbon−carbon bond between the 5-phenyl ring and the uracil. Although variable temperature NMR experiments did not result in coalescence of the two sets of signals at 90 °C in D_2O, this finding was confirmed by HPLC analysis. On a chiral column, these two rotameric diastereoisomers were easily separated at room temperature. The atropisomeric properties and their significance will be further discussed separately.[24]

Species Selectivity and Functional Antagonism of Selected Compounds. Compounds from this series also bound to the monkey GnRH receptor with good affinity, although typically a 10-fold or more reduction was observed (Table 4). However, none of the compounds in this study bound well to rGnRH-R, and the best affinity was about 3 μM (*R*-**13b**, *R*-**13d**, and **24s**).

Table 5. Pharmacokinetic Characterization of Uracils in Cynomolgus Monkeys[a]

compd	CL (ml/(min·kg))	V_d (L/kg)	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	AUC (ng/(mL·kg))	F (%)
***R*-13b**	12.6	3.4	3.1	3.0	737	2392	16
***S*-14a**	13.5	3.6	3.1	5.5	98	912	22
19a	16.9	4.9	3.3	2.3	380	1126	9.9
19b	15.5	4.6	3.5	4.0	420	3280	32

[a] Dosed at 10 mg/kg for both po and iv administrations.

For example, ***R*-13b** exhibited K_i values of 0.56, 3.5, and 3000 nM at the human, monkey, and rat GnRH receptors, respectively.

Compounds ***R*-13b**, **19a**, **19b**, and **24s** possessed good functional antagonism at the hGnRH-R. The IC_{50} values for these compounds ranged from 2.8 to 6.2 nM in inhibition of GnRH-stimulated calcium flux in RBL cells expressing the hGnRH-R.

Pharmacokinetics. The pharmacokinetic profiles of selected compounds were determined in cynomolgus monkeys to study their oral exposure. Compound **19b** gave, after a 10 mg/kg dose intravenously, a plasma clearance (CL) of 15.5 mL/(min·kg), a volume distribution (V_d) of 4.6 L/kg, and a terminal half-life ($t_{1/2}$) of 3.5 h. After oral administration of **19b** at a dose of 10 mg/kg, the plasma concentration reached maximal 420 ng/mL at 4 h, the plasma level area under the curve (AUC) was 3280 ng/mL·h. The oral bioavailability calculated from these data was 32%. These results confirmed that adequate exposure of compounds of this class could be obtained in the cynomolgus monkeys after oral administration. Similarly, both compounds **19a** and ***R*-13b** had good exposure after an oral dose of 10 mg/kg. Thus, the C_{max} values for **19a** and ***R*-13b** were 380 and 737 ng/mL, respectively, and the oral AUC values were 1126 and 2392 ng/mL·h, respectively (Table 5).

In Vivo Studies with Compound *R*-13b. Because all uracil derivatives synthesized herein lack the necessary high binding affinity to the rat receptor, in vivo pharmacology from selected compounds was characterized in castrated male cynomolgus macaques. Three monkeys each received various doses of ***R*-13b**, vehicle, or other GnRH antagonists as part of the evaluation of multiple advanced compounds, and circulating luteinizing hormone (LH) concentrations were measured. Data from the ***R*-13b** (at 40 mg/kg po, at 10 mg/kg iv, and with vehicle iv) are presented in Figure 3. Suppression of circulating LH was observed with nadirs ranging from 33% to 43% of the individual predose concentrations at 4−8 h after administration. Suppression by the 40 mg/kg oral dose was roughly equivalent to the 10 mg/kg intravenous dose. After 24 h, LH levels returned to baseline, demonstrating that the suppression was reversible.

Conclusion

We have reported on the design and synthesis of a series of uracil compounds bearing a 2-aminoalkyl group at the 3-position as potent antagonists of the hGnRH-R. Significant improvements in antagonist activity, as well as pharmacokinetic properties, for this class have been achieved by incorporation of a phenyl group at the 3-side-chain of the uracil core. SAR study at the 1-position also results in identification of electron-deficient benzyl groups as the most favored at this position. This series of compounds have lower molecular weight (***R*-13b**, MW = 495) than most known GnRH antagonists. Compounds from this class also exhibited good oral bioavailability in cynomolgus monkeys (32% for **19b**). Compound ***R*-13b** was characterized to possess good binding affinity with a K_i of 0.56 nM and potent functional antagonism with an IC_{50} of 3.0 nM in inhibition of GnRH-stimulated calcium flux at the hGnRH-R. This compound was moderately lipophilic with a log *D* value of 2.4. Its hydrochloride salt was readily soluble in water (>10 mg/mL). Compound ***R*-13b** also possessed a K_i value of 3.5 nM at the monkey receptor, and it significantly suppressed the plasma luteinizing hormone level by oral administration in this species. The ability of this compound to suppress circulating LH concentrations following oral administration to postmenopausal women will be reported elsewhere.

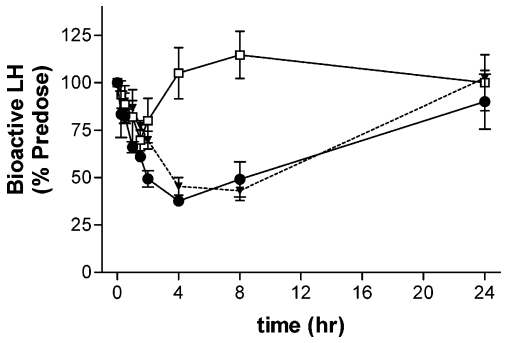


Figure 3. Suppression of plasma LH concentrations in castrated male cynomolgus macaques after administration of ***R*-13b** at 40 mg/kg orally (●), at 10 mg/kg intravenously (▼), or with intravenous vehicle (□). Values shown are the mean ± SEM of bioactive LH levels expressed as a percentage of pretreatment LH levels for each of three individual animals.

Experimental Section

Chemistry. Proton, carbon, and fluorine NMR spectra were recorded on Varian spectrometer (Mercury) using TMS as the internal standard and $CDCl_3$ as the solvent except where indicated. LC−MS analyses were performed on a Perkin-Elmer Sciex API-100 mass spectrometer using the electrospray ionization technique or on a SpectraSystem P4000 HPLC system coupled with a Finnigan LCD/Deca mass spectrometer using the electrospray ionization technique. HRMS ESI was carried out on a Bruker 4.7T BIOAPEX FTMS mass spectrometer. All compounds were characterized by LC−MS and proton NMR (300 MHz). All commercially available reagents were used without further purification.

3-[(2*R*)-Amino-2-phenylethyl]-1-(2,6-difluorobenzyl)-6-methyl-5-(2-fluoro-3-methoxyphenyl)pyrimidin-2,4-dione Hydrochloride (*R*-13b). To a solution of triphenylphosphine (104.1 g, 397 mmol), 1-(2,6-difluorobenzyl)-5-bromo-6-methyluracil (**10**, 87.7 g, 265 mmol) and Boc-*R*-phenylglycinol (66.0 g, 278 mmol) in THF (3.5L) under nitrogen atmosphere, was added diethyl azodicarboxylate (69.2 g, 397 mmol). The reaction mixture was stirred at room temperature for 16 h. The solvent was removed under reduced pressure, and the crude was purified by column chromatography on silica gel (hexane/EtOAc = 2:1) to afford 3-[(2*R*)-(*tert*-butoxycarbonylamino)-2-phenylethyl]-1-(2,6-difluorobenzyl)-5-bromo-6-methyluracil **11b** as a white solid (80.0 g, 55%).

In a pressure vessel, the bromide (**11b**, 2.20 g, 4.0 mmol) was dissolved in a 45:5:50 v/v/v mixture of benzene, EtOH, and 1,2-dimethoxyethane (80 mL). To that, 2-fluoro-3-methoxyphenylboronic acid (816 mg, 4.8 mmol) was added, followed by an aqueous saturated solution of $Ba(OH)_2$ (28.6 mL). The resulting heterogeneous mixture was degassed by N_2 bubbling

for 30 min. Tetrakis(triphenylphosphine)palladium (462 mg, 0.4 mmol) was added, and the vessel was tightly capped and immersed in an oil bath with the mixture being stirred at 80 °C. The reaction mixture was maintained at that temperature for 15 h, after which time the reaction was checked by TLC and LC−MS. Only a small amount of starting material remained. The reaction mixture was cooled, diluted with EtOAc, and washed with H_2O. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography on silica gel, eluting with a 4:1 v/v mixture of hexanes and ethyl acetate. The *R*-**13b** free base was obtained as a white foam (1.32 g, 2.2 mmol, 55%). The corresponding hydrochloride salt was obtained as a white solid by dissolving the free base in CH_2Cl_2 and treating with HCl (4.0 M solution in dioxane, 1 equiv), followed by ether. ^{1}H NMR: δ 2.04 and 2.06 (s, 3H), 3.81 and 3.84 (s, 3H), 3.89 and 4.42 (d, J = 12.0 Hz, 1H), 4.13 and 4.41 (m, 1H), 4.46 and 4.57 (dd, J = 10.0, 14.0 Hz, 1H), 4.79 and 5.10 (d, J = 16.4 Hz, 1H), 5.23 and 5.37 (d, J = 16.4 Hz, 1H), 6.80 and 6.86 (t, J = 8.0 Hz, 2H), 6.91 (m, 1H), 7.03 and 7.13 (t, J = 7.2 Hz, 1H), 7.22 (m, 2H), 7.15−7.26 (m, 3H), 7.28 (t, J = 7.6 Hz, 2H), 7.44 7.49 (d, J = 7.2 Hz, 2H). ^{13}C NMR: δ 17.58 and 17.65, 39.13 and 39.26, 45.6 and 46.1, 54.1 and 54.3, 56.1 and 56.2, 107.97 and 108.03, 111.7 and 111.9 (AA′XX′, 2C), 112.0 and 112.2 (t, J = 6.7 Hz), 113.2 and 113.4, 122.2 and 122.4 (d, J = 12.8 Hz), 123.8 and 124.8 (d, J = 3.8 Hz), 124.2 and 124.6, 127.1 and 127.2 (2C), 128.7 and 128.8 (2C), 129.5 and 129.6, 129.7 and 129.8, 134.1 and 134.3, 147.6 and 147.8 (d, J = 10.6 Hz), 149.9 and 150.3 (d, J = 244 Hz), 151.3 and 151.5, 151.6 and 152.3, 161.1 and 161.2 (dd, J = 7.6, 248 Hz, 2C), 161.4 and 162.9. ^{19}F NMR: δ −113.3 and −113.5 (t, J = 7.7 Hz, 2F), −134.0 and −1134.7 (m). MS, *m/z*: 496 (MH^+). Anal. ($C_{27}H_{24}F_3N_3O_3$·HCl·H_2O) C, H, N.

Sample for X-ray Crystal Structure. ***R*-13b** (free base, 1 g) was dissolved in a mixture of methanol (5 mL) and water (12 mL) with heating. Upon cooling to room temperature, the sample turned milky and was further treated with a few drops of methanol until clear. The sample vial was capped loosely and placed in a hood to evaporate slowly.

3-[(2*R*)-Dimethylamino-2-phenylethyl]-1-(2,6-difluorobenzyl)-6-methyl-5-(2-fluoro-3-methoxyphenyl)-pyrimidin-2,4-dione Hydrochloride (*R*-15b). 3-[(2*R*)-Amino-2-phenylethyl]-1-(2,6-difluorobenzyl)-6-methyl-5-(2-fluoro-3-methoxyphenyl)pyrimidin-2,4-dione (*R*-**13b**, 450 mg, 0.91 mmol) was dissolved in CH_2Cl_2 (20 mL) and treated with formaldehyde (200 μL of a 37% solution in H_2O). The resulting mixture was stirred at room temperature for 10 min. Sodium triacetoxyborohydride (1.33 g, 6.3 mmol) was added portionwise, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated in vacuo, diluted with EtOAc, and washed with H_2O, aqueous saturated $NaHCO_3$, and brine. The organic layer was dried over anhydrous $MgSO_4$ and filtered. Evaporation gave a residue that was purified by column on silica gel, eluting with a 1:1 v/v mixture of hexanes and EtOAc. The free base was isolated as a white foam (282 mg, 0.54 mmol, 59%). This was dissolved in CH_2Cl_2 (5 mL) and treated with HCl (1 mL of a 4.0 M solution in dioxane). After 1 h, the mixture was evaporated to dryness and triturated with Et_2O to give the hydrochloride salt as a white solid (256 mg, 0.46 mmol). ^{1}H NMR: δ 2.15 and 2.16 (s, 3H), 2.68−2.79 (m, 6H), 3.89 (s, 3H), 4.94 and 4.52 (m, 1H), 4.82−5.50 (m, 4H), 6.86−7.49 (m, 11H), 12.33 and 12.40 (brs, 1H). ^{19}F NMR: δ − 114.2 and − 114.1 (s, 2F), −136.2 and −136.0 (s, 1F). ^{13}C NMR: δ 17.9, 38.3 and 38.9, 39.4 and 39.7, 41.4 and 41.9, 43.8 and 43.9, 56.4, 66.8 and 67.0, 108.4 and 108.5, 111.9 (m, 2C), 112.1 (d, J = 16.7 Hz), 113.5, 122.4 and 122.5 (d, J = 11.1 Hz). MS, *m/z*: 524 (MH^+). Anal. ($C_{29}H_{28}F_3N_3O_3$·HCl·$1.3H_2O$) C, H, N.

3-[(2*S*)-Cyclobutylaminopropyl]-1-(2,6-difluorobenzyl)-6-methyl-5-(2-fluoro-3-methoxyphenyl)pyrimidin-2,4-dione Hydrochloride (*S*-16a). To *S*-**13f**[14] (216 mg, 0.5 mmol) in dichloroethane (10 mL) was added cyclobutanone (0.6 mmol), and the mixture was stirred at room temperature for 10 min. Then $NaBH(OAc)_3$ (255 mg, 1.2 mmol) was added, and the mixture was further stirred for 10 h and then diluted with DCM, washed with water, dried (sodium sulfate), and evaporated. The crude product was purified by flash chromatography (silica, 20−50% EtOAc/hexanes).

The above free base was converted to the hydrochloride salt by dissolution in dichloromethane and addition of 1 N HCl solution in ether, followed by removal of the solvent under vacuum to provide the HCl salt as an amorphous solid. ^{1}H NMR (DMSO-d_6): δ 1.12 (d, J = 5.1 Hz, 3H), 1.75 (m, 2H), 2.14 (m, 2H), 2.18 (s, 3H), 2.22 (m, 2H), 3.38 (m, 1H), 3.82 (m, 1H), 3.86 (s, 3H), 4.05 (m, 2H), 5.27 (s, 2H), 6.77 (m, 1H), 7.08−7.22 (m, 4H), 7.42 (m, 1H)9.10 (brs, 2H). ^{19}F NMR: δ −115.0 (t, J = 7.7 Hz, 2F), −135.3 and −135.4 (t, J = 4.5 Hz, 1F). ^{13}C NMR: δ 14.3, 14.7 and 14.8, 17.5, 26.3 and 26.4, 26.5, 38.8, 42.6, 48.9, 49.9, 56.0, 106.9, 111.9 (m, 2C), 112.1 (t, J = 17 Hz), 113.5, 122.2 (d, J = 13.6 Hz), 123.6, 124.0, 130.1 (t, J = 10.7 Hz), 147.4 and 147.5, 149.5 (d, J = 242 Hz), 151.1 and 151.2, 151.2 and 151.3, 106.6 (dd, J = 7.6, 245.8 Hz, 2C), 160.6. MS, *m/z*: 488 (MH^+). Anal. ($C_{25}H_{28}F_3N_3O_3$·HCl·$0.5H_2O$) C, H, N.

3-[(2*R*)-Amino-2-phenylethyl]-1-(2,6-difluorobenzyl)-6-methyl-5-(3,4-methylenedioxyphenyl)pyrimidin-2,4-dione Hydrochloride (19a). 3-[(2*R*)-(*tert*-Butoxycarbonylamino)-2-phenylethyl]-1-(2,6-difluorobenzyl)-5-bromo-6-methyluracil **11b** (22.6 g, 41 mmol), 3-methoxyphenylboronic acid (7.5 g, 49 mmol), and saturated barium hydroxide solution (280 mL) were added to a mixture solvent (benzene/EtOH/DME = 10:1:11, 800 mL). The reaction mixture was degassed with nitrogen, and tetrakis(triphenylphosphine)palladium (4.7 g, 4 mmol) was added. The reaction mixture was then heated at 80 °C for 18 h, cooled to room temperature, and filtered through a pad of Celite. The organic layer was separated and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/EtOAc = 3:1) to yield 3-[(2*R*)-(*tert*-butoxycarbonylamino)-2-phenylethyl]-1-(2,6-difluorobenzyl)-5-(3-methoxyphenyl)-6-methyluracil (22.0 g, 93%).

The above compound was treated with 1 equiv of 4.0 M HCl in dioxane (100 mL) for 30 min. The solvent was removed in vacuo and the residue was treated with ether to precipitate the title compound as a white solid (14.7 g, 75%). ^{1}H NMR: δ 2.10 (s, 3H), 3.99 (m, 1H), 4.52 (m, 2H), 5.18 (s, 2H), 5.91 (s, 1H), 5.96 (s, 1H), 6.60 (m, 2H), 6.86 (m, 3H), 7.23 (m, 2H), 7.33 (m, 2H), 7.59 (d, J = 7.5 Hz, 2H), 8.70 (brs, 3H). ^{19}F NMR ($CFCl_3$): δ −115.0 (s). MS, *m/z*: 478 (MH^+). Anal. ($C_{27}H_{23}F_2N_3O_4$·HCl·$1.4H_2O$) C, H, N.

3-[(2*R*)-Methylamino-2-phenylethyl]-1-(2,6-difluorobenzyl)-6-methyl-5-(3,4-methylenedioxyphenyl)pyrimidin-2,4-dione Hydrochloride (20b). A solution of BH_3/THF (20 mL of a 1.5 M solution in THF, 29.4 mmol, 2 equiv) was added dropwise in neat form to d-(*N*-Boc-*N*-methyl)phenylglycine (3.90 g, 14.7 mmol) at 0 °C under an inert atmosphere of N_2. After 15 min, the ice/water bath was removed and the mixture was allowed to reach room temperature. After 1 h, the reaction mixture was cooled to 0 °C and the reaction was carefully quenched by addition of H_2O (20 mL). The resulting mixture was extracted with EtOAc, and the organic layer was separated and washed with a 10% aqueous citric acid solution and brine. The organics were dried ($MgSO_4$), filtered, and evaporated. The crude (2*R*)-(*N*-Boc-*N*-methyl)phenylglycinol was isolated as a white solid, and it was used without any further purification, 2.67 g (72% yield). ^{1}H NMR: δ 1.43 (s, 9H), 2.69 (br s, 3H), 4.09−4.02 (m, 2H), 5.30 (br, 1H), 7.38−7.22 (m, 5H).

To a suspension of 1-(2,6-difluorobenzyl)-5-bromo-6-methyluracil (**10**, 3.35 g, 10.1 mmol), the above alcohol (2.67 g, 10.6 mmol), and triphenylphosphine (4.0 g, 15.2 mmol) in THF (150 mL) under N_2 at room temperature, was added DEAD (2.40 mL, 15.2 mmol) dropwise. The initial yellow color was discharged, and a clear, colorless solution resulted. This was stirred at room temperature for 15 h, and then the solvent was removed in vacuo. The crude residue was purified by column chromatography on silica gel, eluting with a 3:1 v/v mixture of hexanes and ethyl acetate. 3-[(2*R*)-[*N*-(*tert*-Butoxycarbonyl)-*N*-methylamino)-2-phenylethyl]-1-(2,6-difluoroben-

zyl)-5-bromo-6-methyluracil *R*-**12b** was isolated as a white solid (2.91 g, 51%). ^{1}H NMR: δ 1.39 (s, 9H), 2.51 (br, 6H), 4.33−4.21 (m, 1H), 4.86 (q, J = 12.3 Hz, 1H), 5.29 (q, J = 14.1 Hz, 1H), 5.33 (q, J = 15.7 Hz, 1H), 5.85 (m, 1H), 6.93−6.89 (m, 2H), 7.43−7.27 (m, 6H). MS, *m/z*: 464 (MH^+ − Boc).

In a pressure vessel, 3-[(2*R*)-(*tert*-butoxycarbonylamino)-2-phenylethyl]-1-(2,6-difluorobenzyl)-5-bromo-6-methyluracil (226 mg, 0.40 mmol) was dissolved in a 45:5:50 v/v mixture of benzene, EtOH, and 1,2-dimethoxyethane (8 mL). To that, 3,4-methylenedioxyphenylboronic acid (80 mg, 0.48 mmol) was added followed by an aqueous saturated solution of $Ba(OH)_2$ (3 mL). The resulting heterogeneous mixture was degassed by N_2 bubbling for 30 min. Tetrakis(triphenylphosphine)-palladium (46 mg, 0.04 mmol) was added, and the vessel was tightly capped and immersed in an oil bath at 100 °C. The reaction mixture was maintained at that temperature for 22 h, after which time the reaction was deemed complete by TLC and LC−MS. The reaction mixture was cooled, diluted with EtOAc, and washed with H_2O. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography on silica gel, eluting with a 3:1 v/v mixture of hexanes and ethyl acetate. The product was obtained as a yellow foam (85 mg, 0.14 mmol, 35%).

The above compound (85 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (1.5 mL) and treated with trifluoroacetic acid (0.75 mL). The resulting mixture was stirred for 1.5 h at room temperature and then concentrated in vacuo. The residue was partitioned between EtOAc and an aqueous $NaHCO_3$ saturated solution. The organics were separated, washed with brine, dried ($MgSO_4$), filtered, and evaporated. The residue was purified by column chromatography on silica gel, eluting with EtOAc. The free base was obtained as a white foam (52 mg, 0.10 mmol, 71%). This was dissolved in CH_2Cl_2 (1 mL) and treated with HCl (3 mL of a 1.0 M solution in Et_2O). After 1 h, the volatiles were removed in vacuo and the residue was triturated with Et_2O (10 mL) to give the hydrochloride salt as a white solid (50 mg). ^{1}H NMR: δ 2.13 (s, 3H), 2.44 (s, 3H), 4.25 (d, J = 13.2 Hz, 1H), 4.60 (m, 1H), 4.78 (d, J = 10.4 Hz, 1H), 5.25 (brs, 2H), 5.89 (s, 2H), 6.71 (m, 2H), 6.85 (t, J = 6.3 Hz, 2H), 7.23 (m, 1H), 7.35 (m, 3H), 7.53 (m, 2H), 9.16 (brs, 1H), 10.3 (brs, 1H). ^{19}F NMR: δ −115.1. MS, *m/z*: 506 (MH^+). Anal. ($C_{28}H_{25}F_2N_3O_4$·HCl·1.5H_2O) C, H, N.

3-[(2*R*)-Amino-2-phenylethyl]-6-methyl-5-(2-fluorophenyl)oxazine-2,4-dione Trifluoroacetate (23). To a stirred solution of 2′-fluorophenylacetone (7.6 g, 50 mmol) in ether (50 mL) was added dropwise chlorosulfonyl isocyanate (16.2 g, 115 mmol) at room temperature. The yellowish solution was stirred overnight, poured onto ice (100 g), and basified with sodium carbonate. The product was extracted with ethyl acetate (2 × 200 mL), and the extract was washed with water and brine, dried over magnesium sulfate, and concentrated in vacuo to give a yellow residue (9.5 g, proton NMR, about 70% product). The crude product was crystallized from ether/hexanes to give 6-methyl-5-(2-fluorophenyl)oxazine-2,4-dione (**21**) as a yellowish solid (3.6 g, 33% yield). ^{1}H NMR: δ 2.14 (s, 3H), 7.16 (t, J = 9.0 Hz, 1H), 7.24 (m, 2H), 7.41 (m, 1H), 9.20 (brs, 1H).

DEAD (348 mg, 1.2 mmol) was added to a solution of the above oxazine **21** (221 mg, 1.0 mmol), triphenylphosphine (314 mg, 1.2 mmol), and (*R*)-*N*-Boc-phenylglycinol (249 mg, 1.05 mmol) in dry THF (5 mL). The mixture was stirred at room temperature for 2 h, concentrated, and purified by chromatography on silica gel with 1:3 ethyl acetate/hexanes to give 3-[2(*R*)-*tert*-butoxycarbonylamino-2-phenylethyl]-6-methyl-5-(2-fluorophenyl)oxazine-2,4-dione (**22**) (380 mg, 87%) as a white solid. ^{1}H NMR: δ 1.39 (s, 9H), 2.14 (s, 3H), 4.02 (m, 1H), 4.28 (m, 1H), 5.21 (brs, 1H), 5.30 (m, 1H), 7.38 (m, 9H). MS, *m/z*: 341 (MH^+ − BuOCO).

Compound **22** (30 mg) was treated with trifluoroacetic acid (1 mL) at room temperature for 30 min. Concentration in vacuo gave the title compound as a colorless oil in quantitative yield. ^{1}H NMR: δ 2.05 and 2.08 (s, 3H), 4.10 (m, 1H), 4.45 (m, 1H), 4.62 (m, 1H), 7.15 (m, 3H), 7.40 (m, 6H), 8.20 (brs, 3H). MS, *m/z*: 341 (MH^+).

3-[2(*R*)-Amino-2-phenylethyl]-6-methyl-5-(2-fluorophenyl)-1-(2-methoxybenzyl)uracil Trifluoroacetate (24o). A mixture of **22** (29 mg) and 2-methoxybenzylamine (0.15 mL) was heated in a sealed reaction vial at 100 °C for 1 h. Chromatography on silica gel with 1:2 ethyl acetate/hexanes gave 3-[2(*R*)-*tert*-butoxycarbonylamino-2-phenylethyl]-6-methyl-5-(2-fluorophenyl)-1-(2-methoxybenzyl)uracil as a colorless oil. ^{1}H NMR: δ 1.40 (s, 9H), 2.04 (s, 3H), 3.87 (s, 3H), 4.18 (m, 1H), 4.44 (m, 1H), 5.22 (m, 2H), 5.65 (brs, 1H), 5.78 (m, 1H), 6.85−7.42 (m, 13H). MS, *m/z*: 460 (MH^+ − BuOCO).

The above compound (20 mg) was treated with trifluoroacetic acid (1 mL) at room temperature for 30 min. Concentration in vacuo gave the title compound as a colorless oil in quantitative yield. ^{1}H NMR: δ 2.04 (s, 3H), 3.82 and 3.85 (s, 3H), 4.20 (m, 1H), 4.62 (m, 2H), 5.10 (m, 2H), 6.82−7.40 (m, 13H), 8.05 (brs, 3H). MS, *m/z*: 460 (MH^+). HRMS (CI − CH_4) calcd for $C_{27}H_{26}FN_3O_3$ (MH^+): 460.203 65. Observed: 460.202 10.

3-[(2*R*)-Acetamido-2-phenylethyl]-1-(2,6-difluorobenzyl)-6-methyl-5-(3,4-methylenedioxyphenyl)pyrimidin-2,4-dione (25). A solution of 3-[(2*R*)-amino-2-phenylethyl]-1-(2,6-difluorobenzyl)-6-methyl-5-(3,4-methylenedioxyphenyl)pyrimidin-2,4-dione (**19b**, 400 mg, 0.81 mmol) in acetic anhydride (3 mL) was stirred at room temperature overnight. Volatiles were evaporated, and the residue was dried under vacuum overnight at 60 °C. The crude material was then dissolved in dichloromethane (5 mL) and the product was precipitated with ether/hexane (1/1 volume) to obtain the title compound as a white solid (406 mg, 93%). ^{1}H NMR (DMSO-d_6): δ 1.72 (s, 3H), 2.17 (s, 1H), 4.00 (dd, J = 5.6, 12.8 Hz, 1H), 4.09 (dd, J = 9.2, 12.8 Hz, 1H), 5.16 (d, J = 16.4 Hz, 1H), 5.23 (d, J = 16.4 Hz, 1H), 5.27 (m, 1H), 6.04 (s, 2H), 6.54 (d, J = 7.2 Hz, 1H), 6.59 (s, 1H), 6.92 (d, J = 8.4 Hz, 1H), 7.11 (t, J = 8.4 Hz, 2H), 7.26 (m, 5H), 7.42 (m, 1H), 8.21 (d, J = 9.2 Hz, 1H). ^{13}C NMR: δ 17.6, 22.4, 45.5, 50.2, 101.0, 108.0, 111.0, 111.8 (m, 2C), 112.4 (t, J = 16.7 Hz), 112.7, 124.2, 126.6 (2C), 127.0, 127.9, 128.1, 129.8 (t, J = 9.9 Hz), 140.1, 146.5, 146.9, 149.4, 150.9, 160.6 (dd, J = 7.6, 245.7 Hz, 2C), 161.2, 168.7. ^{19}F NMR: δ −114.8 (t, J = 7.0 Hz). MS, *m/z*: 534 (MH^+). Anal. ($C_{29}H_{25}F_2N_3O_5$) C, H, N.

Pharmacokinetics of Uracil Compounds. A total of 10 mg/kg test articles were administrated to postsurgery monkeys via oral (po) and intravenous (iv) routes. For iv dosing, a formulated (water or 5% chromophore) dosing solution was administered as an intravenous infusion over a period of 15 min. Blood was collected from an accessible vein at predose, 0.25, 0.33, 0.5, 1, 1.5, 4, 8, and 24 h after dosing. For oral dosing, a sterile water solution was administered with a nasogastric gavage. Blood was collected at predose, 0.25, 0.5, 1, 1.5, 2, 4, 8, and 24 h after dosing. Plasma sample was harvested from blood by centrifugation within 30 min after collection. All samples were stored at −70 °C or below until analysis.

The bioanalytical method applied for the measurement of test article in plasma along with added internal standard consisted of protein precipitation from 50 μL of plasma with 200 μL of acetonitrile. The processed samples were then centrifuged to recover the supernatant. The supernatant was dried in a vacuum and then reconstituted in acetonitrile/water solutions before being introduced into an LC−MS/MS system for analysis. The analytical system consisted of a Waters 2790 HPLC module coupled to a Micromass Quattro-LC system. An Agilent Zorbax SB-C18 (5 μm, 4.6 mm × 5.0 mm) column provided separations for sample analysis. The lower limit of quantification (LLOQ) for the analytical method was 5 ng/mL of test article in plasma. All pharmacokinetic parameters were calculated from noncompartmental models using the WinNonlin program, version 3.2. Oral bioavailability was calculated from the dose-adjusted area under the curve (AUC) from the oral route over the dose-adjusted AUC from the iv route.

In Vivo Efficacy in Cynomolgus Monkeys. Complete orchiectomy was performed on six male cynomolgus macaques

approximately 3.7−6.5 years of age, and animals were allowed to recover for at least 4 weeks prior to dosing. Animals were not sedated for dosing but were temporarily restrained outside their cages. Oral doses were administered via nasogastric gavage, and blood samples were collected immediately prior to and 0.25, 0.5, 1, 1.5, 2, 4, 8, and 24 h after dosing. Intravenous doses were infused over a 15 min period, and samples were collected immediately prior to and 0.25, 0.33, 0.5, 1, 1.5, 4, 8, and 24 h after the initiation of the infusion. Bioactive LH concentrations in serum samples were measured at the Oregon Regional Primate Center using a previously reported mouse Leydig cell bioassay, which could detect as little as 3 ng LH/mL using cynomolgus LH RP-1 as the reference preparation.[25]

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Supporting Information Available: Synthesis and analytical data of *S*-**13a**,**b**, *R*-**13c**−**e**, *S*-**14a**, *R*-**14b**, *S*-**15a**−**c**, *R*-**15e**, **19b**−**h**, **20a**,**c,** and **24a**−**n**,**p**−**w**. This material is available free of charge via the Internet at http://pubs.acs.org.

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