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Synthesis and biological evaluation of 3-(2-aminoethyl) uracil derivatives as gonadotropin-releasing hormone (GnRH) receptor antagonists

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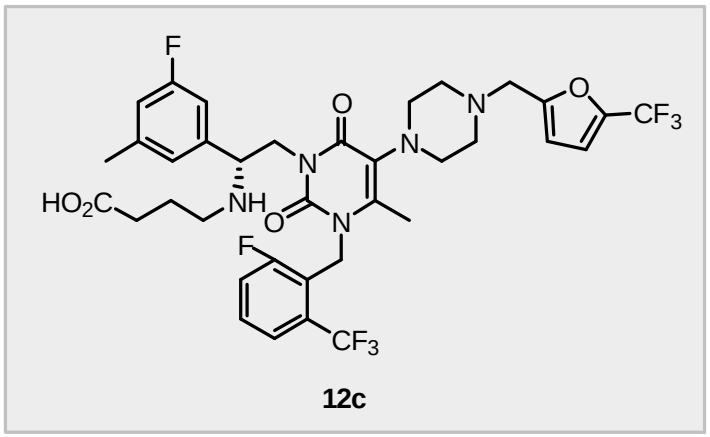
Graphical Abstract



12c

NFAT inhibition IC_{50}	9.9 nM
max. LH inhibition (%, h)	82%, 8 h
LH inhibition (24 h)	69%

Synthesis and biological evaluation of 3-(2-aminoethyl) uracil derivatives as gonadotropin-releasing hormone (GnRH) receptor antagonists

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Keywords:

Gonadotropin-releasing hormone receptor, GnRH antagonist, luteinizing hormone, endometriosis

Abstract

We investigated a series of uracil analogues by introducing various substituents on the phenyl ring of the *N*-3 aminoethyl side chain and evaluated their antagonistic activity against human gonadotropin-releasing hormone (GnRH) receptors. Analogues with substituents at the ortho or meta position demonstrated potent in vitro antagonistic activity. Specifically, the introduction of a 2-OMe group enhanced nuclear factor of activated T-cells (NFAT) inhibition up to 6-fold compared to the unsubstituted analogue. We identified compound **12c** as a highly potent GnRH antagonist with moderate CYP inhibition. Compound **12c** showed potent and prolonged LH suppression after a single dose was orally administered in castrated monkeys compared to a known antagonist, Elagolix. We believe that our SAR study offers useful insights to design GnRH antagonists as a potential treatment option for endometriosis.

1. Introduction

Endometriosis is a progressive disease mainly affecting premenopausal women, with a prevalence rate of 5 - 10% [1]. The disease is histologically characterized by the presence of endometrial tissue outside of the uterine cavity where the tissue grows in response to the cyclic rhythm of ovarian sex hormones [2]. Depending on the affected site, patients suffer from pelvic pain, dysmenorrhea, dyspareunia and infertility [3]. Surgery is a preferred treatment option that can improve pregnancy rate and relieve the endometriosis-associated pain. However, the high recurrence of endometriosis leads to reoperation in 50% of the patients within 5 years [4], requiring continuous management of the patients until they reach menopause. Suppression of ovulation and/or estrogens by the administration of combined oral contraceptive pills (OCPs) can prevent recurring endometriosis and maintain the endometrial tissue as thin and compact [1]. However, OCPs are associated with side effects such as breakthrough bleeding, weight gain, fluid retention, depression and venous thromboembolism. Other agents such as progestational agents, which are similar to combined OCPs in mechanistic ways, also exhibit similar adverse effects such as venous thromboembolism. Human gonadotropin-releasing hormone (GnRH) receptor agonists are another therapeutic option to maintain a hypoestrogenic state by suppressing the hypothalamic-pituitary-ovarian axis. However, with an excessively low estrogen level, they cause serious side effects such as reduction of bone mineral density, which limits the period of their use or necessitates an additional “add-back therapy” with low dose estrogen [1].

According to the estrogen threshold hypothesis proposed by Barbieri, tissues vary in their sensitivity to estrogen. For example, an extremely low estrogen level induces bone loss; however, only a slight elevation of estrogen can restore bone turnover without stimulating the growth of endometriotic lesions. Such a difference suggests an optimal therapeutic window of 30-45 pg/ml estradiol for endometriosis which induces atrophy of endometriotic cells without any impact on bone loss [5]. Given the hierarchy of organ response to estradiol, GnRH antagonists are considered to be a better alternative due to their partial suppression of estradiol. The hypothesis has been

verified by recent clinical trials for an oral GnRH antagonist [6]. In phase III trials, small-molecule GnRH antagonists appear to have advantages over GnRH agonists in terms of convenience in the dosing regimen and control of estrogen level.

Elagolix (**1a**) is an orally available GnRH antagonist currently in phase III trials for the treatment of endometriosis and uterine fibroids (**Figure 1**). Elagolix demonstrated improved potency and CYP3A4 inhibition compared to its parent compound, NBI-42902 (**1b**) [7, 8]. While elagolix is the most advanced compound developed to date, given its modest oral bioavailability and rapid metabolism [9], expanding the compound library with improved pharmacokinetic parameters will aid in finding better clinical candidates. We previously developed a series of uracil derivatives containing the *N*-substituted piperazines at the 5-position and identified SKI2496 (**1c**) as a promising candidate with an improved oral bioavailability [10]. As a continued effort to diversify GnRH antagonists based on a uracil scaffold, we decided to shift our focus on the phenyl ring at the *N*-3 position of the uracil core. While previously reported SAR studies indicated that the 3-aminoethyl group is particularly important for metabolic stability [11, 12], relatively few analogues have been investigated regarding the SAR of the phenyl ring, probably due to synthetic difficulties. In this study, we synthesized a series of uracil derivatives with various nonaromatic or substituted aromatic rings at the 3-position of the uracil core that also contained the 5-piperazinyl substituents. We evaluated the biological activity of the compound library by measuring the binding affinity and NFAT promoter inhibition for GnRH receptors in vitro. In addition, we determined the pharmacokinetic and pharmacodynamic profiles of the selected compound in animal models to assess in vivo antagonistic effects including luteinizing hormone (LH) suppression and species sensitivity.

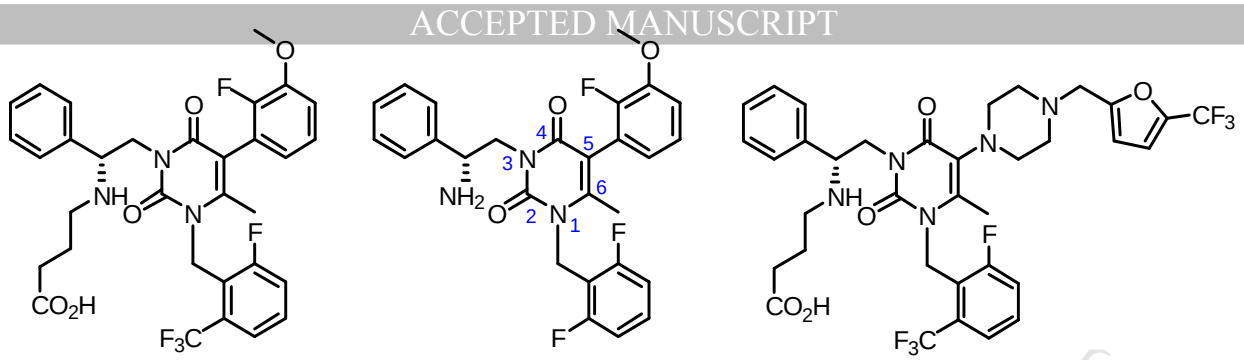


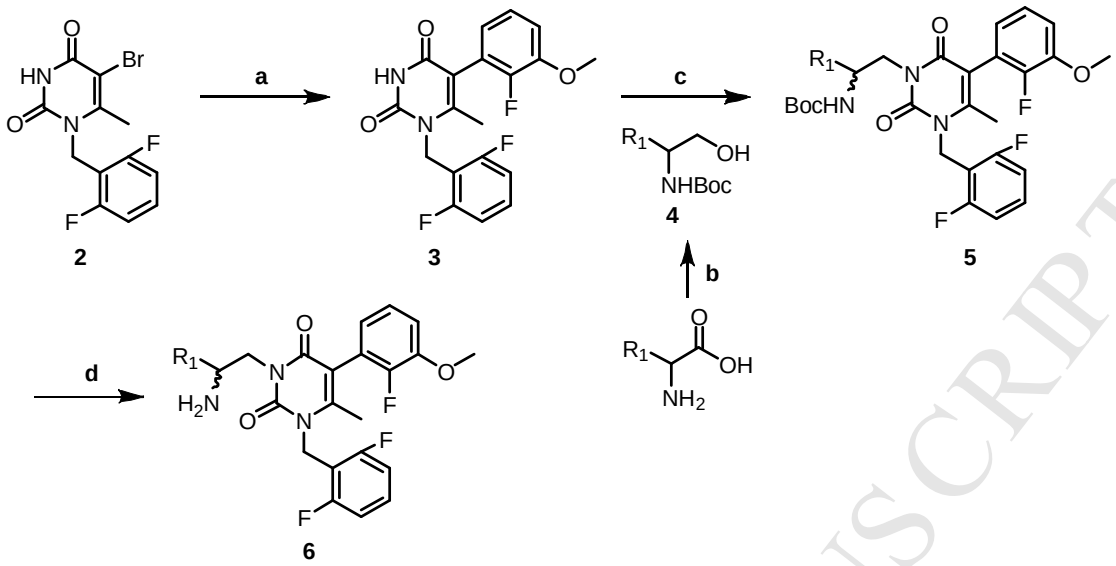
Figure 1. Examples of small-molecule GnRH antagonists

2. Results and discussion

2.1 Chemistry

Syntheses of 3-(2-aminoethyl) uracil derivatives began with compound **2**, which was prepared according to previously described procedures [13]. After *N*-PMB protection of compound **2**, the Suzuki coupling reaction of the 5-bromine with 2-fluoro-3-methoxyphenylboronic acid was performed, followed by deprotection of the *N*-PMB group using aluminum chloride to yield compound **3**. To diversify the *N*-3 position of the uracil core, we synthesized the *N*-Boc-protected amino alcohol fragment **4** by reducing commercially available unnatural amino acids and protecting the α-amino group. The amino alcohol fragment **4** was then introduced into the intermediate **3** by performing the Mitsunobu reaction. Subsequent deprotection of the *N*-Boc group yielded the corresponding amine **6** (**Scheme 1**).

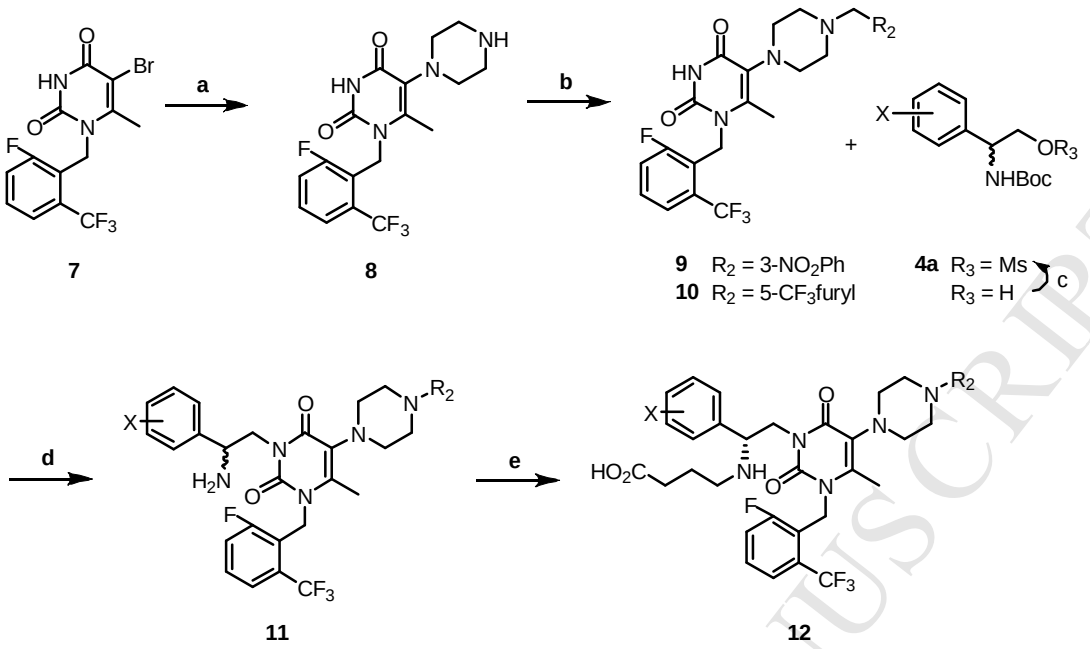
Scheme 1. Synthesis of 3-(2-2-aminoethyl) uracil derivatives



Reagents and coniditions: (a) i) p-methoxybenzyl chloride, K_2CO_3, DMF, 60 ^oC, 3 h, 56%, ii) 2-fluoro-3-methoxyphenylboronic acid, $Pd(PPh_3)_4$, saturated aq. $Ba(OH)_2$, benzene:EtOH:DME = 45:5:50, 85 ^oC, 2 days, 55%, iii) $AlCl_3$, anisole, r.t, 3 h, 93%; (b) i) $LiAlH_4$, THF, 70~80 ^oC, 3~4 h, 37~58%, ii) $(Boc)_2O$, diisopropylethylamine, CH_2Cl_2, r.t., overnight, 22~86%; (c) PBu_3, DEAD, DMF, 80 ^oC, 10~42%; (d) TFA, CH_2Cl_2, r.t., 3h, 52~81%

To synthesize the 5-piperazinyl uracil derivatives, substitution of *N*-benzylpiperazine at the 5-position of compound **7**, followed by removal of the benzyl group yielded compound **8**. Alkylation of **8** with the proper halide afforded two intermediates **9** and **10**, which were subjected to *N*-alkylation with various mesylates **4a** and subsequent Boc deprotection to give the corresponding amines **11**. The amine compounds were further alkylated with ethyl 4-bromobutyrate, followed by hydrolysis with aqueous sodium hydroxide to yield the acids **12** (Scheme 2).

Scheme 2. Synthesis of 5-(4-piperazinyl) uracil derivatives



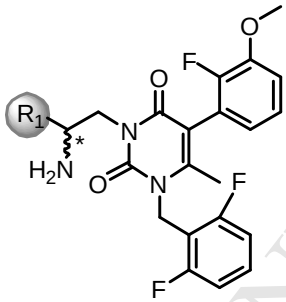
Reagents and conditions: (a) i) benzylpiperazine, MeCN, 120 ^{o}C, 2 h, microwave, 48%, ii) Pd/C, H_2, MeOH/CH_2Cl_2, r.t., 3 h, 87% (b) R_2CH_2Br, DIPEA, CH_2Cl_2, 2 h, r.t, 77~89%; (c) MsCl, TEA, CH_2Cl_2, 100%; (d) i) K_2CO_3, DMF, 70 ^{o}C, overnight, 65%, ii) TFA, CH_2Cl_2, r.t., 3 h, 65~85%; (e) i) $Br(CH_2)_3CO_2Et$, DIPEA, NaI, MeCN, 95 ^{o}C, overnight, 33%, ii) 1N-NaOH, EtOH, 50 ^{o}C, overnight, 79%

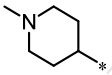
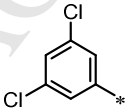
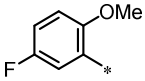
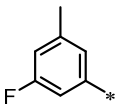
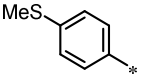
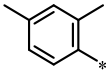
2.2 Structure-Activity Relationship

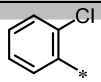
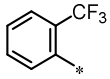
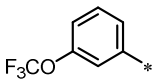
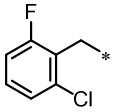
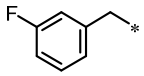
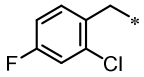
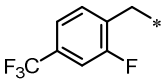
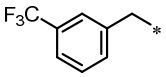
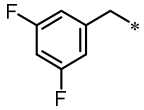
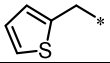
To evaluate the biological activity of the synthesized uracil analogues, we determined their in vitro binding affinities for the human GnRH receptors using the radiolabeled peptide, [^{125}I]D-Trp^{6}-LHRH. We first wanted to determine whether the modification of the phenyl ring at the *N*-3 position of the uracil core affected the binding affinity. As described in **Table 1**, all of the heterocyclic analogues (**6a-6g**) showed reduced binding affinities, regardless of aromaticity, except for the 2-furyl analogue (**6f**). As demonstrated by two enantiomers of the 2-furyl derivatives (**6f, 6g**), stereochemistry is also an important factor at this specific position. Analogues containing a phenyl ring (**6h-6o**) varied in binding affinity depending on the substituents. For example, compounds with at least one electron-withdrawing group (**6h-6j**, and **6m**) demonstrated comparable activities to that

of compound **1a**, except 2-trifluoromethyl and 3-fluoromethoxy analogues (**6n, 6o**). Compound **6i**, which had 2-OMe and 5-F substituents, demonstrated the most potent binding affinity in this series (IC_{50} = 0.29 nM). Given that **6i** was tested as a racemic mixture, we believe that the specific stereoisomer would be more potent. On the other hand, benzyl analogues (**6p-6v**) generally demonstrated weaker binding affinities, with IC_{50} values ranging from 9 to 62.3 nM.

Table 1. SAR of 3-(2-aminoethyl) uracil compounds



Compound	R_1	Stereochemistry	*h*GnRH-R binding IC_{50} (nM)
1a			0.59
6a		R	> 1000
6b		R	> 100
6c		RS	49.5
6d		RS	>1000
6e		RS	69.1
6f		S	2.3
6g		R	73.3
6h		RS	1.21
6i		RS	0.29
6j		RS	0.74
6k		RS	20.4
6l		RS	5.4

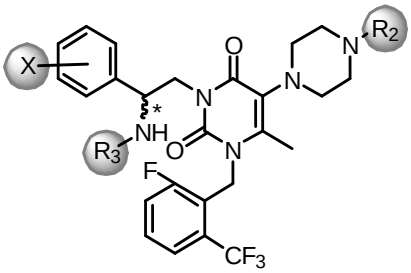
6m		RS	0.38
6n		RS	19.1
6o		RS	7.5
6p		RS	16
6q		RS	46
6r		RS	62.3
6s		RS	11.3
6t		RS	9
6u		RS	6.2
6v		RS	17.2

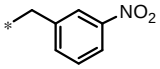
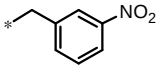
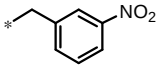
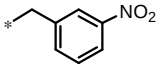
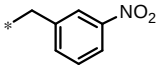
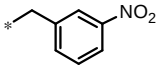
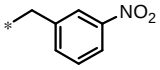
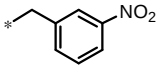
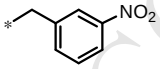
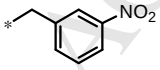
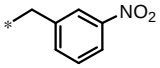
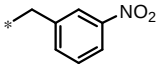
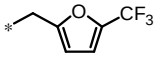
Because several analogues in this series demonstrated potent binding affinity that was comparable to **1a**, we next wanted to incorporate these modifications with the 5-piperazinyluracil moiety that showed superior potency and bioavailability in our previous study [10]. We selected 4-(3-nitrobenzyl)-piperazinyl (**11a-11l**) and 4-((5-(trifluoromethyl)-furan-2-yl)methyl)piperazinyl) groups (**11m-11q**, and **12a-12c**) for further derivatization of the 5-position. We additionally replaced the 1-(2,6-difluoro)benzyl group at the *N*-1 position with a 1-(2-fluoro-6-trifluoromethyl)benzyl group, which was present in compound **1a** and led to a more potent antagonistic effect than that observed for **1b** [14]. For these derivatives (compounds **11a-11q** and **12a-12c**), we assessed the antagonistic activity by performing a reporter gene assay that measures the inhibition of NFAT (nuclear factor of activated T-cells) activation. As described in **Table 2**, among the analogues with 4-(3-nitrobenzyl)-piperazinyl group (**11a-11l**), compounds **11i** (X = 2-methoxy, 5-F) and **11k** (X = 3-methyl, 5-F) showed 5- to 7-fold higher activities than **11a** (X = H).

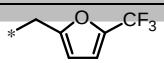
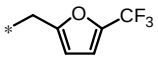
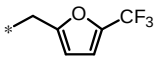
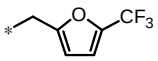
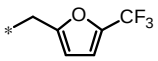
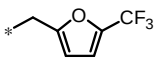
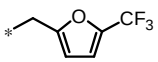
It is interesting to note that compounds with these particular substituents in the previous series (**6i** and **6j**) also demonstrated potent in vitro binding affinities. While most of the compounds were tested as a racemic mixture, it appears that the (R)-stereoisomers (**11i** and **11k**) are more potent than the (S)-stereoisomer (**11j** and **11l**). The four substituents showing the most potent NFAT inhibition, such as 2-OMe, 2-OH, (2-OMe, 5-F) and (3-Me, 5-F) were additionally incorporated into the 5-(4-((5-(trifluoromethyl)-furan-2-yl)methyl)piperazinyl)uracil core as (R)-stereoisomers (compounds **11m-11q**), and these derivatives demonstrated potent NFAT inhibition, having IC_{50} values in the low nanomolar range (1.54 – 15.2 nM). Specifically, compounds **11n**, **11o**, and **11p** exhibited equal or higher activities than the unsubstituted analogue (**11m**, IC_{50} = 8.96 nM) while **11n** (X = 2-OMe) was the most potent (IC_{50} = 1.54 nM).

On the other hand, when these potent compounds were tested for CYP inhibition, they also strongly inhibited the CYP3A4 enzyme. Given that elagolix (**1a**) was modified from NBI-42902 (**1b**) to improve the CYP inhibition, this result is not surprising. Therefore, we added a butyric acid group at the amine group of the *N*-3 side chain, which is the same functional group as that in elagolix. The strategy, when applied to our compounds, was also effective, significantly reducing the CYP3A4 inhibition. While compounds **12a** (X = 2-OMe) and **12b** (X = 2-OMe, 5-F) significantly lost their antagonistic activities, compound **12c** (X = 3-Me, 5-F) maintained its activity and demonstrated relatively weak CYP3A4 inhibition (30% at 10 μM), which is comparable to the unsubstituted analogue (21% at 10 μM) in our previous study [10]. Therefore, we decided to focus on **12c** for further in vivo studies.

Table 2. SAR of 5-(4-piperazinyl) uracil derivatives



Compound	R_2	R_3	X	Stereo-chemistry	hGnRHR luciferase IC_{50} (nM)	CYP3A4 inh. % (1μM /10μM)
11a		-H	-H	*R*	165	ND
11b		-H	2-Me	*RS*	1066	ND
11c		-H	2-OMe	*R*	45	ND
11d		-H	2-OH	*R*	89	ND
11e		-H	2-F	*RS*	142	ND
11f		-H	3-Me	*RS*	160	ND
11g		-H	4-F	*R*	1682	ND
11h		-H	3-F	*RS*	144	ND
11i		-H	2-OMe,5-F	*R*	32	ND
11j		-H	2-OMe,5-F	*S*	26%*	ND
11k		-H	3-Me,5-F	*R*	22	ND
11l		-H	3-Me,5-F	*S*	35%*	ND
11m		-H	H	*R*	8.96	ND

11n		-H	2-OMe	*R*	1.54	88 / ND
11o		-H	2-OMe,5-F	*R*	3.03	89 / ND
11p		-H	3-Me,5-F	*R*	8.40	90 / ND
11q		-H	2-OH	*R*	15.2	ND
12a		-$(CH_2)_3CO_2H$	2-OMe	*R*	65.2	ND
12b		-$(CH_2)_3CO_2H$	2-OMe,5-F	R	38.5	ND / 28
12c		-$(CH_2)_3CO_2H$	3-Me,5-F	R	9.9	ND / 30

* assayed at 0.4 μM

2.3. Interspecies selectivity

Some known small-molecule GnRH antagonists, including compounds **1a** and **1b**, were reported to be highly specific to the human GnRH receptors [15, 16], which limits the use of certain animal species for the in vivo evaluation of efficacy. We performed a competitive binding assay and NFAT promoter inhibition assay to examine the species sensitivity of compound **12c** (**Table 3**). Compound **12c** was more specific to the human GnRH receptors compared to those of monkeys and rats. The binding affinity for the human GnRH receptors was 12-fold greater than that for the monkey receptors, while the NFAT promotor inhibitory activity for the human GnRH receptors was 34-fold higher than that for the rat receptors.

Table 3. Specificities for human, monkey, and rat GnRH receptors

Compounds IC_{50} (nM)	**Competitive binding ([^{125}I]-DTrp^6-LHRH)**		**Inhibition of NFAT reporter activity**	
	human	**monkey**	**human**	**rat**
1a (Elagolix)	0.58	3.5	1.6	590
1c	0.46	3.8	6.3	279
12c	0.33	4.2	4.9	170

2.4 In vivo activity in castrated monkeys

To evaluate the functional antagonism of compound **12c** in vivo, we measured LH (luteinizing hormone) suppression in castrated monkeys after the administration of compound **12c** [17]. A single dose of compound **12c** (30 mg/kg, po) was administered to castrated male cynomolgus monkeys, and the blood samples were collected at the designated time points to analyze circulating LH concentrations using a radioimmunoassay (RIA). As described in **Figure 2**, maximal LH suppression (82% of basal level) was achieved at 8 h after the administration of **12c**, which was maintained for 24 h. Considering that compound **1a** and **1b** demonstrated maximal suppression of 75% and 50% at 8 h and completely recovered the basal level after 32 h and 20 h respectively [18], compound **12c** appeared to exert more potent and prolonged antagonistic activity. In addition, we measured the plasma levels of compound **12c** as described in **Table 4**. Compound **12c** showed a relatively high plasma level with a C_{max} of 6.0 μg/mL and an AUC of 37.1 μg h mL^{-1}, which was also observed with the 5-piperazinyl uracil analogue in our previous study [10], explaining the prolonged antagonistic activity of **12c**.

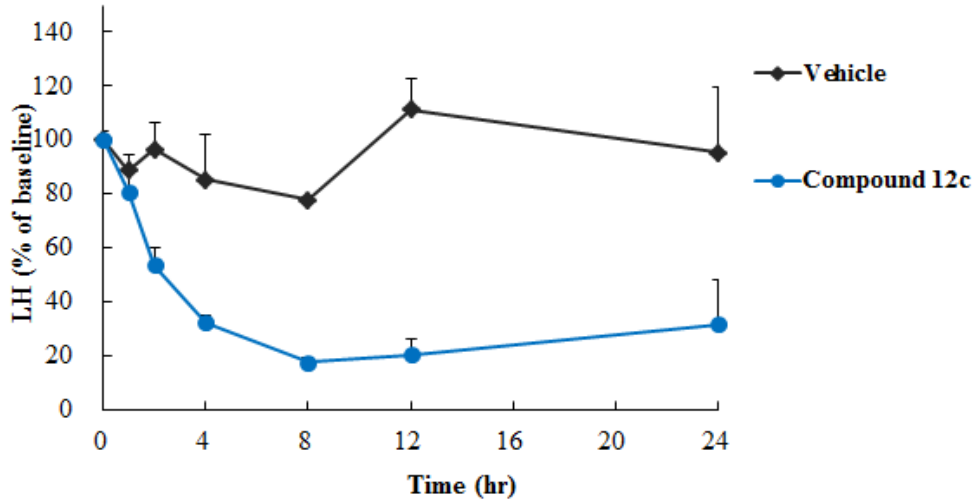


Figure 2. Suppression of plasma LH concentrations in castrated male cynomolgus monkeys after oral administration of compound **12c** at 30 mg/kg. Values shown are the mean ±SEM of bioactive LH levels expressed as a percentage of pretreatment LH levels for each of three individual animals.

Table 4. Pharmacokinetic parameters of compound 12c in castrated monkeys after oral administration (30 mg/kg)

Compound	C_{max} (μM)	AUC_{inf} (μM*hr)	T_{max} (hr)
12c	6.0±2.9	37.1±20.7	2.3±1.5

3. Conclusion

In this study, we synthesized a series of uracil analogues as orally available GnRH antagonists. By introducing various substituents in the phenyl ring of the *N*-3 side chain, we aimed to explore potency and pharmacokinetic properties of these newly synthesized analogues. In addition, we incorporated these results into the 5-piperazinyluracil core, which exhibited potent activity and a favorable pharmacokinetic profile in our previous study. When the aromatic substituents were introduced at the ortho or meta position, the in vitro antagonistic activity was improved. In particular, the introduction of a 2-OMe group enhanced NFAT inhibition up to 6-fold compared to the unsubstituted analogue. However, those analogues appeared to inhibit the CYP3A4 enzyme strongly, which can be overcome by adding a butyric acid group at the side chain amine group of the *N*-3 position. We identified compound **12c**, which is a highly potent GnRH antagonist with moderate CYP inhibition. The antagonistic activity of **12c** was specific to human GnRH receptors, showing 12-fold and 34-fold higher activities than those detected towards monkey and rat receptors respectively. When tested in castrated monkeys, compound **12c** demonstrated more profound and prolonged suppression in LH levels in castrated monkeys than Elagolix, while it reached the maximum effect in shorter time (8 h) than the previously reported compound **1c** (12 h) [10]. We believe that our study offers useful insights to design orally available GnRH antagonists with favorable pharmacokinetic properties.

4. Experimental

4.1. Chemistry

^{1}H NMR and ^{13}C NMR spectra were recored on a Varian Unity 300, Jeol Oxford 600 at 300 and 150 MHz. Chemical shifts were given in ppm using tetramethylsilane as the reference standard, and coupling constants (J) are given in hertz (Hz). Mass spectra were recorded on a Thermo LCQ DECA XP instrument. Chromatographic separations were carried out on silica gel (Kieselgel 60, 230−400 mesh, Merck) or basic silica gel (Chromatorex NHDM1020, 100- 200 mesh, Fuji Silysia Chemical Ltd.) using the indicated eluents. Yields were not optimized. All final compounds were assessed for purity by high performance liquid chromatography (HPLC) on Agilent 1120 Compact LC (G4288A) system via the following conditions. Column: Agilent TC-C18 column (4.6 mm × 250 mm, 5 μm). Mobile phase A: 0.78% NH_4OAc in water (v/v). Mobile phase B: MeCN. Gradient: 65.0% water/ 35.0% MeCN linear to 10% water/ 90% MeCN in 30 min. Wavelength: 272 nM. Flow: 1.0 mL/min. According to the HPLC analyses, all final compounds showed a purity of ≥95%.

4.1.1. Procedure for 5-phenyluracil preparation

*4.1.1.1. 1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-6-methylpyrimidine-2,4(1H,3H) -dione (**3**).* To a suspension of **2** (10 g, 30.2 mmol) and K_2CO_3 (8.35 g, 60.4 mmol) in DMF (125 mL) was added p-methoxybenzyl chloride (4.39 mL, 31.7 mmol). After the mixture was stirred at 60 °C for 3 h, it was cooled to room temperature. The reaction mixture was diluted with EtOAc (90 mL) and washed with saturated NH_4Cl (270 mL) and brine (100 mL), respectively. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel using hexane/EtOAc/CH_2Cl_2 (1:4.5:1) as eluent to give red solid, followd by recrystallization using EtOAc and hexane to yield 5-bromo-1-(2,6-difluorobenzyl)-3-(4-methoxybenzyl)-6-methylpyrimidine-2,4(1H,3H)-dione (7.65 g, 56%) as white solid. The mixture of the *N*-protected bromide (2.8 g. 6.21 mmol), 2-fluoro-3-methoxyphenylboronic acid (1.27 g, 7.45 mmol) and saturated barium hydroxide (1.08 g, 0.93 mmol) in benzene : EtOH : dimethoxyethane (45:5:50) (90 mL) was bubbled under nitrogen for 30 min and then treated with $Pd(PPh_3)_4$, followed by stirring at 85 °C for 2 days. The reaction mixture was cooled to room temperature, diluted with

EtOAc (90 mL) and then washed with water (90 mL) and brine (90 mL), respectively. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography on amine silica gel using hexane/EtOAc (4:1) as eluent to obtain 1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-3-(4-methoxybenzyl)-6-methylpyrimidine-2,4(1H,3H)-dione (1.7 g, 55%) as white solid. The uracil (3.4 g, 6.85 mmol) in anisole (50 mL) was treated with aluminum chloride (4.66 g, 34.2 mmol) under ice bath, followed by warming to room temperature and stirring for 3h. The resulting red mixture was added dropwise to saturated $NaHCO_3$ (70 mL) to give white suspension. EtOAc (100 mL) was added and the organic layer was taken up. The inorganic layer was extracted with CH_2Cl_2/ MeOH (10:1) (100 mL) three times. The combined organic layer was dissolved with CH_2Cl_2/ MeOH (10:1) (700 mL) and dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting solid was triturated with EtOAc (20 mL) for 30min, then filtered. The filtered residue was dried in vauco at 40 °C overnight to obtain compound **3** (2.41 g, 93%) as white solid. mp 72 ^{o}C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.57 (s, 1H), 7.51 – 7.39 (m, 1H), 7.24 – 7.10 (m, 4H), 6.80 – 6.73 (m, 1H), 5.25 (s, 2H), 3.89 (s, 3H), 2.12 (s, 3H).

*4.1.2. General Procedure for N-Boc protected aminoalcohol preparation (**4**).*

The suspension of amino acids (1 mmol) in THF (0.5 mL) was treated with $LiAlH_4$ (2 mmol) slowly under ice bath. The mixture was heated to 70~80 °C and stirred for 3hrs. After it was cooled under ice bath, 4 drops of water was added, followed by treatment of potassium carbonate. The resulting suspension was filtered, then the filtrate solution was concentrated in vacuo. The residue was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (20:1~10:1) as eluent to gain the corresponding amino alcohols. The suspension of amino alcohols (1 mmol) in CH_2Cl_2 (4 mL) were treated with DIPEA (3 mmol) and $(Boc)_2O$ (3 mmol), followed by stirring at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 (4 mL) and washed with satuated NH_4Cl (4 mL) followed by concentration of the organic layer. The residue was purified by column chromatography on silica gel using hexane/EtOAc/CH_2Cl_2 (2:1:0.5) as eluent or triturated

with the eluent in cases of poor solubility.

*4.1.3. General Procedure for Alkylation and Deprotection (**6**).*

The suspension of compound **3** (1.1 mmol) and compound **4** (1 mmol) in DMF (9 mL) were treated with PBu_3 (1.5 mmol) and DEAD (1.5 mmol), then stirred at 80 °C for 3 h. The reaction mixture was cooled to room temperature, diluted with CH_2Cl_2 (9 mL) and then washed with water (9 mL), followed by concentration of the organic layer. The residue was purified by two rounds of column chromatographies on silica gel and subsequently amine silica gel using hexane/EtOAc/CH_2Cl_2 (3:1:0.7~7:1:1) as eluent to gain compound **5**. TFA (10 mL) was added slowly to the solution of compound **5** (1 mmol) in CH_2Cl_2 (100 mL) and then stirred for 3h at room temperature. The reaction mixture was quenched with saturated $NaHCO_3$ under ice bath, which was extracted with CH_2Cl_2 three times. The combined organic layer was concentrated, followed by column chromatographies on silica gel and subsequently amine silica gel using CH_2Cl_2/MeOH (30:1~10:1) as eluent.

*4.1.3.1. 3-((2R)-2-amino-2-(tetrahydrofuran-3-yl)ethyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6a**).* Yield 20%, colorless oil, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36-7.20 (m, 1H), 7.13-7.05 (m, 1H), 7.03-6.87 (m, 3H), 6.82-6.76 (m, 1H), 5.45-5.20 (m, 2H), 4.13-3.55 (m, 9H), 3.20-2.99 (m, 1H), 2.31-2.11 (m, 4H), 2.09-1.95 (m, 1H), 1.85-1.70 (m, 1H). MS (ESI) *m/z* 490 (MH^+). Anal. HPLC 95% (R_t = 10.59 min).

*4.1.3.2. (R)-3-(2-amino-2-(tetrahydro-2H-pyran-4-yl)ethyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro -3-methoxyphenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6b**).* Yield 8%, white foam, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.23 (m, 1H), 7.10 (td, J = 8.0, 1.4 Hz, 1H), 7.01-6.86 (m, 3H), 6.83-6.76 (m, 1H), 5.41-5.23 (m, 2H), 4.07-3.94 (m, 4H), 3.89 (s, 3H), 3.42-3.28 (m, 2H), 3.04-2.93 (m, 1H), 2.15 (s, 3H), 1.72 (d, J = 11.7 Hz, 2H), 1.62-1.36 (m, 4H). MS (ESI) *m/z* 504 (MH^+). Anal. HPLC 97%

(R_t = 10.14 min).

*4.1.3.3. 3-(2-amino-2-(tetrahydro-2H-thiopyran-4-yl)ethyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro -3-methoxyphenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6c**).* Yield 15%, white foam, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.23 (m, 1H), 7.14-7.06 (m, 1H), 7.01-6.87 (m, 3H), 6.82-6.76 (m, 1H), 5.40-5.22 (m, 2H), 4.14-3.93 (m, 2H), 3.89 (s, 3H), 3.00 (dt, J = 8.6, 4.4 Hz, 1H), 2.76-2.55 (m, 4H), 2.15 (m, 4H), 2.05-2.93 (m, 1H), 1.73-1.30 (m, 3H). MS (ESI) *m/z* 520 (MH^+). Anal. HPLC 95% (R_t = 10.96 min).

*4.1.3.4. 3-(2-amino-2-(1-methylpiperidin-4-yl)ethyl)-5-(2-fluoro-3-methoxyphenyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methylpyrimidine-2,4(1H,3H)-dione compound (**6d**)*. Yield 34%. white foam, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.22 (m, 1H), 7.14-7.06 (m, 1H), 7.01-6.86 (m, 3H), 6.88-6.75 (m, 1H), 5.40-5.22 (m, 2H),), 4.09-3.94 (m, 2H), 3.89 (s, 3H), 3.07-2.88 (m, 4H), 2.29 (s, 3H), 2.15 (s, 3H), 2.04-1.19 (m, 5H). MS (ESI) *m/z* 517 (MH^+). Anal. HPLC 97% (R_t = 14.45 min).

*4.1.3.5. 3-(2-amino-2-(pyridin-3-yl)ethyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxy phenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6e**).* Yield 10%, colorless oil, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.61 (dd, J = 4.9, 2.3 Hz, 1H), 8.49 (dt, J = 4.9, 1.8 Hz, 1H), 7.78 (dq, J = 7.3, 2.3 Hz, 1H), 7.35-7.20 (m, 2H), 7.15-7.05 (m, 1H), 7.02-6.87 (m, 3H), 6.81 (ddd, J = 7.7, 6.0, 1.6 Hz, 0.5H), 6.72 (ddd, J = 7.8, 6.1, 1.6 Hz, 0.5H), 5.40-5.19 (m, 2H), 4.50-4.42 (m, 1H), 4.26 (ddd, J = 13.0, 8.8, 6.8 Hz, 1H), 4.15 (ddd, J = 12.9, 7.3, 5.4 Hz, 1H), 3.89 (s, 3H), 2.15 (s, 3H). MS (ESI) *m/z* 497 (MH^+). Anal. HPLC 99% (R_t = 14.52 min).

*4.1.3.6. (S)-3-(2-amino-2-(furan-2-yl)ethyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxy phenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6f**).* Yield 41%, colorless oil, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.21 (m, 2H), 7.13-7.05 (m, 1H), 7.01-6.86 (m, 3H), 6.85-6.75 (m, 1H), 6.28-6.24 (m, 1H),

6.16 (dq, J = 3.3, 0.7 Hz, 1H), 5.38-5.20 (m, 2H), 4.45-4.28 (m, 2H), 4.22 (ddd, J = 12.3, 5.9, 5.1 Hz, 1H), 3.89 (s, 3H), 2.16 (m, 3H). MS (ESI) *m/z* 486 (MH^+). Anal. HPLC 95% (R_t = 11.78 min).

*4.1.3.7. (R)-3-(2-amino-2-(furan-2-yl)ethyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxy phenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6g**).* Yield 38%, white foam, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.21 (m, 2H), 7.13-7.05 (m, 1H), 7.01-6.86 (m, 3H), 6.85-6.75 (m, 1H), 6.28-6.24 (m, 1H), 6.16 (dq, J = 3.3, 0.7 Hz, 1H), 5.38-5.20 (m, 2H), 4.45-4.28 (m, 2H), 4.22 (ddd, J = 12.3, 5.9, 5.1 Hz, 1H), 3.89 (s, 3H), 2.16 (m, 3H). MS (ESI) *m/z* 486 (MH^+). Anal. HPLC 96% (R_t = 11.55 min).

*4.1.3.8. 3-(2-amino-2-(3,5-dichlorophenyl)ethyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6h**).* Yield 12%, white foam, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.21 (m, 4H), 7.13-7.05 (m, 1H), 7.01-6.86 (m, 3H), 6.85-6.75 (m, 1H), 5.33-5.26 (m, 2H), 4.45-4.33 (m, 1H), 4.25-4.11 (m, 1H), 4.09-4.01 (m, 1H), 3.89 (s, 3H), 2.16 (s, 3H). MS (ESI) *m/z* 564 (MH^+). Anal. HPLC 99% (R_t = 16.53 min).

*4.1.3.9. 3-(2-amino-2-(5-fluoro-2-methoxyphenyl)ethyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6i**).* Yield 20%, white foam, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.21 (m, 1H), 7.13-7.05 (m, 1H), 7.01-6.71 (m, 7H), 5.33-5.26 (m, 2H), 4.53-4.45 (m, 1H), 4.38-4.26 (m, 1H), 4.22-4.20 (m, 1H), 3.88 (s, 3H), 3.80 (s, 3H), 2.12 (s, 3H). MS (ESI) *m/z* 544 (MH^+). Anal. HPLC 99% (R_t = 12.72 min).

*4.1.3.10. 3-(2-amino-2-(3-fluoro-5-methylphenyl)ethyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6j**).* Yield 9%, white solid, mp 70 ^{o}C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.21 (m, 1H), 7.13-7.05 (m, 1H), 7.01-6.71 (m, 7H), 5.33-5.26 (m, 2H), 4.45-4.32 (m, 1H), 4.27-4.17 (m, 1H), 4.09-4.05 (m, 1H), 3.89 (s, 3H), 2.30 (s, 3H), 2.16 (s, 3H). MS (ESI) *m/z* 528 (MH^+). Anal. HPLC 99% (R_t = 14.25 min).

*4.1.3.11. 3-(2-amino-2-(4-(methylthio)phenyl)ethyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6k**).* Yield 7%, white foam, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40-7.19 (m, 5H), 7.13-7.05 (m, 1H), 7.01-6.71 (m, 4H), 5.36-5.27 (m, 2H), 4.45-4.32 (m, 1H), 4.27-4.17 (m, 1H), 4.12-4.06 (m, 1H), 3.89 (s, 3H), 2.46 (s, 3H), 2.15 (s, 3H). MS (ESI) *m/z* 542 (MH^+). Anal. HPLC 98% (R_t = 13.90 min).

*4.1.3.12. 3-(2-amino-2-(2,4-dimethylphenyl)ethyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6l**).* Yield 11%, white solid, mp 68 ^{o}C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40-7.19 (m, 2H), 7.13-7.05 (m, 1H), 7.03-6.80 (m, 6H), 5.36-5.27 (m, 2H), 4.63-4.56 (m, 1H), 4.25-4.17 (m, 1H), 4.02-3.98 (m, 1H), 3.89 (s, 3H), 2.40 (s, 3H), 2.28 (s, 3H), 2.14 (s, 3H). MS (ESI) *m/z* 524 (MH^+). Anal. HPLC 95% (R_t = 14.62 min).

*4.1.3.13. 3-(2-amino-2-(2-chlorophenyl)ethyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxy phenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6m**).* Yield 6%, white solid, mp 72 ^{o}C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54-7.51 (m, 1H), 7.37-7.07 (m, 5H), 6.98-6.75 (m, 4H), 5.36-5.27 (m, 2H), 4.87-4.84 (m, 1H), 4.32-4.22 (m, 2H), 3.89 (s, 3H), 2.14 (s, 3H). MS (ESI) *m/z* 530 (MH^+). Anal. HPLC 96% (R_t = 13.59 min).

*4.1.3.14. 3-(2-amino-2-(2-(trifluoromethyl)phenyl)ethyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6n**).* Yield 8%, white foam, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82-7.79 (m, 1H), 7.59-7.51 (m, 2H), 7.39-7.24 (m, 2H), 7.17-7.05 (m, 1H), 6.98-6.75 (m, 4H), 5.36-5.27 (m, 2H), 4.85-4.75 (m, 1H), 4.45-4.22 (m, 2H), 3.89 (s, 3H), 2.13 (m, 3H). MS (ESI) *m/z* 564 (MH^+). Anal. HPLC 96% (R_t = 14.63 min).

4.1.3.15. 3-(2-amino-2-(3-(trifluoromethoxy)phenyl)ethyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-

*methoxyphenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6o**).* Yield 28%, yellow foam, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49-6.75 (m, 10H), 5.36-5.08 (m, 2H), 4.65-4.30 (m, 2H), 4.08-3.82 (m, 4H), 2.09 (s, 3H). MS (ESI) *m/z* 580 (MH^+). Anal. HPLC 98% (R_t = 15.96 min).

*4.1.3.16. 3-(2-amino-3-(2-chloro-6-fluorophenyl)propyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6p**).* Yield 17%, white foam, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27-6.75 (m, 9H), 5.36-5.25 (m, 2H), 4.21-4.06 (m, 2H), 3.59-3.46 (m, 1H), 2.95-2.74 (m, 2H), 2.15 (s, 3H). MS (ESI) *m/z* 562 (MH^+). Anal. HPLC 97% (R_t = 15.03 min).

*4.1.3.17. 3-(2-amino-3-(3-fluorophenyl)propyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxy phenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6q**).* Yield 48%, white foam, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.19 (m, 2H), 7.12-7.07 (m, 1H), 6.99-6.79 (m, 7H), 5.39-5.25 (m, 2H), 4.04 (d, , J = 3.9Hz, 2H), 3.88 (s, 3H), 3.51-3.46 (m, 1H), 2.88-2.82 (m, 1H), 2.59-2.56 (m, 1H), 2.14 (s, 3H). MS (ESI) *m/z* 528 (MH^+). Anal. HPLC 98% (R_t = 13.91 min).

*4.1.3.18. 3-(2-amino-3-(2-chloro-4-fluorophenyl)propyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6r**).* Yield 29%, white foam, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.24 (m, 2H), 7.16-7.09 (m, 2H), 7.02-6.90 (m, 4H), 6.85-6.80 (m, 1H), 5.37-5.25 (m, 2H), 4.09 (d, J = 4.2Hz, 2H), 3.91 (s, 3H), 3.53-3.46 (m, 1H), 3.02-2.91 (m, 1H), 2.75-2.67 (m, 1H), 2.18 (s, 3H). MS (ESI) *m/z* 562 (MH^+). Anal. HPLC 98% (R_t = 15.43 min).

*4.1.3.19. 3-(2-amino-3-(2-fluoro-4-(trifluoromethyl)phenyl)propyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6s**).* Yield 7%, white solid, mp 60 ^{o}C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.24 (m, 2H), 7.19-6.77 (m, 7H), 5.43-5.25 (m, 2H), 4.09 (d, J = 4.2Hz, 2H), 3.91 (s, 3H), 3.53-3.43 (m, 1H), 2.87-2.82 (m, 1H), 2.75-2.67 (m, 1H), 2.59-2.51 (m, 1H), 2.18 (s, 3H). MS (ESI) *m/z* 596 (MH^+). Anal. HPLC 99% (R_t = 14.53 min).

*4.1.3.20. 3-(2-amino-3-(3-(trifluoromethyl)phenyl)propyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6t**).* Yield 8%, white solid, mp 62 ^{o}C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.12 (m, 5H), 7.08-6.84 (m, 5H), 5.43-5.25 (m, 2H), 4.21-4.08 (m, 2H), 3.91 (s, 3H), 3.57-3.50 (m, 1H), 3.02-2.82 (m, 2H), 2.18 (s, 3H). MS (ESI) *m/z* 578 (MH^+). Anal. HPLC 99% (R_t = 15.14 min).

*4.1.3.21. 3-(2-amino-3-(3,5-difluorophenyl)propyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6u**).* Yield 5%, white solid, mp 65 ^{o}C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.12 (m, 3H), 7.10-6.78 (m, 6H), 5.43-5.25 (m, 2H), 4.12 (d, J = 4.2Hz, 2H), 3.91 (s, 3H), 3.57-3.44 (m, 1H), 2.98-2.82 (m, 1H), 2.58-2.49 (m, 1H), 2.18 (s, 3H). MS (ESI) *m/z* 546 (MH^+). Anal. HPLC 99% (R_t = 15.06 min).

*4.1.3.22. 3-(2-amino-3-(thiophen-2-yl)propyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxy phenyl)-6-methylpyrimidine-2,4(1H,3H)-dione (**6v**).* Yield 38%, white foam, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.07 (m, 3H), 6.99-6.78 (m, 6H), 5.39-5.24 (m, 2H), 4.06 (d, J = 6.6 Hz, 2H), 3.88 (s, 3H), 3.54-3.44 (m, 1H), 3.10-3.02 (m, 1H), 2.87-2.75 (m, 1H), 2.15 (s, 3H). MS (ESI) *m/z* 516 (MH^+). Anal. HPLC 99% (R_t = 13.19 min).

4.1.4. Procedure for 5-piperazinyluracil preparation

*4.1.4.1. 1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione (**8**).* The mixture of compound **7** (1.50 g, 3.94 mmol) and 1-benzylpiperazine (5.5 mL, 31.5 mmol) in acetonitrile (1 mL) was reacted under . microwave irradiation at 120 °C for 1.5 h. The mixture was concentrated, followed by purification using silica gel chromatography (hexane/EtOAc/CH_2Cl_2, 1:2:1) to afford 4-benzyl piperazine adduct as a white solid (1.48 g, 79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.15 (m, 2H), 2.31 (s, 3H), 2.50 (m, 2H), 2.78 (m, 2H), 3.52 (s, 3H),

3.58 (m, 2H), 5.36 (s, 2H), 7.19-7.41 (m, 7H), 7.53 (m, 1H). A solution of the above compound (1.48 g, 3.11 mmol) dissolved in CH_2Cl_2/MeOH (1:1, 10 mL) was hydrogenated over 10% Pd/C (280 mg) under atmospheric pressure at room temperature for 5 h. The mixture was filtered through celite, followed by concentration of the filtrate solution. The residue was purified using column chromatography using amine silica gel (CH_2Cl_2/MeOH, 10:1) to yield compound **8** as white solid (934 mg, 78%). mp 78 ^oC, ^1H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, J = 7.8 Hz, 1H), 7.41 (m, 1H), 7.24 (m, 1H), 5.38 (s, 2H), 3.43 (m, 2H), 2.96 (m, 2H), 2.81 (m, 2H), 2.52 (m, 2H), 2.33 (s, 3H).

*4.1.5. General Procedure for Alkylation (**9, 10**).*

A solution of compound **8** (1 mmol) and *N,N*-diisopropylethylamine (5 mmol), in 1,2-dichloroethane (10 mL) was treated with 3-nitrobenzyl bromide (1.5 mmol), then stirred at 50 °C for 2 h. The mixture was cooled to room temperature and washed with saturated ammonium chloride solution and concentrated in vacuo. The residue was purified by column chromatography using silica gel (CH_2Cl_2/MeOH, 30:1).

*4.1.5.1. 1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(3-nitrobenzyl)piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione (**9**).* Yield 77%, yellowish solid, mp 74 ^oC, ^1H NMR (300 MHz, $CDCl_3$) *δ* 8.57 (s, 1H), 8.16 (s, 1H), 8.10 (d, J = 8.1 Hz, 1H), 7.77 (d, J = 7.5 Hz, 1H), 7.41 (m, 1H), 7.55-7.46 (m, 2H), 7.23 (m, 1H), 5.37 (s, 2H), 3.66-3.60 (m, 4H), 2.75 (m, 2H), 2.52 (m, 2H), 2.32 (s, 3H), 2.20 (m, 2H).

*4.1.5.2. 1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-((5-(trifluoromethyl)furan-2-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione (**10**).* Yield 89%, white solid, mp 83 ^oC, ^1H NMR (300 MHz, $CDCl_3$) *δ* 8.57 (s, 1H), 7.54 (d, J = 7.8 Hz, 1H), 7.41 (m, 1H), 7.24 (m, 1H), 6.72 (d, J = 3.0 Hz, 1H), 6.29 (d, J = 3.0 Hz, 1H), 5.40 (s, 2H), 3.72-3.49 (m, 4H), 2.81 (m, 2H), 2.54 (m, 2H), 2.27 (s, 3H), 2.26 (m, 2H).

*4.1.6. General Procedure for Alkylation and Deprotection (**11**).*

To a solution of compound **4** (1 mmol) in CH_2Cl_2 (3 mL) were added trimethylamine (1.3 mmol) and methansulfonylchloride (1.1 mmol). After stirring at room temperature for 30 min, the mixture was diluted with dichloromethane and washed with saturated sodium bicarbonate solution. The organic layer was dried over sodium sulfate and filtered. After concentration, the filtrate was dried in vacuo to give compound **4a**. The mixture of compound **9** or **10** (1 mmol), the mesylate **4a** (2.5mmol) and K_2CO_3 (5mmol) in DMF (10 mL) was stirred at 70 °C overnight. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate, and washed with saturated ammonium chloride solution. The organic layer was concentrated, then the residue was purified using silica gel and amine silica gel chromatography (hexane/EtOAc, 2:1). The alkylated compound (1 mmol) in CH_2Cl_2 (40 mL) was treated with TFA (2 mL), stirring at room temperature for 3 h. The reaction mixture was neutralized with saturated $NaHCO_3$ and extracted with CH_2Cl_2 twice. The organic layer was concentrated, then purified using silica gel chromatography (CH_2Cl_2/MeOH, 15:1~20:1).

*4.1.6.1. (R)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)-benzyl)-6-methyl-5-(4-(3-nitrobenzyl)piperazin-1-yl)pyrimidine-2,4-(1H,3H)-dione (**11a**).* Yield 50%, yellowish solid, mp 71 ^{o}C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (t, J = 1.8 Hz, 1H), 8.11 (dd, J = 8.1, 1.3 Hz, 1H), 7.77 (d, J = 7.7 Hz, 1H), 7.55 (d, J = 7.8 Hz, 1H), 7.48 (t, J = 7.9 Hz, 1H), 7.44-7.29 (m, 5H), 7.26-7.18 (m, 2H), 5.41 (s, 2H), 4.37 (dd, J = 9.5, 4.9 Hz, 1H), 4.21 (dd, J = 12.9, 9.5 Hz, 1H), 4.06 (dd, J = 12.9, 4.9 Hz, 1H), 3.70-3.51 (m, 4H), 2.75 (d, J = 10.4 Hz, 2H), 2.49 (m, 2H), 2.34 (s, 3H), 2.21 (m, 2H). MS (ESI) *m/z* 641 (MH^+). Anal. HPLC 99% (R_t = 19.36 min).

*4.1.6.2. 3-(2-amino-2-(o-tolyl)ethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(3-nitrobenzyl)piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione (**11b**).* Yield 25%, white foam, ^{1}H NMR

(300 MHz, $CDCl_3$) δ 8.17 (d, J = 1.8 Hz, 1H), 8.11 (dd, J = 7.2, 1.2 Hz, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.55-7.46 (m, 3H), 7.25-7.12 (m, 4H), 5.42 (s, 2H), 4.63 (dd, J = 9.9, 4.5 Hz, 1H), 4.21 (dd, J = 13.2, 9.9 Hz, 1H), 3.96 (dd, J = 13.2, 4.5Hz, 1H), 3.78-3.60 (m, 4H), 2.75 (d, J = 9.9 Hz, 2H), 2.51 (m, 2H), 2.46 (s, 3H), 2.32 (s, 3H), 2.25-2.18 (m, 2H). MS (ESI) *m/z* 655 (MH^+). Anal. HPLC 99% (R_t = 20.59 min).

*4.1.6.3. (R)-3-(2-amino-2-(2-methoxyphenyl)ethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(3-nitrobenzyl)piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione (**11c**).* Yield 34%, white foam, ^1H NMR (300 MHz, $CDCl_3$) δ 8.17 (s, 1H), 8.09 (dd, J = 7.2, 1.2 Hz, 1H), 7.75 (d, J = 7.5 Hz, 1H), 7.55 - 7.45 (m, 2H), 7.44-7.29 (m, 1H), 7.25-7.16 (m, 2H), 6.90-6.83 (m, 2H), 5.39 (s, 2H), 4.47-4.31 (m, 2H), 4.21 (dd, J = 12.6, 5.4 Hz, 1H), 3.86 (s, 3H), 3.70-3.49 (m, 4H), 2.73 (d, J = 10.2 Hz, 2H), 2.52-2.37 (m, 2H), 2.30 (s, 3H), 2.25-2.12 (m, 2H). MS (ESI) *m/z* 671 (MH^+). Anal. HPLC 99% (R_t = 19.49 min).

*4.1.6.4. 3-(2-amino-2-(2-fluorophenyl)ethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(3-nitrobenzyl)piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione (**11e**).* Yield 28%, white foam, ^1H NMR (300 MHz, $CDCl_3$) δ 8.16 (s, 1H), 8.09 (dd, J = 7.2, 1.2 Hz, 1H), 7.75 (d, J = 7.5 Hz, 1H), 7.55-7.37 (m, 4H), 7.24-7.16 (m, 2H), 7.12-6.96 (m, 2H), 5.39 (s, 2H), 4.59 (dd, J = 8.7, 6.0 Hz, 1H), 4.26 (dd, J = 12.9, 8.7 Hz, 1H), 4.16 (dd, J = 12.9, 5.7 Hz, 1H), 3.70-3.49 (m, 4H), 2.73 (d, J = 9.3 Hz, 2H), 2.52-2.41 (m, 2H), 2.30 (s, 3H), 2.25-2.12 (m, 2H). MS (ESI) *m/z* 659 (MH^+). Anal. HPLC 99% (R_t = 19.60 min).

*4.1.6.5. 3-(2-amino-2-(m-tolyl)ethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(3-nitrobenzyl)piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione (**11f**).* Yield 37%, white foam, ^1H NMR (300 MHz, $CDCl_3$) δ 8.17 (d, J = 1.5 Hz, 1H), 8.10 (dd, J = 8.1, 1.3 Hz, 1H), 7.76 (d, J = 7.5 Hz, 1H), 7.55-7.45 (m, 2H), 7.44-7.29 (m, 1H), 7.26-7.18 (m, 4H), 7.07-7.04 (m, 1H), 5.41 (s, 2H), 4.32

(dd, J = 9.9, 4.2 Hz, 1H), 4.21 (dd, J = 12.9, 9.9 Hz, 1H), 4.02 (dd, J = 12.6, 4.2Hz, 1H), 3.70-3.52 (m, 4H), 2.75 (d, J = 9.9 Hz, 2H), 2.57-2.45 (m, 2H), 2.33 (s, 3H), 2.30-2.21 (m, 2H). MS (ESI) *m/z* 655 (MH^+). Anal. HPLC 98% (R_t = 21.03 min).

*4.1.6.6. (R)-3-(2-amino-2-(4-fluorophenyl)ethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(3-nitrobenzyl)piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione (**11g**).* Yield 53%, white foam, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (s, 1H), 8.10 (dd, J = 8.1, 1.2 Hz, 1H), 7.75 (d, J = 7.5 Hz, 1H), 7.55-7.36 (m, 3H), 7.31-7.18 (m, 3H), 7.13-7.09 (m, 1H), 7.94-6.88 (m, 1H), 5.39 (s, 2H), 4.36 (dd, J = 8.7, 5.4 Hz, 1H), 4.21-4.03 (m, 2H), 3.68-3.50 (m, 4H), 2.74 (d, J = 9.9 Hz, 2H), 2.53-2.42 (m, 2H), 2.33 (s, 3H), 2.25-2.18 (m, 2H). MS (ESI) *m/z* 659 (MH^+). Anal. HPLC 98% (R_t = 20.03 min).

*4.1.6.7. 3-(2-amino-2-(3-fluorophenyl)ethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(3-nitrobenzyl)piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione (**11h**).* Yield 54%, white foam, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (d, J = 1.5 Hz, 1H), 8.10 (dd, J = 8.1, 1.3 Hz, 1H), 7.76 (d, J = 7.5 Hz, 1H), 7.55-7.45 (m, 2H), 7.44-7.29 (m, 1H), 7.26-7.18 (m, 4H), 7.07-7.04 (m, 1H), 5.41 (s, 2H), 4.32 (dd, J = 9.9, 4.2 Hz, 1H), 4.21 (dd, J = 12.9, 9.9 Hz, 1H), 4.02 (dd, J = 12.6, 4.2Hz, 1H), 3.70-3.52 (m, 4H), 2.75 (d, J = 9.9 Hz, 2H), 2.57-2.45 (m, 2H), 2.33 (s, 3H), 2.30-2.21 (m, 2H). MS (ESI) *m/z* 659 (MH^+). Anal. HPLC 98% (R_t = 20.45 min).

*4.1.6.8. (R)-3-(2-amino-2-(5-fluoro-2-methoxyphenyl)ethyl)-1-(2-fluoro-6-(trifluoromethyl) benzyl)-6-methyl-5-(4-(3-nitrobenzyl)piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione (**11i**).* Yield 33%, white foam, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (s, 1H), 8.11 (dd, J = 7.8, 1.2 Hz, 1H), 7.76 (d, J = 7.5 Hz, 1H), 7.55-7.46 (m, 2H), 7.45-7.36 (m, 1H), 7.22-7.19 (m, 1H), 6.97 (dd, J = 9.3, 3.0Hz, 1H), 6.89-6.82 (m, 1H),), 6.75 (dd, J = 9.0, 4.5Hz, 1H), 5.38 (s, 2H), 4.45 (m, 1H), 4.29-4.25 (m, 2H), 3.85 (s, 3H), 3.60 (s, 2H), 3.72-3.49 (m, 2H), 2.75-2.72 (m, 2H), 2.51-2.40 (m, 2H), 2.31 (s, 3H), 2.30-2.16 (m, 2H). MS (ESI) *m/z* 689 (MH^+). Anal. HPLC 99% (R_t = 20.19 min).

*4.1.6.9. (S)-3-(2-amino-2-(5-fluoro-2-methoxyphenyl)ethyl)-1-(2-fluoro-6-(trifluoromethyl) benzyl)-6-methyl-5-(4-(3-nitrobenzyl)piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione (**11j**).* Yield 25%, white foam, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (s, 1H), 8.11 (dd, J = 7.8, 1.2 Hz, 1H), 7.76 (d, J = 7.5 Hz, 1H), 7.55-7.46 (m, 2H), 7.45-7.36 (m, 1H), 7.22-7.19 (m, 1H), 6.97 (dd, J = 9.3, 3.0 Hz, 1H), 6.89-6.82 (m, 1H), 6.75 (dd, J = 9.0, 4.5Hz, 1H), 5.38 (s, 2H), 4.45 (m, 1H), 4.29-4.25 (m, 2H), 3.85 (s, 3H), 3.60 (s, 2H), 3.72-3.49 (m, 2H), 2.75-2.72 (m, 2H), 2.51-2.40 (m, 2H), 2.31 (s, 3H), 2.30-2.16 (m, 2H). MS (ESI) *m/z* 689 (MH^+). Anal. HPLC 97% (R_t = 20.14 min).

*4.1.6.10. (R)-3-(2-amino-2-(3-fluoro-5-methylphenyl)ethyl)-1-(2-fluoro-6-(trifluoromethyl) benzyl)-6-methyl-5-(4-(3-nitrobenzyl)piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione (**11k**).* Yield 34%, white foam, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (s, 1H), 8.11 (dd, J = 7.8, 1.2 Hz, 1H), 7.77 (d, J = 7.8 Hz, 1H), 7.56-7.46 (m, 2H), 7.45-7.36 (m, 1H), 7.22-7.19 (m, 1H), 7.02 (s, 1H), 6.92 (d, J = 9.9 Hz, 1H), 6.75 (d, J = 9.3 Hz, 1H), 5.50-5.35 (m, 2H), 4.33 (dd, J = 9.3, 4.8 Hz, 1H), 4.18 (dd, J = 12.6, 9.3 Hz, 1H), 3.99 (dd, J = 12.9, 4.8 Hz, 1H), 3.70-3.55 (m, 4H), 2.81-2.69 (m, 2H), 2.57-2.43 (m, 2H), 2.34 (s, 3H), 2.33 (s, 3H), 2.30-2.17 (m, 2H). MS (ESI) *m/z* 673 (MH^+). Anal. HPLC 95% (R_t = 21.78 min).

*4.1.6.11. (S)-3-(2-amino-2-(3-fluoro-5-methylphenyl)ethyl)-1-(2-fluoro-6-(trifluoromethyl) benzyl)-6-methyl-5-(4-(3-nitrobenzyl)piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione (**11l**).* Yield 22%, white foam, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (s, 1H), 8.11 (dd, J = 7.8, 1.2 Hz, 1H), 7.77 (d, J = 7.8 Hz, 1H), 7.56-7.46 (m, 2H), 7.45-7.36 (m, 1H), 7.22-7.19 (m, 1H), 7.02 (s, 1H), 6.92 (d, J = 9.9 Hz, 1H), 6.75 (d, J = 9.3 Hz, 1H), 5.50-5.35 (m, 2H), 4.33 (dd, J = 9.3, 4.8 Hz, 1H), 4.18 (dd, J = 12.6, 9.3 Hz, 1H), 3.99 (dd, J = 12.9, 4.8 Hz, 1H), 3.70-3.55 (m, 4H), 2.81-2.69 (m, 2H), 2.57-2.43 (m, 2H), 2.34 (s, 3H), 2.33 (s, 3H), 2.30-2.17 (m, 2H). MS (ESI) *m/z* 673 (MH^+). Anal. HPLC 97% (R_t = 21.74 min).

*4.1.6.12. (R)-3-(2-amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-((5-(trifluoromethyl)furan-2-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione (**11m**).* Yield 74%, white foam, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57-7.51 (m, 1H), 7.44-7.35 (m, 3H), 7.35-7.28 (m, 2H), 7.26-7.17 (m, 2H), 6.73 (dq, J = 3.7, 1.2 Hz, 1H), 6.30 (dd, J = 3.4, 0.9 Hz, 1H), 5.40 (s, 2H), 4.36 (dd, J = 9.4, 4.9 Hz, 1H), 4.21 (dd, J = 12.9, 9.5 Hz, 1H), 4.06 (dd, J = 12.9, 4.9 Hz, 1H), 3.70-3.49 (m, 4H), 2.80 (d, J = 10.4 Hz, 2H), 2.49 (t, J =14.3 Hz, 2H), 2.34-2.16 (m, 5H). MS (ESI) *m/z* 654 (MH^+). Anal. HPLC 99% (R_t = 20.50 min).

*4.1.6.13. (R)-3-(2-amino-2-(2-methoxyphenyl)ethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-((5-(trifluoromethyl)furan-2-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione (**11n**).* Yield 32%, colorless oil, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, J = 7.8 Hz, 1H), 7.41-7.34 (m, 1H), 7.24-7.16 (m, 3H), 6.90-6.82 (m, 2H), 6.72 (d, J = 3.0 Hz, 1H), 6.29 (d, J = 3.0 Hz, 1H), 6.29 (d, J = 3.3 Hz, 1H), 5.38 (s, 2H), 4.46 (dd, J = 8.7, 5.4 Hz, 1H), 4.36 (dd, J = 12.6, 9.0 Hz, 1H), 4.20 (dd, J = 12.6, 5.4 Hz, 1H), 3.86 (s, 3H), 3.60 (s, 2H), 3.72-3.49 (m, 2H), 2.81-2.74 (m, 2H), 2.51-2.37 (m, 2H), 2.27 (s, 3H), 2.26 – 2.19 (m, 2H). MS (ESI) *m/z* 684 (MH^+). Anal. HPLC 98% (R_t = 20.58 min).

*4.1.6.14. (R)-3-(2-amino-2-(5-fluoro-2-methoxyphenyl)ethyl)-1-(2-fluoro-6-(trifluoromethyl) benzyl)-6-methyl-5-(4-((5-(trifluoromethyl)furan-2-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione (**11o**).* Yield 39%, colorless oil, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, J = 7.8 Hz, 1H), 7.45-7.35 (m, 1H), 7.25-7.18 (m, 1H), 6.94 (dd, J = 9.3, 3.0Hz, 1H), 6.89-6.81 (m, 1H), 6.76-6.71 (m, 2H), 6.29 (d, J = 3.3 Hz, 1H), 5.37 (s, 2H), 4.48-4.25 (m, 1H), 4.29-4.23 (m, 2H), 3.85 (s, 3H), 3.60 (s, 2H), 3.72-3.49 (m, 2H), 2.81-2.74 (m, 2H), 2.51-2.37 (m, 2H), 2.28 (s, 3H), 2.26-2.16 (m, 2H). MS (ESI) *m/z* 702 (MH^+). Anal. HPLC 96% (R_t = 20.96 min).

*4.1.6.15. (R)-3-(2-amino-2-(3-fluoro-5-methylphenyl)ethyl)-1-(2-fluoro-6-(trifluoromethyl) benzyl)-6-methyl-5-(4-((5-(trifluoromethyl)furan-2-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione (**11p**).* Yield 26%, colorless oil, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, J = 7.8 Hz, 1H), 7.45-7.36 (m, 1H), 7.22-7.19 (m, 1H), 7.01 (s, 1H), 6.90 (d, J = 9.6 Hz, 1H), 6.75-6.72 (m, 2H), 6.29 (d, J = 3.0 Hz, 1H), 5.48-5.35 (m, 2H), 4.31 (dd, J = 9.3, 4.5 Hz, 1H), 4.18 (dd, J = 13.2, 9.3 Hz, 1H), 4.02 (dd, J = 13.2, 4.8 Hz, 1H), 3.70-3.51 (m, 4H), 2.81-2.69 (m, 2H), 2.57-2.43 (m, 2H), 2.32 (s, 6H), 2.31-2.17 (m, 2H). MS (ESI) *m/z* 702 (MH^+). Anal. HPLC 98% (R_t = 22.28 min).

*4.1.7. General Procedure for Demethylation and Hydrolysis (**11d, 11q**).*

The mixture of compound **9** or **10** (1 mmol), the mesylate (2.5 mmol) and K_2CO_3 (5 mmol) in DMF (10 mL) was stirred at 70 °C overnight. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate, and washed with saturated ammonium chloride solution. The organic layer was concentrated, then the residue was purified using silica gel and amine silica gel chromatography (hexane/EtOAc, 2:1). A solution of the alkylated compound (1 mmol) in anhydrous dichloroethane (15 mL) was chilled to −78 °C and treated with 1 M boron tribromide (5 mmol) in dichloromethane slowly under a nitrogen atmosphere. Then, the mixture was stirred at 40 °C for 48 h. Methanol (15 mL) was added to the reaction mixture at ambient temperature and then stirred for 20 min. The mixture was concentrated under reduced pressure and the residue was diluted in dichloromethane and washed with saturated $NaHCO_3$ solution. The organic layer was dried over sodium sulfate and filtered. After concentration of the organic layer, the residue was purified using silica gel chromatography (CH_2Cl_2/MeOH, 10:1).

*4.1.7.1. (R)-3-(2-Amino-2-(2-hydroxyphenyl)ethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(3-nitrobenzyl)piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione **(11d).*** Yield 80%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (m, 1H), 8.11 (m, 1H), 7.76 (m, 1H), 7.56 (d, *J* = 7.9 Hz, 1H), 7.49 (t, *J* = 7.9 Hz, 1H), 7.42 (m, 1H), 7.27-7.20 (m, 1H), 7.13 (m, 1H), 7.08 (d, *J* = 7.6 Hz, 1H),

6.82 (dd, *J* = 8.1, 1.2 Hz, 1H), 6.76 (td, *J* = 7.4, 1.3 Hz, 1H), 5.56-5.31 (m, 2H), 4.52 (dd, *J* = 8.7, 3.4 Hz, 1H), 4.42 (dd, *J* = 12.9, 8.7 Hz, 1H), 4.20 (dd, *J* = 12.9, 3.4 Hz, 1H), 3.67-3.51 (m, 4H), 2.74 (m, 2H), 2.48 (m, 2H), 2.34 (s, 3H), 2.22 (m, 2H). MS (ESI) *m/z* 657 (MH^+). Anal. HPLC 99% (R_t = 20.01 min).

4.1.7.2. (R)-3-(2-amino-2-(2-hydroxyphenyl)ethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-((5-(trifluoromethyl)furan-2-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1H,3H)-dione ***(11q)***. Yield 30%, colorless oil, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57-7.51 (m, 1H), 7.44-7.35 (m, 1H), 7.26-7.19 (m, 1H), 7.17-7.04 (m, 2H), 6.84-6.71 (m, 3H), 6.31-6.28 (m, 1H), 5.53-5.32 (m, 2H), 4.51 (dd, J = 8.7, 3.6 Hz, 1H), 4.41 (dd, J = 13.2, 8.4 Hz, 1H), 4.19 (dd, J = 12.8, 3.4 Hz, 1H), 3.70-3.49 (m, 4H), 2.89-2.72 (m, 2H), 2.57-2.43 (m, 2H), 2.34-2.16 (m, 5H). MS (ESI) *m/z* 669 (MH^+). Anal. HPLC 99% (R_t = 21.30 min).

*4.1.8. General Procedure for Alkylation and Hydrolysis (**12**).*

A solution of compound **11** (1 mmol) in acetonitrile (2 mL) were treated with *N*,*N*-diisopropylethylamine (1 mmol), sodium iodide (3 mmol) and 4-bromobutyric acid ethyl ester (1.2 mmol), followed by stirring at 95 °C overnight. Then, it was cooled to ambient temperature, diluted with dichloromethane, and washed with saturated $NaHCO_3$ solution. The organic layer was concentrated and the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 35:1) to obtain the ester compound. A solution of the ester (1 mmol) dissolved in ethanol (3.5 mL)/water (2.5 mL) was slowly added 1 N NaOH (10 mmol). After the mixture was stirred at 60 °C for 3 h, it was cooled to ambient temperature and concentrated under reduced pressure. The residue was neutralized with 0.2 N HCl and extracted with dichloromethane. After the organic layer was concentrated, the residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (10:1~7:1) as eluent to afford compound **12**.

*4.1.8.1. (R)-4-((2-(3-(2-fluoro-6-(trifluoromethyl)benzyl)-4-methyl-2,6-dioxo-5-(4-((5-(trifluoromethyl)furan-2-yl)methyl)piperazin-1-yl)-2,3-dihydropyrimidin-1(6H)-yl)-1-(2-methoxyphenyl)ethyl) amino)butanoic acid (**12a**).* Yield 26%, white solid, mp 90 ^{o}C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, J = 7.7 Hz, 1H), 7.42-7.27 (m, 2H), 7.24-7.15 (m, 2H), 6.97-6.89 (m, 2H), 6.72 (dd, J = 3.3, 1.3 Hz, 1H), 6.29 (d, J = 3.4 Hz, 1H), 5.48-5.32 (m, 2H), 4.74 (dd, J = 13.4, 10.3 Hz, 1H), 4.42 (dd, J = 10.3, 4.4 Hz, 1H), 4.03 (dd, J = 13.3, 4.6 Hz, 1H), 3.92 (s, 3H), 3.63-3.50 (m, 4H), 2.85-2.30 (m, 10H), 2.27 (s, 3H), 1.79-1.53 (m, 2H). MS (ESI) *m/z* 770 (MH^+). Anal. HPLC 99% (R_t = 16.45 min).

*4.1.8.2. (R)-4-((1-(5-fluoro-2-methoxyphenyl)-2-(3-(2-fluoro-6-(trifluoromethyl)benzyl)-4-methyl-2,6-dioxo-5-(4-((5-(trifluoromethyl)furan-2-yl)methyl)piperazin-1-yl)-2,3-dihydropyrimidin-1(6H)-yl)ethyl)amino)butanoic acid (**12b**).* Yield 25%, white solid, mp 88 ^{o}C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, J = 7.9 Hz, 1H), 7.44-7.34 (m, 1H), 7.26-7.17 (m, 1H), 7.02-6.89 (m, 2H), 6.87-6.80 (m, 1H),), 6.75-6.71 (m, 1H), 6.30 (d, J = 3.5 Hz, 1H), 5.39 (s, 2H), 4.65 (dd, J = 13.2, 9.4 Hz, 1H), 4.39 (dd, J = 9.0, 4.8 Hz, 1H), 4.12 (dd, J = 13.2, 5.0 Hz, 1H), 3.91 (s, 3H), 3.62 (s, 2H), 3.59-3.49 (m, 2H), 2.85-2.75 (m, 3H), 2.67-2.40 (m, 5H), 2.33 (s, 3H), 2.30-2.16 (m, 2H), 1.84-1.59 (m, 2H). MS (ESI) *m/z* 788 (MH^+). Anal. HPLC 98% (R_t = 18.29 min).

*4.1.8.3. (R)-4-((1-(3-fluoro-5-methylphenyl)-2-(3-(2-fluoro-6-(trifluoromethyl)benzyl)-4-methyl-2,6-dioxo-5-(4-((5-(trifluoromethyl)furan-2-yl)methyl)piperazin-1-yl)-2,3-dihydropyrimidin-1(6H)-yl)ethyl)amino)butanoic acid (**12c**).* Yield 31%, white solid, mp 83 ^{o}C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, J = 7.9 Hz, 1H), 7.44-7.33 (m, 1H), 7.21 (dd, J = 11.6, 8.3 Hz, 1H), 6.95 (s, 1H), 6.88-6.78 (m, 2H), 6.74-6.70 (dd, J = 3.0, 1.2 Hz, 1H), 6.29 (d, J = 3.3 Hz, 1H), 5.39 (s, 2H), 4.34 (dd, J = 13.2, 10.2 Hz, 1H), 4.20 (dd, J = 10.5, 3.9 Hz, 1H), 3.99 (dd, J = 12.6, 4.2 Hz, 1H), 3.64-3.50 (m, 4H), 2.87-2.74 (m, 2H), 2.74-2.17 (m, 11H), 1.78-1.52 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) 175.58, 163.93, 161.82, 161.38, 155.10, 151.93, 151.55, 141.27, 140.93, 129.63, 129.17, 124.65,

123.83, 122.97, 122.33, 120.98, 120.80, 120.17, 118.04, 115.76, 115.61, 112.28, 109.37, 60.19, 54.52 (2C), 53.59 (2C), 49.82, 46.72, 45.69, 43.10, 35.31, 23.74, 21.30, 14.56. MS (ESI) *m/z* 772 (MH^+).

The phosphate salt of compound **12c** was prepared as follows: a solution of compound **12c** (1.68 g, 2.18 mmol) in dichloromethane (15 mL) was added to 1 M phosphoric acid in ethanol (5.45 mL, 5.45 mmol) and then stirred for 10 min. The solution was added dropwise to ethyl ether (150 mL) and stirred for 30 min. The suspension was filtered and washed with ethyl ether. The filtered solid was dried in vacuo. Yield 98%, white solid, mp 106 ^oC, purity 99.8%. solubility (in H_2O) = 94.56 mg/mL. ^1H NMR (300 MHz, CD3OD) δ 7.62 (d, J = 7.8 Hz, 1H), 7.57-7.47 (m, 1H), 7.33 (dd, J = 12.0, 8.2 Hz, 1H), 7.09-6.93 (m, 4H), 6.82-6.73 (m, 1H), 5.37 (s, 2H), 4.64-4.58 (m, 1H), 4.53-4.33 (m, 2H), 4.21-4.08 (m, 1H), 3.80-3.55 (m, 2H), 3.24-3.07 (m, 2H), 3.02-2.56 (m, 6H), 2.46-2.29 (m, 8H), 1.89 (m, 2H). Anal. HPLC 98% (R_t = 16.60 min).

4.2. Solubility

Water solubility of **12c** was determined experimentally using the following procedure at 25 °C. (1) Standard preparation: 5 mg of **12c** was weighed and diluted with distilled water in a 10 mL volumetric flask. Subsequently, it was diluted with mobile phase to obtain a solution having known concentrations of about 5 μg/mL, 25 μg/mL, 50 μg/mL, 250 μg/mL, and 500 ug per mL, respectively. (2) Sample preparation: excessive compound **12c** was added in 1 mL of distilled water into polypropylene tube, which was shaken at 100 rpm at 37 °C for 24 hrs. After centrifuging tube at 13,000 rpm for 5 min, the supernatant was taken and diluted with diluent. (3) Sample analysis: Following the HPLC condition for purity, aliquots were analyzed. Solubility was calculated by using standard calibration curve. If the area is out of calibration curve range, sample will be diluted additionally.

4.3. In vitro Assays

4.3.1. Binding Assays

Receptor binding assays were performed as described previously.[13] CHO-K1 cells stably transfected with human GnRH receptor (4 ng/μL, PerkinElmer) or HEK293 cells transiently transfected with monkey GnRH receptor was used for binding assay. GnRH receptor preparation was incubated with [^{125}I]D-Trp^{6}-LHRH (0.2 nM/50 μL/well) and test/reference compounds in various concentrations for 1 h at 27 °C. Reaction mixtures were then filtered onto the filter paper (Filtermat A, PerkinElmer). The filter was allowed to completely dry and solid scintillant (Meltilex A, PerkinElmer) was added before counting radioactivity with Microbeta2 TriLux (4PM tubes/2 detector, PerkinElmer). Counts from each well were converted to % inhibition values according to the formula. % inhibition = [1-(compound-NSB)/(TB-NSB) x 100

4.3.2. In vitro Functional Assays

Antagonistic effect of test compounds on NFAT activation was assessed using luciferase assay according to the previously reported protocol.[13] HEK293 cells stably or transiently transfected with pcDNA3.1-human/rat-GnRHR and pGL4-NFATpromoter AP-1-luc were pretreated with test compounds for an hour. The cells were subsequently stimulated with 20 nM or 1 nM GnRH and incubated for 6 h at 37 °C. Luciferase activity as a result of NFAT promoter activation was determined afterward from cell lysates. Inhibition of reporter gene activity was calculated as percentage of maximal agonist-induced Luc activity. The experiments were performed in triplicate.

4.3.3. CYP3A4 Inhibition Assay

Inhibition activity of CYP3A4 was measured by incubating 3 μmol/L BOMR substrate (Vivid CYP3A4 Red substrate) with 5 nmol/L CYP3A4 derived from recombinant baculovirus (Life Technologies) in the presence of 10 μmol/L of each test compound for 30 min at 37 °C. The conversion into resorufin (red standard of Vivid CYP3A4 Red) was measured by fluorescence.

4.4. In vivo Assays

4.4.1. LH suppression in Cynomolgus Monkeys

Phosphoric acid salt of compound **12c** (30 mg/kg as a base) dissolved in saline was administrated to fasted castrated cynomolgus monkeys (4−5 years old, n = 3) by a nasogastric gavage. Blood samples were collected 0, 1, 2, 4, 8, 12, 24 after administration followed by centrifugation. LH concentrations in serum samples were measured using Radioimmunoassay (RIA). All animal experiment protocols were approved by Frontier Bioscience Institutional Animal Care and Use Committee (IACUC).

4.4.2. Pharmacokinetics in Cynomolgus Monkeys

Phosphoric acid salt of compound **12c** (30 mg/kg as a base) dissolved in saline was administrated to fasted castrated cynomolgus monkeys (4-5 years old, n = 3) by a nasogastric gavage. Blood samples were collected 0, 1, 2, 4, 8, 12, 24 after administration (anticoagulant : heparin).The internal standard was added to 50 μL of plasma and then shaken for 10 s. Then, 150 μL of acetonitrile was added and vortexed for 30 s for protein precipitation. It was centrifuged for 5 min, and 80 μL of the supernatant was transferred to the analysis tube for LC/MS/MS analysis. The mass spectrometer was operated in positive ion mode. The HPLC conditions were as follows: column, Shiseido, CAPCELL PAK, C18, MGIII, 5 μm, 2.0 mm I.D x 50 mM; mobile phase, 0.01 mol/L ammonium formate (pH 4.0)/acetonitrile = 4/6; flow rate, 0.25 mL/min; column temperature, 40 °C.

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- A series of 3-(2-aminoethyl) uracil analogs were synthesized as GnRH antagonists.
- Compound **12c** showed highly potent GnRH antagonism with moderate CYP inhibition.
- Compound **12c** exhibited potent and prolonged LH suppression in castrated monkeys.