A Dissertation for the Degree of Doctor of Philosophy in Pharmacy

Synthesis and Biological Evaluation of an Orally Bioavailable Gonadotropin-releasing Hormone (GnRH) Receptor Antagonist

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Part 1

Discovery of an Orally Bioavailable Gonadotropin-releasing Hormone (GnRH) Receptor Antagonist

ABSTRACT

We developed a compound library for orally available gonadotropin-releasing hormone (GnRH) receptor antagonists that were based on a uracil scaffold. Based on in vitro activity and CYP inhibition profile, we selected **18a** (SKI2496) for further in vivo studies. Compound **18a** exhibited more selective antagonistic activity toward the human GnRH receptors over the GnRHRs in monkeys and rats, and this compound also showed inhibitory effects on GnRH-mediated signaling pathways. Pharmacokinetic and pharmacodynamic evaluations of **18a** revealed improved bioavailability and superior gonadotropic suppression activity compared with Elagolix, the most clinically advanced compound. Considering that **18a** exhibited highly potent and selective antagonistic activity toward the *h*GnRHRs along with favorable pharmacokinetic profiles, we believe that **18a** may represent a promising candidate for an orally available hormonal therapy.

Keyword : Gonadotropin-releasing hormone, GnRH receptor antagonist, GnRH, Sex hormone

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1. INTRODUCTION

1.1 GnRH and GnRH receptor

Human gonadotropin-releasing hormone (GnRH) is a key regulator of sexual development and reproductive function.[1] This linear decapeptide hormone is produced in the hypothalamus and stimulates the synthesis and secretion of gonadotropins, including luteinizing hormone (LH) and follicle-stimulating hormone (FSH), by interacting with GnRH receptors (GnRHR) in the pituitary gland. Human GnRHR is a member of the G-protein coupled receptor (GPCR) family and is known to be involved in various signaling processes, including activation of mitogen-activated protein kinase (MAPK) family activity and mobilization of intracellular Ca^{2+}.[2] The signaling processes triggered by GnRHR activation eventually lead to the production of steroid hormones that maintain the reproductive cycle.

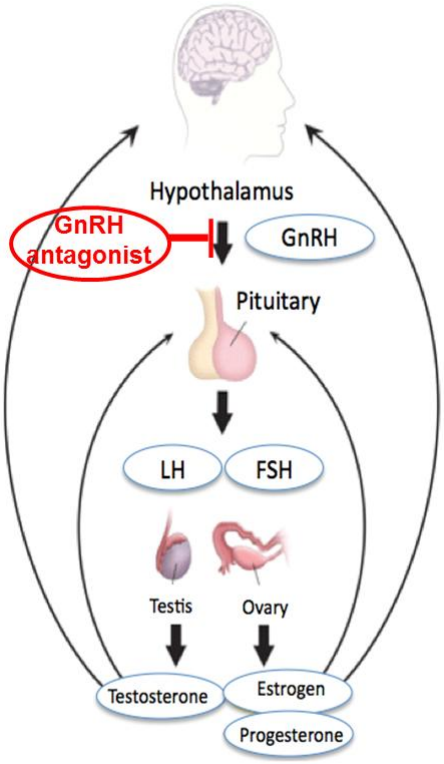


Figure. Hypothalamic Pituitary Gonadal (HPG) axis

1.2 Development of GnRH receptor antagonist

As GnRH plays a critical role in the human reproductive system, GnRH and its analogs have been widely used for the treatment of various steroid hormone-dependent diseases, including endometriosis, uterine fibroids, and prostate cancer.[3] Notably, chronic administration of GnRH analogs can downregulate GnRHRs and results in the complete ablation of steroid hormones; thus, these analogs has been accepted as an effective and safe method to treat hormone-dependent tumors.[4,5]

However, growing concern exists over this particular strategy because of the initial flare effect[6] and the risk of bone loss from long-term use of hormonal agonists.[7,8] To overcome these challenges, several peptide-based GnRH antagonists have been introduced to the market. For example, Degarelix has been approved for the treatment of hormone-sensitive prostate cancer[9] and Cetrorelix is used for infertility.[10] Nevertheless, one problem with currently available agents is that, owing to their peptidic nature, they must be administered by injection, which is associated with various potential side effects. Non-peptidic agents are considered to be preferable because of their superior oral availability and greater specificity.

To date, many non-peptidic compounds have been reported in the literature as GnRH antagonists;[11] however, only a few compounds described in **Figure 1** have been advanced to clinical development. Sufugolix (compound **1a**)[12] exhibited gonadotropin suppression in healthy young men[13] and post-menopausal women by single or repeated dosing,[14] although this compound was eventually discontinued in phase II trials. Relugolix (compound **1b**)[15] has exhibited greater potency and much weaker CYP3A4 inhibition than compound **1a**, and it is currently in phase III clinical trials for uterine fibroids.[16] Compounds with a uracil

scaffold, such as NBI-42902 (**2a**)[17] and Elagolix (**2b**)[18], have shown promising results in phase I trials. Notably, compound **2b** exhibited greater potency and weaker CYP3A4 inhibition compared to compound **2a**, and compound **2b** has advanced to phase III trials for the treatment of endometriosis[19a,b] and uterine fibroids.[20a,b]

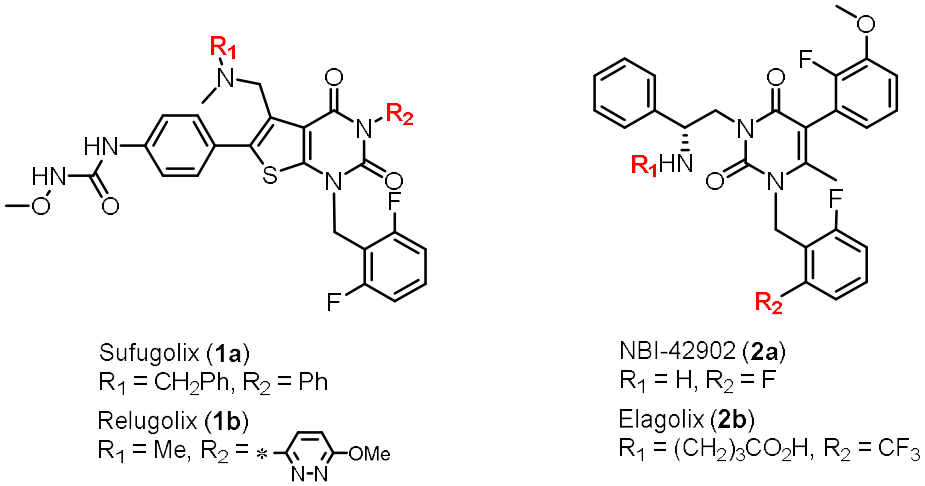


Figure 1. Representative non-peptidic GnRH antagonists in clinical development.

2. AIMS OF OUR STUDIES

Although compound **2b** is the most advanced non-peptidic compound studied to date, this compound appears to exhibit relatively low oral bioavailability (F = 5.8% in rats and 11% in monkeys).[18] According to previously reported structure-activity analyses,[21-24] the uracil group provides a versatile structural scaffold for diverse modifications. The 6-methyl group and electron-deficient aromatic ring at the 1-position are required for a proper orientation needed to achieve an optimal binding interaction. The 3-aminoethyl group is essential for activity and metabolic stability, whereas the 5-phenyl group appears to mostly affect binding affinity. We speculated not only that the 5-position of the uracil moiety is readily available for further modification but also that the structure-activity relationship of non-aromatic substituents at this specific position has not yet been elucidated. Therefore, we sought to design a new series of compounds that contained various non-aromatic heterocycles at the 5-position of the uracil group to yield compounds with improved oral absorption and pharmacodynamic profiles, as shown in **Figure 2**.

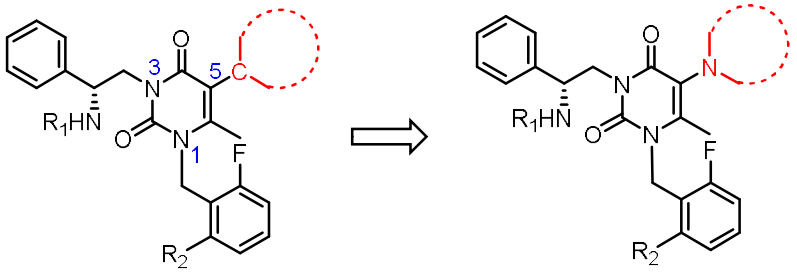


Figure 2. Strategy for modifications at the 5-position of the uracil scaffold.

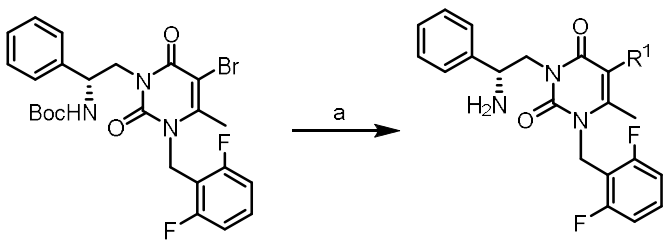
In this present study, we synthesized a library of uracil derivatives that contained cyclic amines, including substituted piperidines and piperazines, at the 5-position. We performed *in vitro* binding and cell-based assays for human GnRHR and CYP inhibition tests to select potent GnRH antagonists that did not significantly inhibit CYP enzymes. Based on the results of these *in vitro* assays, we selected a final candidate, compound **18a** (SKI2496), and then further evaluated its biological effects on downstream cellular signaling pathways. We also examined interspecies differences in binding affinity and activation of GnRHR-specific signaling processes using human-, monkey-, and rat-derived GnRH receptors and cells. Finally, we evaluated the *in vivo* antagonistic effects of compound **18a** in castrated monkeys, along with its pharmacokinetic profiles in rats, dogs and monkeys.

3. RESULTS AND DISCUSSION

3.1 Chemistry

Syntheses of 5-heterocyclic uracil derivatives began with compound **3**, which was prepared in a large quantity according to previously described procedures.[17] Substitutions of the 5-bromine with various heterocyclic amine reagents were performed by microwave irradiation at 120°C, followed by deprotection of the *N*-Boc group in the presence of trifluoroacetic acid to yield the corresponding amines **4** (**Scheme 1**).

Scheme 1. Synthesis of uracils with 5-heterocycles[a]



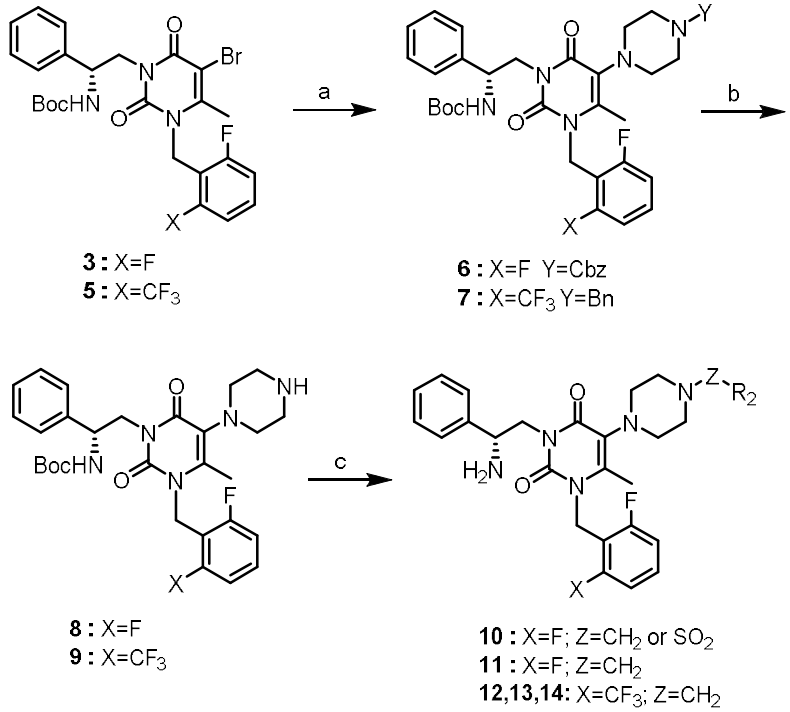
3 **4**

[a] Reagents and conditions: (a) (i) H-R_1, MeCN, microwave, 120°C (ii) TFA, CH_2Cl_2, r.t. 10~98%

To synthesize 5-piperazinyl uracil derivatives, either *N*-benzyl or *N*-Cbz piperazine was used for selective deprotection reactions. These

N-protected piperazines were hydrogenated under Pd/C to yield common intermediates **8** or **9**, and these intermediates were subsequently alkylated with various substituted aryl halides or aldehydes followed by TFA treatment to yield the final compounds **10**, **11**, **12**, **13** and **14** (**Scheme 2**).

Scheme 2. Synthesis of 5-(4-substitutedpiperazinyl)uracils[a]

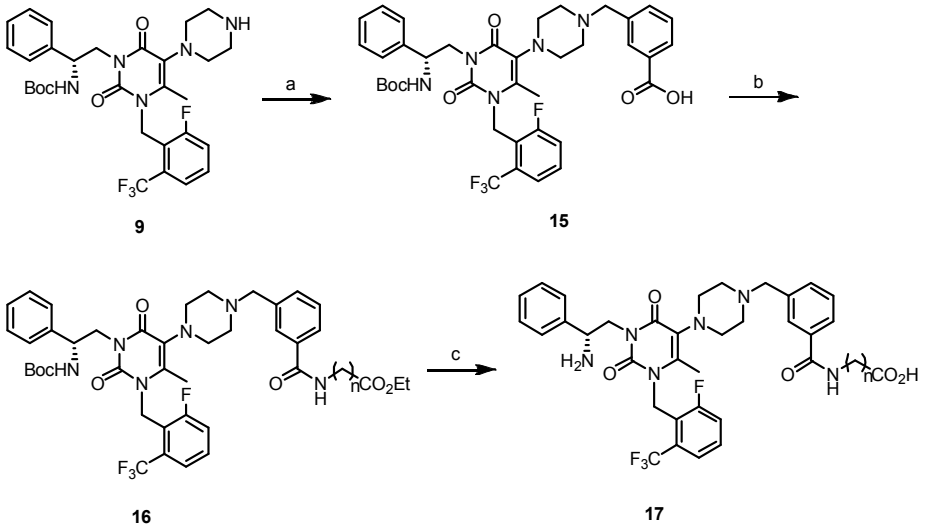


[a] Reagents and conditions: (a) *N*-Cbz-piperazine or *N*-Bn-piperazine, MeCN, microwave, 120°C, 65~83%; (b) Pd/C, H_2, MeOH, 78~95%; (c) (i) [Method A] R_2-CH_2Br or R_2-SO_2Cl, DIPEA, CH_2Cl_2, r.t., [Method B] R_2-CH_2Cl, K_2CO_3, MeCN, 20–60°C, [Method C] R_2-CH_2Br, K_2CO_3, KI, DMF, 80°C, [Method D] R_2-CHO, dichloroethane,

$NaBH(OAc)_3$, r.t., (ii) TFA, CH_2Cl_2, r.t. 7~83%

Among the 5-(4-substitutedpiperazinyl)uracils, acid-containing derivatives, i.e., compound **16**, were alkylated using 3-(bromomethyl)benzoic acid methyl ester, and then were hydrolyzed under basic conditions to yield compound **15**, as shown in **Scheme 3**. Compound **15** was further reacted with amine-containing aliphatic esters to produce the corresponding amide **16**. Hydrolysis, followed by *N*-Boc deprotection of **16**, afforded the desired product **17**.

Scheme 3. Synthesis of uracils with acid-containing 4-benzylpiperazines at the 5-position[a]

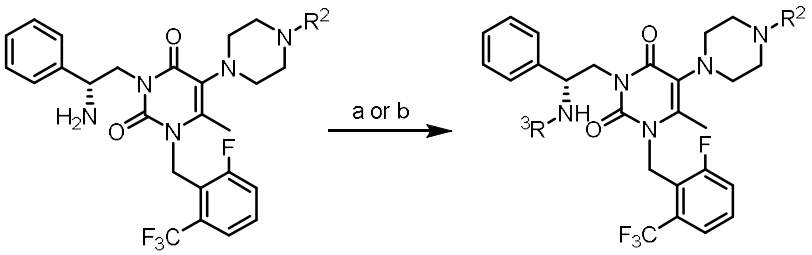


[a]Reagents and conditions: (a) (i) 3-(bromomethyl)benzoic acid methyl ester, TEA, CH_2Cl_2, r.t., (ii) 1N-NaOH, MeOH, 60°C, 89%; (b) HCl · $NH_2(CH_2)_nCO_2Et$, HBTU, DIPEA, DMF, r.t. 95~99%.; (c) (i) 1N-

NaOH, EtOH, r.t, (ii) TFA, CH_2Cl_2, r.t. 21~35%

To conjugate a side chain that contained an aliphatic acid at the 3-aminoethyl group, we followed procedures previously reported by Struthers and co-workers (**Scheme 4**).[18] Alkylation of compound **12** or **14** with ethyl 4-bromobutyrate or phosphonate, followed by hydrolysis with aqueous NaOH yielded the corresponding acids **18** and **19**. Meanwhile, reaction of compound **14** with 1,3-propanesultone at 90°C generated the corresponding sulfonic acid **20**.

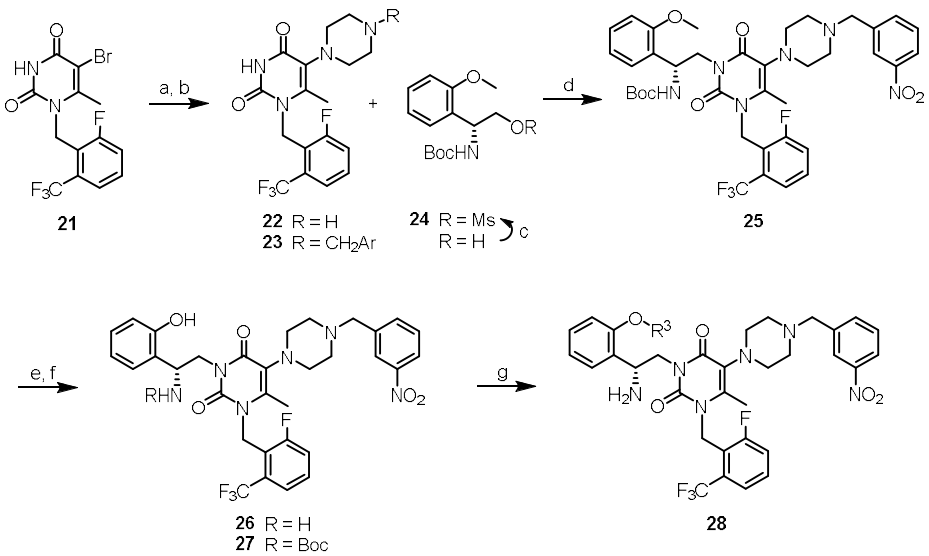
Scheme 4. Synthesis of compounds that incorporate acids at the side chain of 3-phenethylamines[a]



[a] Reagents and conditions: (a) (i) $Br(CH_2)_3CO_2Et$ or $Br(CH_2)_3P(=O)(OEt)_2$, DIPEA, MeCN, 95°C (ii) 1N NaOH, EtOH:H_2O (7:5), 60°C, 13~46% or TMSBr, DCE, 45°C, 30%; (b) 1,3-propanesultone, MeCN, 90°C, 93%

To introduce an acid substituent at the 3-phenethyl group, compound **21** was prepared according to a previously described procedure.[25] Compound **21** was then subjected to microwave irradiation in the presence of 1-benzylpiperazine. Subsequent hydrogenation followed by an alkylation reaction with 3-nitrobenzyl bromide yielded compound **23**. Alkylation of **23** with a mesylate fragment **24** generated compound **25**, which was treated with boron trifluoride to afford **26**. For additional alkylation of the phenolic OH, a Boc group was first reintroduced at the 3-aminoethyl position, and then *O*-alkylation of **27** was performed with a proper *t*-butoxycarbonylalkyl bromide. TFA treatment finally afforded the desired product, compound **28** (**Scheme 5**).

Scheme 5. Synthesis of derivatives containing an acid substituent at the phenyl ring of the 3-phenethyl group[a]



[a] Reagents and conditions: (a) (i) 1-benzylpiperazine, MeCN, microwave, 120°C, 79% (ii) Pd/C, H_2, MeOH:CH_2Cl_2 (1:1), r.t, 78%.; (b) 3-NO_2-benzylbromide, DIPEA, dichloroethane, 50°C, 77%; (c) MsCl, TEA, CH_2Cl_2, quantitative; (d) K_2CO_3, DMF, 70°C, 62% ; (e) BF_3, DCE, -78°C→40°C, 80%; (f) $(Boc)_2O$, TEA, CH_2Cl_2, 40°C, 67%; (g) (i) $Br(CH_2)_nCO_2tBu$, K_2CO_3, DMF, 70°C, (ii) TFA, CH_2Cl_2, r.t. 38~39%

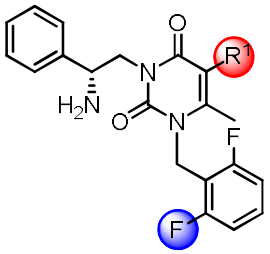
3.2 Structure Activity Relationship.

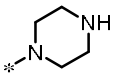
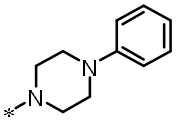
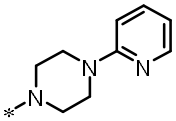
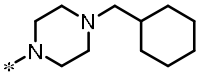
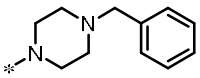
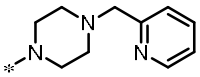
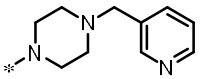
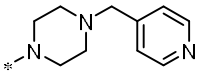
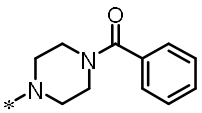
To assess the binding affinity of the synthesized compounds, we performed competitive binding assays using radiolabeled peptide, [^{125}I]D-Trp^6-LHRH, for the human recombinant GnRHRs.[26] Additionally, we evaluated the functional antagonistic activity of the compound by measuring inhibition of reporter gene activity in HEK293

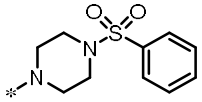
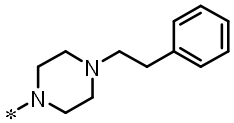
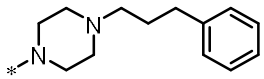
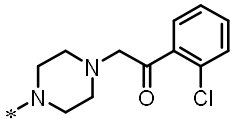
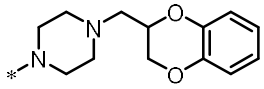
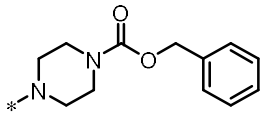
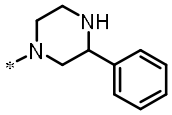
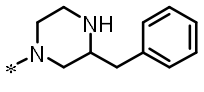
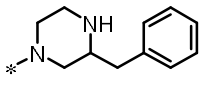
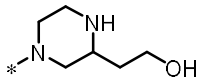
cells transfected with constructs encoding GnRHR and nuclear factor of activated T-cells (NFAT) promoter AP-1-luc, a luciferase assay system, as summarized in **Tables 1**–**5**.

To investigate the structure activity relationship, we first measured the binding affinity of uracil derivatives that contained various aromatic and non-aromatic substituents at the 5-position of the uracil core. As shown in **Table 1**, most of the 5-piperazinyl analogues demonstrated weak or no affinity towards the human GnRH receptors, except for 5-(4-benzyl)piperazinyl (IC_{50} = 12 nM) and 5-[4-(3-pyridinyl)]piperazinyl (IC_{50} = 13.9 nM) analogues (**4e, 10b**). Any changes in the linker properties, such as length, flexibility, or additional functional groups, of these analogues resulted in a significant reduction in activity. When the 5-piperazine group of **4e** was replaced with a piperidine (**4r)**, the resulting compound showed greatly reduced binding affinity, exhibiting 3-fold weaker activity than its piperazinyl counterpart, which indicated that the extra nitrogen atom may participate in hydrogen bonding interactions. The other 5-heterocyclic uracils, including substituted pyrrolidines, a fused azacyclic ring, and morpholine-containing analogues, exhibited little or no activity.

Table 1. SAR of the 5-heterocyclic uracil compounds



Compound	R^1	*h*GnRH-R binding IC_{50} (nM)
2a		0.59
4a		>1000
4b		>1000
4c		>1000
4d		>1000
4e		12
10a		>100
10b		13.9
10c		>100
4f		>100

10d		>100
10e		>100
10f		>100
10g		>100
10h		>100
4g		>100
4h		>100
4i	 (*R*)	>100
4j	 (*S*)	>100
4k		>100
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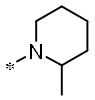
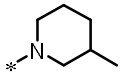
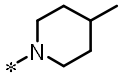
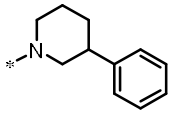
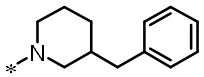
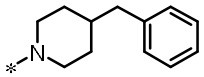
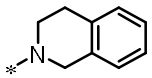
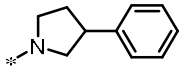
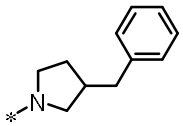
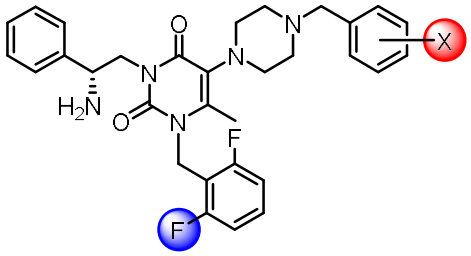
4m		>100
4n		80.3
4o		75.6

4p		>100
4q		>100
4r		34.2
4s		>100
4t		>100
4u		>100

As compound **4e** exhibited the greatest potency among this series, we decided to focus on 5-(4-benzyl)piperazinyl uracils. While keeping the rest of the functional groups unchanged, we introduced diverse substituents into the benzene ring and analyzed the structure-activity relationship, as shown in **Table 2**. Among compounds that contained the ortho-substituted benzenes, it appeared that only a small sized electron-withdrawing group, such as 2-F (**11c**) or 2-CN (**11d**), enhanced the potency, exhibiting IC_{50} values in the low nanomolar range. Electron-withdrawing groups at the meta-position, such as 3-NO_2 (**11i**), 3-F (**11k**), 3-CN (**11l**), 3-Cl (**11n**), 3-CF_3 (**11o**), and 3-OCF_3 (**11p**), generally provided potent activities regardless of the size. Notably,

compound **11o** (IC_{50} = 0.91 nM) demonstrated excellent potency, which was comparable to that of compound **2a**. Interestingly, a combination of an electron-withdrawing group at both positions yielded mixed results; the 2,3-diF substituted compound (**11y**) showed reduced activity (IC_{50} = 10 nM), whereas the 2-F, 3-CF_3 substituted compound (**11z**) demonstrated comparable activity (IC_{50} = 1.1 nM), suggesting that potential dipole-dipole interactions may have affected binding interactions in this region, in addition to electronic factors.

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



Compound	X	hGnRHR binding IC_{50} (nM)
2a		0.59
4e	H	12.0
11a	2-NO_2	13.3
11b	2-Me	73.8
11c	2-F	4.1
11d	2-CN	6.8
11e	2-OMe	>1000
11f	2-Cl	19.1
11g	2-CF_3	65.6
11h	2-OCF_3	>100
11i	3-NO_2	2.9
11j	3-Me	>100
11k	3-F	3.8
11l	3-CN	5.0
11m	3-OMe	14.8
11n	3-Cl	3.1
11o	3-CF_3	0.91

11p	3-OCF_3	1.5
11q	4-NO_2	36.9
11r	4-Me	65.5
11s	4-F	48.6
11t	4-CN	10.0
11u	4-OMe	>100
11v	4-Cl	14.0
11w	4-CF_3	65.7
11x	4-OH	35.3
11y	2,3-diF	10.0
11z	2-F,3-CF_3	1.1

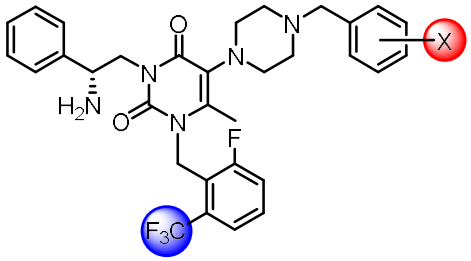
As the 5-(4-benzyl)piperazinyl group with an electron-withdrawing substituent demonstrated comparable activity to that of compound **2a**, we wanted to explore whether we would observe a similar structure-activity relationship by modifying compound **2b**. As shown in **Figure 1**, there are two major differences between **2a** and **2b**; first, compound **2b** has a 1-(2-fluoro-6-trifluoromethyl)benzyl group instead of a 1-(2,6-difluoro)benzyl group; and second, compound **2b** has an extended acid group at the 3-aminoethyl side chain. It has been

reported that replacing the 1-(2,6-difluoro)benzyl group in compound **2a** with a 1-(2-fluoro-6-trifluoromethyl)benzyl group enhanced antagonistic activity by affecting the kinetics of ligand-receptor interactions.[27] Therefore, we switched the 1-(2,6-difluoro)benzyl with 1-(2-fluoro-6-trifluoromethyl)benzyl and also added various substituents at the 5-(4-benzyl)piperazinyl group, as shown in **Table 3**. First, we measured IC_{50} values for human GnRHRs and, as expected, the 1-(2-fluoro-6-trifluoromethylbenzyl) analogues were generally more potent than the corresponding 1-(2,6-difluorobenzyl) analogues, ranging from 1.5-fold (**11p** versus **12e**) to 25-fold (**11y** versus **12r**). Additionally, we determined the inhibitory effects of each compound on NFAT activation. NFAT is a calcium-dependent nuclear transcription factor and its reporter gene assays provide a reliable method to monitor the activation of GPCR signaling pathways, including GnRHR. Surprisingly, all of the analogues showed relatively weak inhibition compared with compound **2b** (IC_{50} = 5.4 nM) in NFAT-responsive luciferase assays. Those

compounds that contained an acetyl or ester substituent (**12m** and **12i**) showed the greatest inhibitory effects in this series (IC_{50} = 16.5 and 23.3 nM, respectively), and compound **12h** (R = 3-CF_3) also showed comparable activity (IC_{50} = 27.9 nM). Compound **12n**, which contains a sulfinyl group, was slightly less potent (IC_{50} = 51.2 nM) than compound **12m**, which contains an acetyl group, whereas a compound with a sulfonate group (**12o**) significantly lost its activity (IC_{50} = 214 nM). Compounds with a 3-amide substituent (**12j, 12k**, and **12l**) showed much weaker activity, and 3-carboxylic acid analogue (**12p**) was inactive. Among those compounds having multiple substituents, compounds **12t** and **12v** exhibited slightly better activity than the other compounds. Notably, compounds **12r** and **12o** showed weak NFAT inhibition (IC_{50} = 266 and 214 nM, respectively) in contrast to their potent binding affinity for GnRHR (IC_{50} = 0.39 and 0.38 nM, respectively). Interestingly, incorporating a 3-(oxazol-2-yl) group into the meta-position of the benzyl ring (**12w**) provided good potency,

which might occur because the oxazolyl group is an isostere of an ester group, so it exhibited activity similar to that of **12i** (R= 3-CO_2Me, IC_{50} = 23.3 nM). Considering the overall SAR shown in **Table 3**, we believe that having a lipophilic electronic-withdrawing group at the meta-position or a combination of the 2-fluoro and a 3-hydrophilic electronic-withdrawing group of the benzyl ring may be beneficial for both binding affinity and NFAT inhibition.

Table 3. SAR of 1-(2-fluoro-6-trifluoromethyl)benzyl derivatives



Compound	X	hGnRHR binding IC_{50} (nM)	NFAT Inhibition IC_{50} (nM)
2a		0.59	87
2b		0.94	5.4
11i		2.9	401
12a	2-F	1	340
12b	2-CN	0.32	351
12c	2-CO_2Me	24.3	>10,000
12d	3-NO_2	0.45	155
12e	3-OCF_3	1	250
12f	3-F	1.7	321
12g	3-CN	0.41	213
12h	3-CF_3	0.52	27.9
12i	3-CO_2Me	0.39	23.3
12j	3-$CONH_2$	1.95	86.5
12k	3-CONHMe	ND	170
12l	3-CONHEt	ND	>300
12m	3-COMe	0.16	16.5
12n	3-SOMe	ND	51.2
12o	3-SO_2Me	0.38	214

12p	3-CO_2H	>100	ND
12q	4-CN	1.16	326
12r	2-F, 3-F	0.39	266
12s	2-F, 3-NO_2	ND	115
12t	2-F, 3-CN	ND	61
12u	2-CN, 3-F	ND	108
12v	2-F, 3-CONH2	ND	42
12w	3-(oxazol-2-yl)	ND	53

ND = not determined

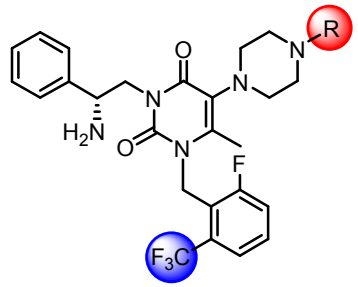
Next, while keeping the 1-(2-fluoro-6-trifluoromethyl)benzyl group at the uracil core, we focused on the 5-(4-heterocyclic piperazinyl) group, and we further investigated the SAR of these compounds, as described in **Table 4**. As previously noted in **Table 1**, compound **10b**, which contains a pyridyl group at the 4-position of 5-piperazine, showed the second greatest activity (IC_{50} = 13.9 nM). Therefore, we decided to evaluate the SAR of the 4-pyridyl derivatives, as well as other 4-heterocyclic derivatives. As expected, the 5-(4-pyridyl) derivative **13a** showed potent binding affinity for hGnRHR (IC_{50} = 2 nM), although its NFAT inhibitory activity appeared to be weak (IC_{50} = 290 nM). We believe that this apparent discrepancy in hGnRHR binding affinity and NFAT inhibition is possibly due to the

slow dissociation kinetics, which was reported with compounds **2a** and **2b** previously.[18] Other 4-pyridyl derivatives exhibited comparable IC_{50} values for hGnRHR ranging from 1.07 to 14.2 nM; however, these compounds also exhibited similarly weak NFAT inhibition, irrespective of the position and electronic properties of the substituent. Moving the position of the pyridyl nitrogen or the additional introduction of hetero atoms did not appear to improve either binding affinity or NFAT inhibition.

Given that the 4-pyridyl group does not appear to significantly improve activity, we next investigated the SAR of the fused bicyclic systems and various five-membered heterocyclic functional groups at the same position. While most compounds with a fused ring system at the 2,3-position of the benzyl group (**13r–13v**) exhibited weak to no activity, compound **13s** was the only exception, as it exhibiting potent binding affinity (IC_{50} = 0.6 nM) but showed weak NFAT inhibition (IC_{50} = 885 nM). When various five-membered heterocycles were introduced, the 2-furyl and 2-thiophenyl analogues (**14f**, **14h**) showed promising potency, which prompted us to incorporate additional substituents. Interestingly, placing an electron-withdrawing group at the 5-position of the furyl ring dramatically enhanced activity. The 2-(5-

trifluoromethyl)furyl analogue (**14k**) showed the greatest activity for both binding affinity (IC_{50} = 0.95 nM) and NFAT inhibition (IC_{50} = 12.6 nM), which is comparable to **2b**. By contrast, replacing the furyl ring with a thiophene (**14r**) or a 1,3,4-oxadiazole (**14s**) decreased activity.

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



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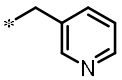
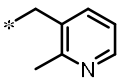
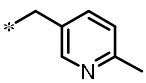
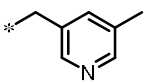
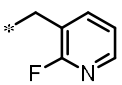
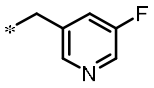
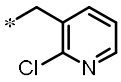
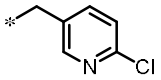
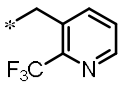
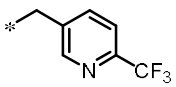
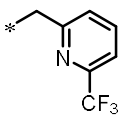
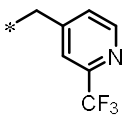
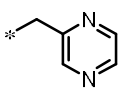
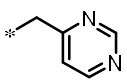
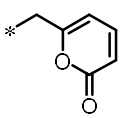
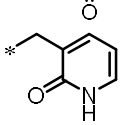
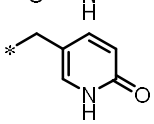
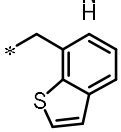
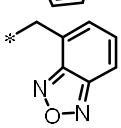
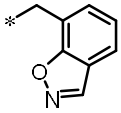
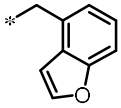
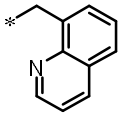
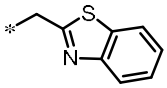
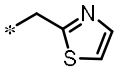
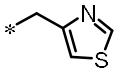
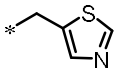
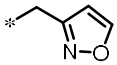
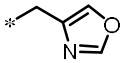
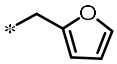
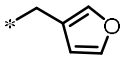
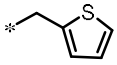
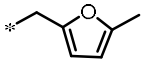
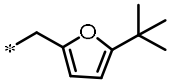
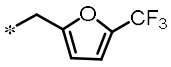
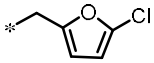
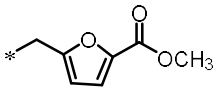
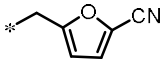
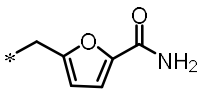
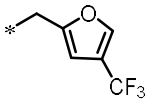
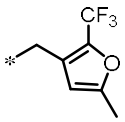
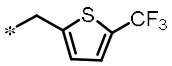
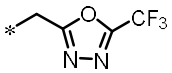
Compound	R	hGnRHR binding IC_{50} (nM)	NFAT inhibition IC_{50} (nM)
2a		0.59	87
2b		0.94	5.4
13a		2	290
13b		5.11	543
13c		14.2	1690
13d		7.18	1180
13e		1.07	193

13f		2.0	1510
13g		2.57	526
13h		3.77	554
13i		ND	>300
13j		ND	>300
13k		ND	217
13l		ND	226
13m		20	2050
13n		ND	>300
13o		ND	>300
13p		>100	ND
13q		28.1	1090
13r		18.2	10230
13s		0.6	885

13t		ND	>300
13u		ND	>300
13v		>100	ND
13w		48	4900
14a		17.7	1660
14b		35.6	2130
14c		8.94	930
14d		14.1	>1000
14e		7.01	>1000
14f		2.76	>1000
14g		6.59	>1000
14h		1.27	>1000
14i		ND	>300
14j		ND	>300
14k		0.95	12.6
14l		0.39	178
14m		0.31	64

14n		0.17	103
14o		ND	>300
14p		ND	>300
14q		>100	>1000
14r		ND	62
14s		ND	>300

ND = not determined

Based on SAR analyses of all tested compounds, we selected three representative compounds among the 1-(2-fluoro-6-trifluoromethyl)benzyl uracil derivatives shown in **Table 3** and **4**. Compounds **13d** (R = 4-(3-nitro)benzyl), **12h** (R = 4-(3-trifluoromethyl)benzyl), and **14k** (R = 4-((5-trifluoromethyl)furan-2-yl)) were first screened for human CYP isozyme inhibition. Unfortunately, all three compounds demonstrated strong CYP3A4 inhibition (89–96%) at a concentration of 10 μM (**Table 5**). As discussed in the introduction and the SAR regarding compounds in **Table 1** and **2**, compound **2a** suffered from CYP3A4 inhibition, whereas compound **2b** was much less problematic. The previously reported SAR studies indicated that when

the primary amine group at the *N*-3 side chain of uracil in **2a** was alkylated with a butyric acid, this structural modification, which is also present in compound **2b**, significantly reduced CYP3A4 inhibition.[18] In addition to the 3-position of the uracil core, the Neurocrine Bioscience research group has reported that the phenyl ring at the 5-position is another viable site for a further modification to reduce CYP3A4 inhibition.[28,29]

Inspired by these approaches, we decided to modify our representative compounds by adding polar groups into three different regions, as shown in **Figure 3**. Because it has been previously reported that the length of the alkyl group at the *N*-3 side chain affects the activity, whereas the butyric acid group appears to provide an optimal distance,[18] we replaced the terminal carboxylic acid group with phosphoric acid and sulfonic acid, while maintaining a propyl linker, shown as group **I** in **Table 5**. Furthermore, we incorporated another carboxylic acid group into the phenyl ring of the *N*-3 side chain (group **II**) and 5-(4-benzyl)piperazinyl group via an amide bond (group **III**).

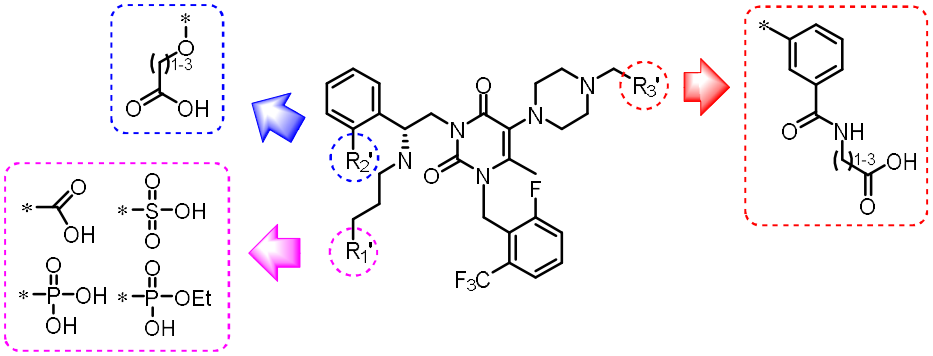


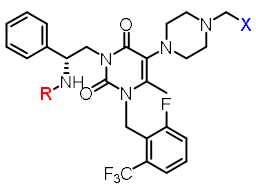
Figure 3. Strategy to overcome CYP3A4 inhibition of piperazinyl compounds

As shown in group **I** of **Table 1**, introducing three different acid groups into compound **14k** significantly reduced CYP3A4 inhibition; this introduction also adversely affected the functional antagonistic activity of all compounds except for compound **18a**, which contained a butyric acid group. This finding again confirmed that the butyric acid group is the most preferable functional group at this position. Similarly, when we added a butyric acid group to compounds **12d** and **12h**, we observed attenuated CYP3A4 inhibition in the modified compounds (**18b** and **18c, respectively**).

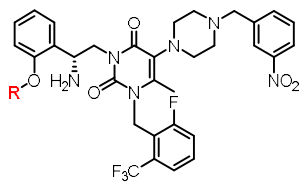
For the compounds belonging to groups **II** and **III**, we maintained the terminal carboxylic acid, but varied the alkyl chain length. A shorter chain was more beneficial for CYP3A4 inhibition in group **II**,

whereas there was little difference by chain length in group **III**. Notably, among these three groups, modifications in group **I** appeared to most efficiently reduce CYP3A4 inhibition. The addition of a carboxylic acid chain in groups **II** and **III** also reduced CYP3A4 inhibition, as demonstrated in compounds **28a, 28b**, and **17c**; however, not as effectively as compounds in group **I**. Overall, we concluded that compound **18a** in group **I** exhibited the most potent GnRH antagonistic activity with low CYP3A4 inhibition. Therefore, we decided to focus on this compound for further studies.

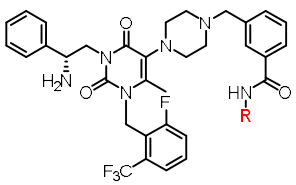
Table 5. SAR of compounds that contained an extended acid side chain



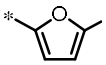
I

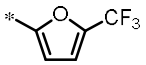
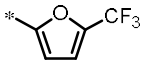
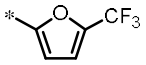
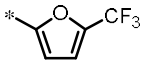
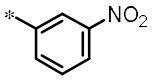
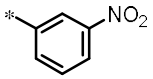
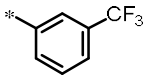
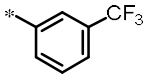


II



III

Compound	Group I/II/III	R	X	Binding inhibition IC_{50} (nM)	NFAT inhibition IC_{50} (nM)	CYP3A4 inhib.% (10 μM)
2b				0.94	5.4	27
14k	I	-H		0.95	12.6	96

18a	I	-$(CH_2)_3CO_2H$		0.44	13.0	21
19a	I	-$(CH_2)_3PO(OEt)(OH)$		1.8	184	45
19b	I	-$(CH_2)_3P(=O)(OH)_2$		1.2	411	16
20	I	-$(CH_2)_3SO_3H$		1.1	122	45
12d	I	-H		0.45	102	89
18b	I	-$(CH_2)_3CO_2H$		1.42	60	17
12h	I	-H		0.33	27.9	96
18c	I	-$(CH_2)_3CO_2H$		0.68	49.5	22
26	II	-H		0.17	89.3	97
28a	II	-CH_2CO_2H		0.23	27.7	30
28b	II	-$CH_2CH_2CH_2CO_2H$		0.14	26.9	57
12j	III	-H		1.95	86.5	93
17a	III	-CH_2CO_2H		>10	>300	74
17b	III	-$CH_2CH_2CO_2H$		1.2	146	72
17c	III	-$CH_2CH_2CH_2CO_2H$		1.1	376	66

3.3 Interspecies Differences and Activation of Signaling Pathways.

Most of the currently available non-peptidyl GnRH antagonists are highly specific to human GnRHR,[15,30,31] so we determined the species specificity of compound **18a** in parallel with that of compound **2b** (Elagolix), as described in **Table 6**. Competitive binding assay data revealed that compound **18a** appeared to be the most specific to human GnRHR, followed by monkey and rat GnRHR; the species specificity of compound **2b** also showed a similar trend, presumably because of their structural similarity. We also observed similar interspecies specificity in the NFAT promoter inhibition assay. The NFAT inhibitory activity of compound **18a** for human GnRHR was 2-fold greater than that of the monkey homolog and 44-fold more potent than of the rat homolog. Compared with compound **2b**, compound **18a** was 4-fold less potent for human receptors, while it was approximately 2-fold more potent for both the monkey and rat receptors.

To further examine the effects of compound **18a** on GnRH-induced downstream cellular responses, we assessed calcium mobilization and ERK1/2 activation upon the treatment of cells with **18a**. Compound **18a** effectively blocked Ca^{2+} flux with an IC_{50} value of

0.76 nM, which is comparable to that of compound **2b** (IC_{50} = 0.86 nM). Regarding ERK1/2 phosphorylation, compound **18a** exhibited an inhibitory activity with an IC_{50} value of 2.9 nM, confirming that compound **18a** inhibited GnRHR activity along with intracellular GnRH-mediated signaling pathways.

Table 6. Summary of *in vitro* binding and cell based assay results for compound 18a and 2b

Compounds IC_{50} (nM)	Competitive binding ([^{125}I]-DTrp^{6}-LHRH)			Inhibition of NFAT reporter activity			Inhibition of ERK activation	Inhibition of Ca^{2+} flux
	Human	Monkey	Rat	Human	Monkey	Rat	Human	Human
18a	0.46	3.7	520	6.32	13.2	279.2	2.9	0.76
2b	0.58	3.5	896	1.63	32.4	590.1	ND	0.86

ND = not determined

3.4 Pharmacokinetic Studies.

The pharmacokinetic properties of compound **18a** were evaluated in rats, dogs, and castrated monkeys. The estimated bioavailability for each animal was 15.6%, 52.6% and 13.2% in rat, dog and castrated monkeys, respectively, when animals were orally administered 10 mg/kg compound **18a** (**Table 7**). Compared with the

previously reported parameters for compound **2b** in rats, compound **18a** exhibited greater oral bioavailability and showed 5-fold higher AUC. Based on these results, we believe that compound **18a** exhibited improved oral exposure compared with compound **2b**.

Table 7. Pharmacokinetic parameters of compound 18a in SD rats, dogs and castrated monkeys after oral administration (10 mg/kg).

Species	C_{max} (μg/mL)	T_{max} (h)	$T_{1/2}$ (h)	AUC_{inf} ((μg·h)/mL)	BA* (%)
SD rat[a]	0.5±0.2	1.6±0.6	1.9±0.6	1.5 ± 0.5	15.6
Beagle dog [b]	2.7±0.3	1.3±0.6	1.7±0.1	9.6±3.3	52.6
Castrated monkey [c]	0.3±0.1	1.7±0.6	3.5±1.9	1.3±0.9	13.2
2b (Rat)[18]	0.34	0.25	0.9	0.29	5.8

[a] male, n=5, 7 weeks old

[b] male, n=3, 17–18 months old and 10–15 kg

[c] male, n=3, 5.5–6.5 yrs. old and 4–4.8 kg

* BA was calculated using the PK parameters from the group administered intravenously with 5 mg/kg (SD rats), 0.3 mg/kg (beagle dog) or 0.5 mg/kg (castrated monkey).

3.5 Animal Studies.

Compound **18a** had a low binding affinity for rat GnRHRs, as described earlier in **Table 6**, so we speculated that rats did not represent a proper animal model for our *in vivo* pharmacodynamic studies. Instead,

we chose castrated monkeys to confirm *in vivo* functional antagonism by measuring gonadotropin suppression after the acute administration of compound **18a**.[26] Following a single dosing of either compound **18a** or compound **2b** (30 mg/kg, po) in castrated male cynomolgus monkeys with elevated levels of gonadotropins, blood samples were collected at scheduled time points for analyses of circulating LH and FSH concentrations using a radioimmunoassay (RIA).

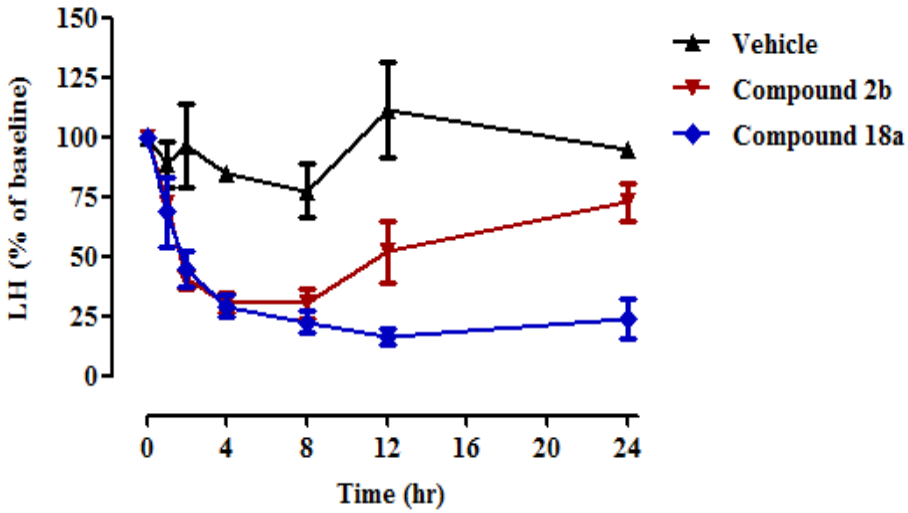


Figure 4. Suppression of serum LH concentrations in castrated male cynomolgus monkeys after oral administration of either compound **18a** or compound **2b** at 30 mg/kg. The time courses are shown with circulating LH levels expressed as a percentage of pretreatment LH levels. On the day of the study, monkeys were administered saline (vehicle), compound **18a** or compound **2b** by nasogastric gavage at doses of 30 mg/kg. Values shown represent the means ± SEM of LH levels, which are expressed as a percentage of pretreatment LH levels for each of three individual animals.

While compound **2b** resulted in maximal LH suppression of 70% at 4–8 h after its administration, after which LH began to return to baseline, compound **18a** reached a maximal LH suppression of 84% at 12 h, which was maintained for up to 24 h. This finding indicated that compound **18a** provided both stronger potency and much longer duration than compound **2b** (**Figure 4**). We believe that the prolonged efficacy on LH suppression by compound **18a** was related to higher exposure of the drug. Overall, compound **18a** resulted in higher AUC and C_{max} values than those values of compound **2b** at 30 mg/kg in castrated monkeys (compound **18a**: AUC of 37 μg·hr/mL and C_{max} of 5.5 μg/mL; compound **2b**: AUC of 3.9 μg·hr/mL and C_{max} of 2.3 μg/mL).

4. CONCLUSION

In this study, we sought to improve the potency and oral exposure of a GnRH antagonist with a uracil scaffold, and we performed SAR studies on the 5-position of the uracil moiety. To this aim, we designed and synthesized a series of 5-(4-benzyl piperazinyl) and the 5-(4-heterocyclic piperazinyl) uracil derivatives. Our SAR analyses revealed that the presence of a lipophilic electron withdrawing group at the meta-position of the 4-benzyl substituent improved the binding affinity and antagonistic activity for GnRHR. Further studies of heteroaromatic rings yielded another promising candidate, compound **14k**, with a 4-((5-trifluoromethyl)furan-2yl))piperazine group at the 5-position. However, these heterocyclic derivatives proved to be potent inhibitors of the enzyme CYP3A4. To resolve this issue, we incorporated an extended acid to three different regions of the uracil scaffold. Compounds modified with a butyric acid not only showed a dramatic reduction of CYP3A4 inhibition but also retained receptor activity comparable to that of the corresponding parent compounds.

Based on our *in vitro* findings, we selected compound **18a** for

further investigation. Species specificity and signaling pathway activation assays indicated that compound **18a** demonstrated selective antagonistic activity towards human GnRH receptors over GnRHRs from monkeys and rats, and compound **18a** showed inhibitory effects on both Ca^{2+} flux and ERK activation in GnRH-mediated signaling pathways. Pharmacokinetic evaluations of compound **18a** in rats and monkeys revealed improved oral exposure compared with those evaluations of compound **2b**. Finally, compound **18a** exhibited superior gonadotropic suppression in castrated monkeys compared with compound **2b**; the stronger and prolonged efficacy of compound **18a** was attributed to its higher oral exposure. Given these favorable *in vitro* and *in vivo* characteristics, compound **18a** has advanced to preclinical toxicological studies for further development. We believe that compound **18a** may not only represent a promising candidate for the treatment of sex hormone-dependent disorders but may also yield useful insights into the development of orally available GnRH analogues in the future.

5. EXPERIMENTAL

5.1 Chemistry

5.1.1 General.

Melting points were determined on a Buchi melting point B-545 apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were measured with a Varian Unity 300 (300MHz) or a Varian Unity Inova (500MHz) or a Jeol Oxford 600 (^{13}C NMR) spectrometer. Chemical shifts were given in ppm units 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. HPLC analyses were performed on an Agilent HP1100 system. Chromatographic separations were carried out on silica gel (Kieselgel 60, 230-400 mesh, Merck) or basic silica gel (Chromatorex NH-DM1020, 100- 200 mesh, Fuji Silysia Chemical Ltd.) using the indicated eluents. Yields were not optimized. The purity (≥ 95%) of each sample was measured by analytical HPLC, and the detailed conditions and results of HPLC analyses were included in Supporting Information. All

animal experiment protocols were approved by Frontier Bioscience Institutional Animal Care and Use Committee (IACUC).

5.1.2 General Procedure for Amination and Deprotection (for compounds 4a - 4u).

A mixture of compound **3** (1 mmol) and amine (15 mmol) in acetonitrile (6 mL) was placed in a microwave vessel and heated at 120 °C under microwave irradiation for 1-5 h with stirring. After cooled to ambient temperature, the resulting solution was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel using Hexane/EtOAc (2:1) as eluent to yield the aminated product. The aminated product was then dissolved in dichloromethane (20 mL), treated with trifluoroacetic acid (2 mL), and stirred at room temperature for 3 h. The resulting solution was neutralized with saturated sodium bicarbonate solution and extracted with dichloromethane. The organic phase was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (20:1) as eluent.

5.1.2.1 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (4a). Yield 44%, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 - 7.18 (m, 6H),

6.90 (t, *J* = 8.3 Hz, 2H), 5.30 - 5.15 (m, 2H), 4.36 (dd, *J* = 9.3, 5.1 Hz, 1H), 4.19 (dd, *J* = 13.0, 9.3 Hz, 1H), 4.05 (dd, *J* = 13.0, 5.1 Hz, 1H), 3.50 - 3.30 (m, 2H), 3.05 - 2.72 (m, 4H), 2.57 - 2.43 (m, 2H), 2.43 (s, 3H). MS (ESI) *m/z* 456 (MH^+).

5.1.2.2 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(4-phenylpiperazin-1-yl)-pyramidine-2,4(1*H*,3*H*)-dione (4b). Yield 20%, off-white foam. ^1H NMR (300 MHz, $CDCl_3$) δ 7.44 - 7.37 (m, 2H), 7.36 - 7.20 (m, 6H), 6.97 - 6.81 (m, 5H), 5.28 and 5.21 (d, *J* = 16.2 Hz, 2H), 4.38 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.22 (dd, *J* = 13.0, 9.4 Hz, 1H), 4.06 (dd, 13.2, 5.1 Hz, 1H), 3.82 - 3.49 (m, 4H), 2.85 (m, 2H), 2.66 (m, 2H), 2.52 and 2.46 (s, 3H). MS (ESI) *m/z* 532 (MH^+).

5.1.2.3 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(4-(pyridin-2-yl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (4c). Yield 40%, colorless oil. ^1H NMR (300 MHz $CDCl_3$) δ 8.18 (ddd, *J* = 4.9, 2.0, 0.9 Hz, 1H), 7.56 - 7.15 (m, 7H), 6.91 (t, *J* = 8.3 Hz, 2H), 6.65 (d, *J* = 8.7 Hz, 1H), 6.59 (ddd, *J* = 7.1, 4.9, 0.8 Hz, 1H), 5.30 - 5.18 (m, 2H), 4.36 (dd, *J* = 9.3, 5.1 Hz, 1H), 4.19 (dd, *J* = 13.0, 9.4 Hz, 1H), 4.25 - 4.13 (m, 2H), 4.05 (dd, *J* = 13.0, 5.1 Hz, 1H), 3.59 (m, 2H), 2.97 (m, 2H), 2.63 (m, 2H), 2.47 (s, 3H). MS (ESI) *m/z* 533 (MH^+).

5.1.2.4 (*R*)-3-(2-Amino-2-phenylethyl)-5-(4-(cyclohexylmethyl) piperazin-1-yl)-1-(2,6-difluorobenzyl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (4d). Yield 10%, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 - 7.38 (m, 2H), 7.33 - 7.19 (m, 4H), 6.89 (m, 2H), 5.26 and 5.19 (d, *J* = 16.2 Hz, 2H), 4.36 (dd, *J* = 9.5, 4.9 Hz, 1H), 4.20 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.05 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.55 (m, 2H), 2.75 (m, 2H), 2.47 (m, 2H), 2.41 (s, 3H), 2.19 - 2.03 (m, 4H), 1.84 - 1.61 (m, 4H), 1.47 (m, 1H), 1.30 - 1.11 (m, 4H), 0.86 (m, 2H). MS (ESI) *m/z* 552 (MH^+).

5.1.2.5 (*R*)-3-(2-Amino-2-phenylethyl)-5-(4-benzylpiperazin-1-yl)-1-(2,6-difluorobenzyl)-6-methyl-pyrimidine-2,4(1*H*,3*H*)-dione (4e). Yield 42%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 - 7.34 (m, 4H), 7.33 - 7.19(m, 7H), 6.89 (m, 2H), 5.27 and 5.19 (d, *J* = 15.9 Hz, 2H), 4.37 (dd, *J* = 9.6, 5.1 Hz, 1H), 4.21 (dd, *J* = 12.9, 9.3 Hz, 1H), 4.05 (dd, *J* = 12.9, 5.1 Hz, 1H), 3.70 - 3.44 (m, 4H), 2.78 (m, 2H), 2.48 (m, 2H), 2.41 (s, 3H), 2.20 (m, 2H). MS (ESI) *m/z* 546 (MH^+).

5.1.2.6 (*R*)-3-(2-Amino-2-phenylethyl)-5-(4-benzoylpiperazin-1-yl)-1-(2,6-difluorobenzyl)-6-methyl-pyrimidine-2,4(1*H*,3*H*)-dione (4f). Yield 24%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 - 7.34 (m, 7H), 7.33 - 7.19(m, 4H), 6.90 (m, 2H), 5.27 and 5.19 (d, *J* = 15.9 Hz,

2H), 4.62 (m, 1H), 4.35 (dd, *J* = 9.3, 5.1 Hz, 1H), 4.19 (dd, *J* = 13.0, 9.3 Hz, 1H), 4.05 (dd, *J* = 13.0, 5.2 Hz, 1H), 3.69 (m, 1H), 3.59 - 3.45 (m, 2H), 3.06 (m, 2H), 2.53 (m, 2H), 2.45 (s, 3H). MS (ESI) *m/z* 560 (MH^+).

5.1.2.7 (*R*)-Benzyl 4-(3-(2-amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazine-1-carboxylate (4g). Yield 44%, colorless oil. NMR (300 MHz, $CDCl_3$) δ 7.40 - 7.27 (m, 10H), 7.26 - 7.19 (m, 1H), 6.90 (t, *J* = 8.3 Hz, 2H), 5.22 (d, *J* = 5.2 Hz, 2H), 5.14 (s, 2H), 4.34 (dd, *J* = 9.2, 5.2 Hz, 1H), 4.23 - 3.99 (m, 4H), 3.55 - 3.34 (m, 2H), 3.04 - 2.87 (m, 2H), 2.58 - 2.40 (m, 2H), 2.44 (s, 3H). MS (ESI) m/z 590 (MH^+).

5.1.2.8 3-((*R*)-2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(3-phenylpiperazin-1-yl)-pyrimidine-2,4(1*H*,3*H*)-dione (4h). Yield 14%, colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ 7.45 - 7.10 (m, 11H), 6.90 (t, *J* = 8.3 Hz, 2H), 5.29 - 5.17 (m, 2H), 4.41 - 4.28 (m, 1H), 4.25 - 4.11 (m, 1H), 4.04 (dt, *J* = 13.0, 4.8 Hz, 1H), 3.83 (d, *J* = 10.5 Hz, 1H), 3.61 - 3.33 (m, 2H), 3.19 - 2.96 (m, 2H), 2.49 (s, 3H), 2.70 - 2.45 (m, 2H). MS (ESI) *m/z* 532 (MH^+).

5.1.2.8 3-((*R*)-2-Amino-2-phenylethyl)-5-((*R*)-3-benzylpiperazin-1-yl)-1-(2,6-difluorobenzyl)-6-methyl-pyrimidine-2,4(1*H*,3*H*)-dione

(4i). Yield 34%, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 - 7.35 (m, 2H), 7.35 - 7.16 (m, 9H), 6.89 (t, *J* = 8.2 Hz, 2H), 5.20 (q, *J* = 16.1 Hz, 2H), 4.36 (dd, *J* = 9.3, 5.0 Hz, 1H), 4.20 (dd, *J* = 12.9, 9.3 Hz, 1H), 4.05 (dd, *J* = 13.0, 5.0 Hz, 1H), 3.38 (t, *J* = 11.2 Hz, 1H), 3.26 (t, *J* = 10.7 Hz, 1H), 3.07 - 2.95 (m, 2H), 2.92 - 2.78 (m, 1H), 2.76 - 2.62 (m, 2H), 2.58 (d, *J* = 11.5 Hz, 1H), 2.45 (d, *J* = 11.5 Hz, 1H), 2.37 (s, 3H). MS (ESI) *m/z* 546 (MH^+).

5.1.2.9 3-((*R*)-2-Amino-2-phenylethyl)-5-((*S*)-3-benzylpiperazin-1-yl)-1-(2,6-difluorobenzyl)-6-methyl-pyrimidine-2,4(1*H*,3*H*)-dione

(4j). Yield 20%, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 (d, *J* = 7.0 Hz, 2H), 7.35 - 7.13 (m, 9H), 6.89 (t, *J* = 8.2 Hz, 2H), 5.19 (s, 2H), 4.37 (dd, *J* = 9.3, 5.0 Hz, 1H), 4.20 (dd, *J* = 13.0, 9.3 Hz, 1H), 4.04 (dd, *J* = 13.0, 5.0 Hz, 1H), 3.45 (t, *J* = 11.5 Hz, 1H), 3.22 (t, *J* = 10.8 Hz, 1H), 3.09 - 2.96 (m, 2H), 2.93 - 2.80 (m, 1H), 2.74 (dd, *J* = 13.5, 6.7 Hz, 1H), 2.63 (dd, *J* = 13.4, 6.9 Hz, 1H), 2.51 (d, *J* = 13.1 Hz, 2H), 2.36 (s, 3H). MS (ESI) *m/z* 546 (MH^+).

5.1.2.10 3-((*R*)-2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-5-(3-(2-hydroxyethyl)piperazin-1-yl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (4k). Yield 14%, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 - 7.48 (m, 2H), 7.42 - 7.21 (m, 4H), 6.89 (t, *J* = 8.3 Hz, 2H), 5.36

(d, *J* = 16.3 Hz, 1H), 5.07 (d, *J* = 16.3 Hz, 1H), 4.76 - 4.48 (m, 2H), 4.07 (d, *J* = 9.4 Hz, 1H), 3.77 - 3.61 (m, 1H), 3.61 - 3.35 (m, 1H), 2.97 - 2.75 (m, 2H), 2.68 - 2.53 (m, 2H), 2.40 (s, 3H), 2.50 - 2.25 (m, 3H), 2.18 - 1.88 (m, 2H). MS (ESI) *m/z* 500 (MH^+).

5.1.2.11 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(piperidin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (4l). Yield 34%, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 - 7.36 (m, 2H), 7.34 - 7.19(m, 4H), 6.89 (m, 2H), 5.25 and 5.19 (d, *J* = 16.2 Hz, 2H), 4.36 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.19 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.04 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.29 (q, *J* = 12.7 Hz, 2H), 2.57 (m, 2H), 2.41 (s, 3H), 1.76 - 1.44 (m, 5H), 1.32 (m, 1H). MS (ESI) *m/z* 455 (MH^+).

5.1.2.12 3-((*R*)-2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(2-methylpiperidin-1-yl)-pyrimidine-2,4(1*H*,3*H*)-dione (4m). Yield 11%, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42–7.34 (m, 2H), 7.33 - 7.17(m, 4H), 6.89 (m, 2H), 5.21 (m, 2H), 4.36 (m, 1H), 4.24 - 4.00 (m, 2H), 3.44 - 3.17 (m, 2H), 2.55 (m, 1H), 2.42 and 2.41 (s, 3H), 1.76 -1.31 (m, 5H), 1.13 (m, 1H), 0.77 and 0.66 (d, *J* = 6.3Hz, 3H). MS (ESI) *m/z* 469 (MH^+).

5.1.2.13 3-((*R*)-2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(3-methylpiperidin-1-yl)-pyrimidine-2,4(1*H*,3*H*)-dione (4n). Yield 46%, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 - 7.36 (m, 2H), 7.33 - 7.19(m, 4H), 6.89 (m, 2H), 5.22 (m, 2H), 4.36 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.10 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.04 (ddd, *J* = 12.9, 4.9, 1.7 Hz, 1H), 3.20 (m, 1H), 2.95 and 2.89 (t, , *J* = 11.1 Hz, 1H), 2.54 (m, 2H), 2.40 (s, 3H), 1.76 - 1.41 (m, 4H), 0.96 (m, 1H), 0.83 (d, *J* = 6.5Hz, 3H). MS (ESI) *m/z* 469 (MH^+).

5.1.2.14 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(4-methylpiperidin-1-yl)-pyrimidine-2,4(1*H*,3*H*)-dione (4o). Yield 62%, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 - 7.36 (m, 2H), 7.34 - 7.19(m, 4H), 6.89 (m, 2H), 5.22 (d, *J* = 15.9 Hz, 2H), 4.36 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.20 (dd, *J* = 13.0, 9.5 Hz, 1H), 4.04 (dd, *J* = 13.0, 4.9Hz, 1H), 3.32 (q, *J* = 12.6 Hz, 2H), 2.56 (t, , *J* = 13.7 Hz, 2H), 2.40 (s, 3H), 1.61 (m, 2H), 1.48 (m, 1H), 1.19 (m, 2H), 0.93 (d, *J* = 6.4 Hz, 3H). MS (ESI) *m/z* 469 (MH^+).

5.1.2.15 3-((*R*)-2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(3-phenylpiperidin-1-yl)-pyrimidine-2,4(1*H*,3*H*)-dione (4p). Yield 64%, white solid, mp = 86 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 - 7.02 (m, 11H), 6.80 (q, *J* = 8.4 Hz, 2H), 5.29 - 5.15 (m, 1H),

5.01 - 4.82 (m, 1H), 4.56 (t, *J* = 10.0 Hz, 1H), 4.25 (m, 1H), 4.00 (m, 1H), 3.42 - 3.19 (m, 2H), 2.83 - 2.56 (m, 3H), 2.40 (s, 3H), 1.93 (d, *J* = 11.9 Hz, 1H), 1.85 - 1.46 (m, 3H). MS (ESI) *m/z* 531 (MH^+).

5.1.2.16 3-((*R*)-2-Amino-2-phenylethyl)-5-(3-benzylpiperidin-1-yl)-1-(2,6-difluorobenzyl)-6-methyl-pyrimidine-2,4(1*H*,3*H*)-dione (4q). Yield 38%, white foam. ^1H NMR (300 MHz, $CDCl_3$) δ 7.41 (d, *J* = 7.5 Hz, 2H), 7.36 - 7.12 (m, 9H), 6.89 (t, *J* = 8.1 Hz, 2H), 5.28 - 5.11 (m, 2H), 4.39 (dd, *J* = 9.3, 4.9 Hz, 1H), 4.26 - 4.15 (m, 1H), 4.06 (dd, *J* = 13.2, 4.6 Hz, 1H), 3.30 - 3.14 (m, 1H), 3.12 - 2.97 (m, 1H), 2.63 - 2.50 (m, 2H), 2.47 (d, *J* = 7.3 Hz, 2H), 2.36 (s, 3H), 1.85 - 1.62 (m, 3H), 1.58 - 1.43 (m, 1H), 1.11 - 0.95 (m, 1H). MS (ESI) *m/z* 545 (MH^+).

5.1.2.17 (*R*)-3-(2-Amino-2-phenylethyl)-5-(4-benzylpiperidin-1-yl)-1-(2,6-difluorobenzyl)-6-methyl-pyrimidine-2,4(1*H*,3*H*)-dione (4r). Yield 98%, white foam. ^1H NMR (300 MHz, $CDCl_3$) δ 7.42 - 7.34 (m, 2H), 7.33 - 7.10(m, 9H), 6.89 (m, 2H), 5.21 (d, *J* = 15.9 Hz, 2H), 4.34(dd, *J* = 9.3, 5.0 Hz, 1H), 4.17 (dd, *J* = 12.9, 9.3 Hz, 1H), 4.03 (dd, *J* = 12.9, 5.1Hz, 1H), 3.28 (dt, *J* = 17.7, 11.8 Hz, 2H), 2.64 - 2.48(m, 4H), 2.40 (s, 3H), 1.69 - 1.55 (m, 3H), 1.25 (m, 2H). MS (ESI) *m/z* 545 (MH^+).

5.1.2.18 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-5-(3,4-dihydroisoquinolin-2(1H)-yl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (4s). Yield 16%, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 - 7.37 (m, 2H), 7.36 - 7.20 (m, 4H), 7.15 - 7.08 (m, 3H), 6.98 (m, 1H), 6.91 (m, 2H), 5.25 (m, 2H), 4.62 (m, 1H), 4.39 (dd, *J* = 9.3, 5.0 Hz, 1H), 4.23 (m, 1H), 4.09 (dd, *J* = 12.9, 5.0Hz, 1H), 3.77 - 3.53 (m, 2H), 3.07 (m, 1H), 2.89 (m, 1H), 2.74 (m, 1H), 2.42 (s, 3H). MS (ESI) *m/z* 503 (MH^+).

5.1.2.19 3-((*R*)-2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(3-phenylpyrrolidin-1-yl)-pyrimidine-2,4(1*H*,3*H*)-dione (4t). Yield 60%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 - 7.38 (m, 2H), 7.35 - 7.16 (m, 9H), 6.90 (t, *J* = 8.2 Hz, 2H), 5.30 - 5.18 (m, 2H), 4.38 (dd, *J* = 9.5, 5.0 Hz, 1H), 4.29 - 4.19 (m, 1H), 4.12 - 4.03 (m, 1H), 3.53 (p, *J* = 8.1 Hz, 1H), 3.35 (q, *J* = 7.8 Hz, 1H), 3.29 - 3.06 (m, 3H), 2.43 (s, 3H), 2.38 - 2.25 (m, 1H), 2.18 - 2.00 (m, 1H). MS (ESI) *m/z* 517 (MH^+).

5.1.2.20 3-((*R*)-2-Amino-2-phenylethyl)-5-(3-benzylpyrrolidin-1-yl)-1-(2,6-difluorobenzyl)-6-methyl-pyrimidine-2,4(1*H*,3*H*)-dione (4u). Yield 60%, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 - 7.36 (m, 2H), 7.35 - 7.14 (m, 9H), 6.89 (t, *J* = 8.3 Hz, 2H), 5.29 - 5.14 (m, 2H),

4.42 - 4.32 (m, 1H), 4.27 - 4.16 (m, 1H), 4.05 (ddd, *J* = 12.9, 4.9, 1.8 Hz, 1H), 3.12 - 2.93 (m, 3H), 2.82 (td, *J* = 7.8, 2.1 Hz, 1H), 2.73 (d, *J* = 7.6 Hz, 2H), 2.63 - 2.51 (m, 1H), 2.37 (s, 3H), 2.07 - 1.92 (m, 1H), 1.77 - 1.65 (m, 1H). MS (ESI) *m/z* 531 (MH^+).

5.1.3 General Procedure for Amination (for compounds 6, 7).

A mixture of compound **3** or **5** (1 mmol) and amine (10 mmol) in acetonitrile (10 mL) was placed in a microwave vessel and heated at 120 °C under microwave irradiation for 2 h with stirring. After cooling to ambient temperature, the resulting solution was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel using Hexane/EtOAc (2:1) as eluent to yield compound **6** or **7**.

5.1.3.1 (*R*)-Benzyl 4-(3-(2-((*tert*-butoxycarbonyl)amino)-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-meth-yl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazine-1-carboxylate (**6**). Yield 65%, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 - 7.19 (m, 11H), 6.96 - 6.82 (m, 2H), 5.72 (d, *J* = 7.2 Hz, 1H), 5.23 (m, 2H), 5.01 (m, 1H), 4.26 (t, *J* = 12.2 Hz, 1H), 4.19 - 3.90 (m, 3H), 3.50 (m, 2H), 2.97 (m, 2H), 2.53 (m, 2H), 2.45 (s, 3H), 1.41 - 1.13 (m, 9H).

5.1.3.2 (*R*)-*tert*-Butyl (2-(5-(4-benzylpiperazin-1-yl)-3-(2-fluoro-6-(trifluoromethyl)benzyl)-4-methyl-2,6-dioxo-2,3-dihydropyrimidin-1(6*H*)-yl)-1-phenylethyl)carbamate (7). Yield 83%, off-white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, *J* = 7.8 Hz, 1H), 7.40 - 7.17 (m, 12H), 5.82 (d, *J* = 6.9 Hz, 1H), 5.42 (m, 2H), 5.08 (m, 1H), 4.29 (m, 1H), 4.16 (m, 1H), 3.62 (m, 2H), 3.54 (s, 2H), 2.79 (m, 2H), 2.48 (m, 2H), 2.35 (3H, s), 2.17 (m, 2H), 1.36 (m, 9H).

5.1.4 General Procedure for Deprotection (for compounds 8, 9).

A solution of compound **6** or **7 (**1 mmol) in Methanol (10 mL) was hydrogenated over 10% Pd/C (18% w/w) under atmospheric pressure at room temperature for 5 h. The mixture was filtered through Celite, and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (8:1) as eluent.

5.1.4.1 (*R*)-*tert*-Butyl (2-(3-(2,6-difluorobenzyl)-4-methyl-2,6-dioxo-5-(piperazin-1-yl)-2,3-dihydropyri-midin-1(6*H*)-yl)-1-phenylethyl)carbamate (8). Yield 78%, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 - 7.18 (m, 6H), 6.89 (m, 2H), 5.79 and 5.61 (m, 1H), 5.42 - 5.08 (m, 2H), 5.02 (m, 1H), 4.27 (t, *J* = 12.3 Hz, 1H), 4.04

(dd, *J* = 13.2, 3.3 Hz, 1H), 3.43 (m, 2H), 2.96 (m, 2H), 2.86 (m, 2H), 2.52 (m, 2H), 2.45 (s, 3H), 1.41 - 1.11 (m, 9H).

5.1.4.2 (*R*)-*tert*-Butyl (2-(3-(2-fluoro-6-(trifluoromethyl)benzyl)-4-methyl-2,6-dioxo-5-(piperazin-1-yl)-2,3-dihydropyrimidin-1(6*H*)-yl)-1-phenylethyl)carbamate (9). Yield 95%, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (m, 1H), 7.40 - 7.17 (m, 7H), 5.78 (d, *J* = 6.6 Hz, 1H), 5.42 (m, 2H), 5.01 (m, 1H), 4.26 (m, 1H), 4.05 (m, 1H), 3.47 (m, 2H), 3.02 - 2.80 (m, 4H), 2.55 (m, 2H), 2.36 (3H, s), 1.36 (m, 9H).

5.1.5 General Procedure for Alkylation and Deprotection (for compounds 10 ~ 14).

5.1.5.1 Method A. (for Compounds 10a-d, 10g, 11a-d, 11f, 11g, 11h, 11j-w, 11y, 11z, 12d, 12e,13a, 13c, 13f, 13i-l, 13n, 13o, 13r-w, 14k, 14o-r). To a solution of the piperazine compound (**8** or **9**, 1 mmol) in dichloromethane (5 mL) were added *N,N*-diisopropylethylamine (2 mmol) and aromatic alkyl bromide or benzenesulfonyl chloride (**10d**, 1.1 mmol), followed by stirring at room temperature for 2 h. The resulting solution was partitioned between dichloromethane and saturated NH_4Cl solution. The organic layer was

separated and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel using hexane/EtOAc (2:1) as eluent. The Boc-protecting group of the resultant product was removed by following the procedure described below as *N*-Boc deprotection.

5.1.5.2 Method B (for Compounds 11j, 11e, 12c, 12a, 12j, 14m, 14s). To a solution of the piperazine compound (**8** or **9**, 1 mmol) in acetonitrile (5 mL) were added potassium carbonate (2 mmol) and aromatic alkyl chloride (1.1 mmol) and stirred at 20 - 60 °C for 3 h. The resulting mixture was diluted with ethyl acetate and washed with water. After concentration of the organic layer, the residue was purified by column chromatography on silica gel using hexane/EtOAc (2:1) as eluent. The Boc-protecting group of the resultant product was removed by following the procedure described below as *N*-Boc deprotection.

5.1.5.3 Method C (for Compounds 10e, 10f, 10h). To a solution of the piperazine compound (**8**, 1 mmol) in DMF (5 mL) were added potassium carbonate (2 mmol), potassium iodide (0.1 mmol) and aromatic alkyl bromide (1.3 mmol) and then stirred at 80 °C overnight. The resulting mixture was diluted with ethyl acetate and washed with

water. After concentration of the organic layer, the residue was purified by column chromatography on silica gel using hexane/EtOAc/CH_2Cl_2 (1:1:1) as eluent. The Boc-protecting group of the resultant product was removed by following the procedure described below as *N*-Boc deprotection.

5.1.5.4 Method D (for Compounds 11x, 13b, 13d, 13e, 13g, 13h, 13m, 13p, 13q, 14a-j, 14l, 14n). To a solution of piperazine compound (**8** or **9**, 1 mmol) in dichloroethane (5 mL) was added aromatic aldehyde (1.5 mmol) and stirred at room temperature for 20 min. The mixture was cooled to 0 °C and treated with sodium triacetoxyborohydride (2 mmol), followed by stirring at room temperature overnight. The resulting mixture was partitioned between ethyl acetate and saturated sodium bicarbonate solution. The organic layer was separated and concentrated. The residue was purified by column chromatography on silica gel using hexane/EtOAc (2:1) as eluent. The Boc-protecting group of the resultant product was removed by following the procedure described below as *N*-Boc deprotection.

5.1.5.5 *N*-Boc Deprotection. A mixture of the 4-substituted piperazine compound (1 mmol) in dichloromethane (10 mL) was treated with trifluoroacetic acid (1 mL) slowly at 0 °C, which was stirred at room temperature for 3 h. The resulting solution was cooled to 0 °C, neutralized with saturated sodium bicarbonate solution and extracted with dichloromethane. The organic phase was dried with Na_2SO_4 and filtered. After concentration *in vacuo*, the residue was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (20:1) as eluent.

5.1.5.5.1 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(4-(pyridin-2-ylmethyl)-piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (10a). Yield 51%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.52 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 1H), 7.66 (td, *J* = 7.6, 1.8 Hz, 1H), 7.57 (dt, *J* = 8.0, 1.2 Hz, 1H), 7.44 - 7.36 (m, 2H), 7.36 - 7.19 (m, 4H), 7.15 (ddd, *J* = 7.3, 4.9, 1.4 Hz, 1H), 6.89 (t, *J* = 8.3 Hz, 2H), 5.29 - 5.14 (m, 2H), 4.36 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.20 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.05 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.69 (s, 2H), 3.77 - 3.48 (m, 2H), 2.89 - 2.66 (m, 2H), 2.62 - 2.39 (m, 2H), 2.43 (s, 3H), 2.39 - 2.22 (m, 2H). MS (ESI) *m/z* 547 (MH^+).

5.1.5.5.2 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(4-(pyridin-3-ylmethyl)-piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (10b). Yield 27%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 - 8.46 (m, 2H), 7.75 (dt, *J* = 7.7, 2.0 Hz, 1H), 7.43 - 7.35 (m, 2H), 7.35 - 7.19 (m, 5H), 6.90 (t, *J* = 8.3 Hz, 2H), 5.29 - 5.15 (m, 2H), 4.36 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.19 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.05 (dd, *J* = 13.0, 5.0 Hz, 1H), 3.68 - 3.46 (m, 2H), 3.53 (s, 2H), 2.85 - 2.66 (m, 2H), 2.57 - 2.38 (m, 2H), 2.42 (s, 3H), 2.31 - 2.11 (m, 2H). MS (ESI) *m/z* 547 (MH^+).

5.1.5.5.3 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(4-(pyridin-4-ylmethyl)-piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (10c). Yield 27%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 - 8.49 (m, 2H), 7.43 - 7.37 (m, 2H), 7.36 - 7.19 (m, 6H), 6.90 (t, *J* = 8.3 Hz, 2H), 5.29 - 5.14 (m, 2H), 4.37 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.20 (dd, *J* = 13.0, 9.4 Hz, 1H), 4.06 (dd, *J* = 12.9, 5.1 Hz, 1H), 3.70 - 3.49 (m, 2H), 3.52 (s, 2H), 2.81 - 2.66 (m, 2H), 2.57 - 2.38 (m, 2H), 2.42 (s, 3H), 2.30 - 2.15 (m, 2H). MS (ESI) *m/z* 547 (MH^+).

5.1.5.5.4 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(4-(phenylsulfonyl)piperazin-1-yl)pyrimidine-2,4(1*H*, 3*H*)-dione (10d). Yield 68%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$)

δ 7.83 - 7.73 (m, 2H), 7.68 - 7.49 (m, 3H), 7.41 - 7.17 (m, 6H), 6.88 (t, *J* = 8.2 Hz, 2H), 5.26 - 5.10 (m, 2H), 4.33 (dd, *J* = 9.2, 5.2 Hz, 1H), 4.16 (dd, *J* = 12.9, 9.3 Hz, 1H), 4.03 (dd, *J* = 13.0, 5.2 Hz, 1H), 3.74 - 3.43 (m, 4H), 2.65 - 2.37 (m, 4H), 2.29 (s, 3H). MS (ESI) m/z 596 (MH^+).

5.1.5.5.5 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(4-phenethylpiperazin-1-yl)-pyrimidine-2,4(1*H*, 3*H*)-dione (10e). Yield 40%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 - 7.16 (m, 11H), 6.90 (t, *J* = 8.3 Hz, 2H), 5.29 - 5.14 (m, 2H), 4.36 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.20 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.05 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.71 - 3.48 (m, 2H), 2.98 - 2.77 (m, 4H), 2.67 - 2.44 (m, 4H), 2.42 (s, 3H), 2.27 - 2.10 (m, 2H). MS (ESI) m/z 560 (MH^+).

5.1.5.5.6 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(4-(3-phenylpropyl)piperazin-1-yl)pyrimidine-2,4(1*H*, 3*H*)-dione (10f). Yield 42%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 - 7.15 (m, 11H), 6.90 (t, *J* = 8.3 Hz, 2H), 5.29 - 5.14 (m, 2H), 4.36 (dd, *J* = 9.5, 5.0 Hz, 1H), 4.20 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.05 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.71 - 3.43 (m, 2H), 2.89 - 2.72 (m, 2H), 2.66 (t, *J* = 8.3 Hz, 2H), 2.60 - 2.45 (m, 2H), 2.41 (s, 3H), 2.37 (t, *J* =

8.3 Hz, 2H), 2.21 - 2.01 (m, 2H), 1.81 (p, *J* = 7.5 Hz, 2H). MS (ESI) m/z 574 (MH^+).

5.1.5.5.7 (*R*)-3-(2-Amino-2-phenylethyl)-5-(4-(2-(2-chlorophenyl)-2-oxoethyl)piperazin-1-yl)-1-(2,6-di-fluorobenzyl)-6-methylpyrimidine-2,4(1*H*, 3*H*)-dione (10g). Yield 34%, yellow foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 - 7.48 (m, 1H), 7.44 - 7.19 (m, 9H), 6.89 (t, *J* = 8.3 Hz, 2H), 5.29 - 5.13 (m, 2H), 4.35 (dd, *J* = 9.5, 4.9 Hz, 1H), 4.19 (dd, *J* = 13.0, 9.5 Hz, 1H), 4.03 (dd, *J* = 13.0, 4.9 Hz, 1H), 3.73 (s, 2H), 3.62 - 3.41 (m, 2H), 2.88 - 2.70 (m, 2H), 2.41 (s, 3H), 2.50 - 2.35 (m, 4H). MS (ESI) m/z 608 (MH^+).

5.1.5.5.8 3-((*R*)-2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-5-(4-((2,3-dihydrobenzo[b][1,4]dioxin-2-yl)methyl)piperazin-1-yl)-6-methylpyrimidine-2,4(1*H*, 3*H*)-dione (10h). Yield 18%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 - 7.19 (m, 6H), 6.97 - 6.78 (m, 6H), 5.29 - 5.15 (m, 2H), 4.41 - 4.25 (m, 3H), 4.19 (dd, *J* = 13.0, 9.4 Hz, 1H), 4.11 - 3.97 (m, 2H), 3.70 - 3.45 (m, 2H), 2.98 - 2.57 (m, 4H), 2.42 (s, 3H) 2.56 - 2.25 (m, 4H). MS (ESI) m/z 604 (MH^+).

5.1.5.5.9 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(4-(2-nitrobenzyl)piperazin-1-yl)pyrimidine-2,4(1*H*, 3*H*)-dione (11a). Yield 60%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ

7.87 (dd, *J* = 8.1, 1.3Hz, 1H), 7.56 (td, *J* = 7.6, 1.3 Hz, 1H), 7.42 - 7.36 (m, *3*H), 7.35 - 7.27 (m, 3H), 7.27 - 7.20(m, 2H), 6.89 (m, 2H), 5.20 (d, *J* = 15.9 Hz, 2H), 4.36 (dd, *J* = 9.5, 5.0 Hz, 1H), 4.20 (dd, *J* = 12.0, 9.5 Hz, 1H), 4.05 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.81 (s, 2H), 3.69 - 3.48(m, 2H), 2.72(m, 2H), 2.54 - 2.44(m, 2H), 2.42 (s, 3H), 2.34 - 2.21 (m, 2H). MS (ESI) *m/z* 591 (MH^+).

5.1.5.5.10 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(4-(2-methylbenzyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (11b). Yield 50%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (m, 2H), 7.32 - 7.15 (m, 5H), 7.14 - 7.07(m, 3H), 6.87 (m, 2H), 5.23 and 5.17 (d, *J* = 15.9 Hz, 2H), 4.33 (dd, *J* = 9.5, 4.9 Hz, 1H), 4.17 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.02 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.49 (s, 2H), 3.59 - 3.41(m, 2H), 2.76 (m, 2H), 2.55 - 2.37(m, 5H), 2.36 (s, 3H), 2.15 (m, 2H). MS (ESI) *m/z* 576 (MH^+).

5.1.5.5.11 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-5-(4-(2-fluorobenzyl)piperazin-1-yl)-6-methylpyrimidine-2,4 (1*H*,3*H*)-dione (11c). Yield 50%, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (td, *J* = 7.5, 1.9 Hz, 1H), 7.39 (m, 2H), 7.34 - 7.18 (m, 5H), 7.11 (td, *J* = 7.5, 1.3 Hz, 1H), 7.01 (ddd, *J* = 9.6, 8.2, 1.3 Hz, 1H), 6.89 (m, 2H), 5.26 and 5.18 (d, *J* = 16.1 Hz, 2H), 4.37 (dd, *J* = 9.4, 4.9 Hz, 1H),

4.21 (dd, *J* = 13.0, 9.5 Hz, 1H), 4.05 (dd, *J* = 13.0, 4.9 Hz, 1H), 3.60 (s, 2H), 3.69 - 3.45 (m, 2H), 2.78 (m, 2H), 2.58 - 2.35 (m, 5H), 2.32 - 2.17 (m, 2H). MS (ESI) *m/z* 564 (MH^+).

5.1.5.5.12 (*R*)-2-((4-(3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)benzonitrile (11d). Yield 58%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (dd, *J* = 7.9, 1.2 Hz, 1H), 7.63 (dd, *J* = 7.7, 1.3 Hz, 1H), 7.57 (td, *J* = 7.6, 1.4 Hz, 1H), 7.44 - 7.38 (m, 2H), 7.38 - 7.20 (m, 5H), 6.91 (m, 2H), 5.28 and 5.21 (d, *J* = 16.1 Hz, 2H), 4.38 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.21 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.06 (dd, *J* = 13.0, 5.0 Hz, 1H), 3.75 (s, 2H), 3.61 (m, 2H), 2.77 (m, 2H), 2.58 - 2.42 (m, 5H), 2.33(m, 2H). MS (ESI) *m/z* 571 (MH^+).

5.1.5.5.13 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-5-(4-(2-methoxybenzyl)piperazin-1-yl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (11e). Yield 35%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (dd, *J* = 7.5, 1.8 Hz, 1H), 7.42 - 7.36 (m, 2H), 7.35 - 7.17(m, 5H), 6.99 - 6.79 (m, 4H), 5.25 and 5.18 (d, *J* = 16.1 Hz, 2H), 4.36 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.19 (dd, *J* = 13.0, 9.4 Hz, 1H), 4.05 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.82 (s, 3H), 3.57 (s, 2H), 3.66 - 3.48(m,

2H), 2.80(m, 2H), 2.55 - 2.37(m, 5H), 2.22 (m, 2H). MS (ESI) *m/z* 576 (MH^+).

5.1.5.5.14 (*R*)-3-(2-Amino-2-phenylethyl)-5-(4-(2-chlorobenzyl) piperazin-1-yl)-1-(2,6-difluorobenzyl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (11f). Yield 32%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (dd, *J* = 7.6, 1.8 Hz, 1H), 7.42-7.35 (m, 2H), 7.35-7.27(m, 3H), 7.27-7.12(m, 4H), 6.89 (m, 2H), 5.26 and 5.19 (d, *J* = 16.1 Hz, 2H), 4.36 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.20 (dd, *J* = 13.0, 9.5 Hz, 1H), 4.05 (dd, *J* = 13.0, 5.0 Hz, 1H), 3.63 (s, 2H), 3.70-3.49(m, 2H), 2.78(m, 2H), 2.57-2.39(m, 5H), 2.28(m, 2H) MS (ESI) *m/z* 580 (MH^+)

5.1.5.5.15 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(4-(2-(trifluoromethyl)benzyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (11g). Yield 76%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, *J* = 7.8Hz, 1H), 7.60 (d, *J* = 7.8 Hz, 1H), 7.51 (t, *J* = 7.8 Hz, 1H), 7.43 - 7.37 (m, 2H), 7.35 - 7.18(m, 5H), 6.89 (m, 2H), 5.26 and 5.19 (d, *J* = 16.1 Hz, 2H), 4.37 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.20 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.06 (dd, *J* = 13.0, 5.0 Hz, 1H), 3.67 (s, 2H), 3.65 - 3.50 (m, 2H), 2.75 (m, 2H), 2.57 - 2.39 (m, 5H), 2.25 (m, 2H). MS (ESI) *m/z* 614 (MH^+).

5.1.5.5.16 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(4-(2-(trifluoromethoxy)benz-yl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (11h). Yield 53%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 - 7.64 (m, 1H), 7.44 - 7.36 (m, 2H), 7.35 - 7.17 (m, 7H), 6.95 - 6.83 (m, 2H), 5.30 - 5.13 (m, 2H), 4.36 (dd, *J* = 9.5, 5.0 Hz, 1H), 4.20 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.05 (dd, *J* = 13.0, 5.0 Hz, 1H), 3.68 - 3.49 (m, 4H), 2.75 (m, 2H), 2.49 (m, 2H), 2.42 (s, 3H), 2.23 (m, 2H). MS (ESI) *m/z* 630 (MH^+).

5.1.5.5.17 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(4-(3-nitrobenzyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (11i). Yield 63%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (m, *J* = 1.9 Hz, 1H), 8.10 (ddd, *J* = 8.2, 2.4, 1.1 Hz, 1H), 7.77 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.48 (t, *J* = 7.9 Hz, 1H), 7.39 (m, 2H), 7.30 (m, 2H), 7.27 - 7.20 (m, 2H), 6.89 (m, 2H), 5.20 (d, *J* = 15.9 Hz, 2H), 4.36 (dd, *J* = 9.5, 5.1 Hz, 1H), 4.19 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.05 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.69 - 3.50 (m, 2H), 3.61 (s, 2H), 2.75 (m, 2H), 2.58 - 2.43 (m, 2H), 2.42 (s, 3H), 2.29 - 2.16 (m, 2H). MS (ESI) *m/z* 591 (MH^+).

5.1.5.5.18 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(4-(3-methylbenzyl)piperazin-1-yl)pyrimidine-

2,4(1*H*,3*H*)-dione (11j). Yield 46%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 (m, 2H), 7.32 - 7.10 (m, 7H), 7.05 (m, 1H), 6.89 (m, 2H), 5.25 and 5.18 (d, *J* = 16.1 Hz, 2H), 4.36 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.19 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.05 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.61 - 3.51(m, 2H), 3.44 (s, 2H), 2.72 (m, 2H), 2.51 - 2.37 (m, 5H), 2.34 (s, 3H), 2.15 (m, 2H). MS (ESI) *m/z* 576 (MH^+).

5.1.5.5.19 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-5-(4-(3-fluorobenzyl)piperazin-1-yl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (11k). Yield 67%, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 (m, 2H), 7.34 - 7.18 (m, 5H), 7.15 - 7.07 (m, 2H), 6.97 - 6.84 (m, 3H), 5.26 and 5.19 (d, *J* = 16.1 Hz, 2H), 4.38 (dd, *J* = 9.5, 4.9 Hz, 1H), 4.21 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.06 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.51 (s, 2H), 3.69 - 3.48 (m, 2H), 2.75 (m, 2H), 2.57 - 2.38 (m, 5H), 2.26 - 2.12 (m, 2H). MS (ESI) *m/z* 564 (MH^+).

5.1.5.5.20 (*R*)-3-((4-(3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)benzonitrile (11l). Yield 58%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (m, 2H), 7.53 (dt, *J* = 7.7, 1.5 Hz, 1H), 7.44 - 7.35 (m, 3H), 7.34 - 7.18 (m, 4H), 6.89 (m, 2H), 5.26 and 5.19 (d, *J* = 16.1 Hz, 2H), 4.36 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.19 (dd, *J* =

12.9, 9.5 Hz, 1H), 4.05 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.75 (s, 2H), 3.69 - 3.48 (m, 2H), 2.72 (m, 2H), 2.56 - 2.39 (m, 5H), 2.18 (m, 2H). MS (ESI) *m/z* 571 (MH^+).

5.1.5.5.21 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-5-(4-(3-methoxybenzyl)piperazin-1-yl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (11m). Yield 39%, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 - 7.35 (m, 2H), 7.34 - 7.17 (m, 5H), 6.96 - 6.83 (m, 4H), 6.78 (ddd, *J* = 8.2, 2.5, 1.1 Hz, 1H), 5.25 and 5.18 (d, *J* = 16.1 Hz, 2H), 4.36 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.19 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.05 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.81 (s, 3H), 3.50 (s, 2H), 3.69 - 3.52 (m, 2H), 2.76 (m, 2H), 2.55 - 2.32 (m, 5H), 2.24 - 2.06 (m, 2H). MS (ESI) *m/z* 576 (MH^+).

5.1.5.5.22 (*R*)-3-(2-Amino-2-phenylethyl)-5-(4-(3-chlorobenzyl)piperazin-1-yl)-1-(2,6-difluorobenzyl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (11n). Yield 57%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 - 7.35 (m, 3H), 7.34 - 7.27 (m, 2H), 7.27 - 7.18 (m, 5H), 6.89 (m, 2H), 5.25 and 5.18 (d, *J* = 16.1 Hz, 2H), 4.36 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.19 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.05 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.48 (s, 2H), 3.68 - 3.50 (m, 2H),

2.74 (m, 2H), 2.55 - 2.37 (m, 5H), 2.16 (m, 2H). MS (ESI) *m/z* 580 (MH^+).

5.1.5.5.23 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(4-(3-(trifluoromethyl)benz-yl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (11o). Yield 45%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 - 7.55 (m, 2H), 7.50 (m, 1H), 7.45 - 7.36 (m, 3H), 7.35 - 7.18 (m, 4H), 6.89 (m, 2H), 5.25 and 5.19 (d, *J* = 16.1 Hz, 2H), 4.36 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.20 (dd, *J* = 13.0, 9.5 Hz, 1H), 4.05 (dd, *J* = 13.0, 5.0 Hz, 1H), 3.69 - 3.48 (m, 4H), 2.74 (m, 2H), 2.56 - 2.37 (m, 5H), 2.18 (m, 2H). MS (ESI) *m/z* 614 (MH^+).

5.1.5.5.24 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(4-(3-(trifluoromethoxy)benz-yl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (11p). Yield 55%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 (d, *J* = 6.9 Hz, 2H), 7.39 - 7.23 (m, 7H), 7.15 - 7.10 (m, 1H), 6.90 (m, 2H), 5.22 (m, 2H), 4.35 (dd, *J* = 9.5, 4.9 Hz, 1H), 4.20 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.05 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.53 (s, 2H), 3.69 - 3.54 (m, 2H), 2.75 (m, 2H), 2.52 - 2.38 (m, 5H), 2.18 (m, 2H). MS (ESI) *m/z* 630 (MH^+).

5.1.5.5.25 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(4-(4-nitrobenzyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-

dione (11q). Yield 56%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (m, 2H), 7.53 (m, 2H), 7.37 (m, 2H), 7.28 (m, 2H), 7.25 - 7.16 (m, 2H), 6.87 (m, 2H), 5.23 and 5.17 (d, *J* = 15.9 Hz, 2H), 4.34 (dd, *J* = 9.4, 5.0Hz, 1H), 4.17 (dd, *J* = 13.0, 9.4 Hz, 1H), 4.03 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.67 - 3.48 (m, 2H), 3.59 (s, 2H), 2.71 (m, 2H), 2.53 - 2.43 (m, 2H), 2.40 (s, 3H), 2.20 (m, 2H). MS (ESI) *m/z* 591 (MH^+).

5.1.5.5.26 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(4-(4-methylbenzyl)-piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (11r). Yield 30%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 (m, 2H), 7.32 - 7.18 (m, 6H), 7.11 (m, 2H), 6.89 (m, 2H), 5.25 and 5.18 (d, *J* = 16.2 Hz, 2H), 4.35 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.19 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.05 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.67 - 3.51 (m, 2H), 3.48 (s, 2H), 2.75 (m, 2H), 2.55 - 2.37 (m, 5H), 2.33 (s, 3H), 2.14 (m, 2H). MS (ESI) *m/z* 576 (MH^+).

5.1.5.5.27 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-5-(4-(4-fluorobenzyl)piperazin-1-yl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (11s). Yield 53%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 (m, 2H), 7.34 - 7.27 (m, 4H), 7.25 - 7.18 (m, 2H), 6.98 (m, 2H), 6.89 (m, 2H), 5.25 and 5.18 (d, *J* = 16.1 Hz, 2H), 4.35 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.19 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.05 (dd, *J* = 12.9, 5.0 Hz,

1H), 3.69 - 3.45 (m, 2H), 3.48 (s, 2H), 2.72 (m, 2H), 2.54 - 2.36 (m, 5H), 2.14 (m, 2H). MS (ESI) *m/z* 564 (MH^+).

5.1.5.5.28 (*R*)-4-((4-(3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)benzonitrile (11t). Yield 58%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (m, 2H), 7.49 (m, 2H), 7.39 (m, 2H), 7.34 - 7.18 (m, 4H), 6.91 (m, 2H), 5.25 and 5.18 (d, *J* = 16.1 Hz, 2H), 4.36 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.19 (dd, *J* = 13.0, 9.4 Hz, 1H), 4.05 (dd, *J* = 12.9, 5.0Hz, 1H), 3.69 - 3.47 (m, 2H), 3.56 (s, 2H), 2.71 (m, 2H), 2.56 - 2.44 (m, 2H), 2.43 (s, 3H), 2.18 (m, 2H). MS (ESI) *m/z* 571 (MH^+).

5.1.5.5.29 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-5-(4-(4-methoxybenzyl)piperazin-1-yl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (11u). Yield 22%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 - 7.35 (m, 2H), 7.34 - 7.18 (m, 6H), 6.94 - 6.81 (m, 4H), 5.25 and 5.18 (d, *J* = 16.1 Hz, 2H), 4.35 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.19 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.04 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.80 (s, 3H), 3.46 (s, 2H), 3.69 - 3.52 (m, 2H), 2.76 (m, 2H), 2.55 - 2.32 (m, 5H), 2.20 - 2.06 (m, 2H). MS (ESI) *m/z* 576 (MH^+).

5.1.5.5.30 (*R*)-3-(2-Amino-2-phenylethyl)-5-(4-(4-chlorobenzyl)piperazin-1-yl)-1-(2,6-difluorobenzyl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (11v). Yield 38%, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 - 7.35 (m, 2H), 7.34 - 7.18 (m, 8H), 6.89 (m, 2H), 5.25 and 5.18 (d, *J* = 16.1 Hz, 2H), 4.36 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.19 (dd, *J* = 13.0, 9.4 Hz, 1H), 4.05 (dd, *J* = 12.9, 5.0Hz, 1H), 3.66 - 3.49 (m, 2H), 3.48 (s, 2H), 2.73 (m, 2H), 2.53 - 2.37 (m, 5H), 2.16 (m, 2H). MS (ESI) *m/z* 580 (MH^+).

5.1.5.5.31 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-6-methyl-5-(4-(4-(trifluoromethyl)benz-yl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (11w). Yield 31%, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (m, 2H), 7.48 (m, 2H), 7.38 (m, 2H), 7.34 - 7.18 (m, 4H), 6.89 (m, 2H), 5.26 and 5.18 (d, *J* = 16.1 Hz, 2H), 4.37 (dd, *J* = 9.5, 4.9 Hz, 1H), 4.21 (dd, *J* = 13.0, 9.5 Hz, 1H), 4.05 (dd, *J* = 13.0, 5.0Hz, 1H), 3.68 - 3.49(m, 2H), 3.57 (s, 2H), 2.74 (m, 2H), 2.56 - 2.39 (m, 5H), 2.20 (m, 2H). MS (ESI) *m/z* 614 (MH^+).

5.1.5.5.32 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-5-(4-(4-hydroxybenzyl)piperazin-1-yl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (11x). Yield 14%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (d, *J* = 6.9 Hz, 2H), 7.39 - 7.17 (m, 6H), 6.89 (m,

2H), 6.71 (d, *J* = 8.7 Hz, 2H), 5.22 (s, 2H), 4.39 (dd, *J* = 9.5, 4.9 Hz, 1H), 4.21 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.03 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.50 (m, 2H), 3.43 (s, 2H), 2.71 (m, 2H), 2.40 - 2.34 (m, 5H), 2.10 (m, 2H). MS (ESI) *m/z* 562 (MH^+).

5.1.5.5.33 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-5-(4-(2,3-difluorobenzyl)piperazin-1-yl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (11y). Yield 41%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 - 7.35 (m, 2H), 7.35 - 7.18 (m, 5H), 7.09 - 6.97 (m, 2H), 6.89 (m, 2H), 5.25 and 5.18 (d, *J* = 16.1 Hz, 2H), 4.35 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.19 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.04 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.67 - 3.46 (m, 4H), 2.76 (m, 2H), 2.56 - 2.36 (m, 5H), 2.24 (m, 2H). MS (ESI) *m/z* 582 (MH^+).

5.1.5.5.34 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2,6-difluorobenzyl)-5-(4-(2-fluoro-3-(trifluoromethyl)benzyl)-piperazin-1-yl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (11z). Yield 50%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (t, *J* = 6.9 Hz, 1H), 7.48 (t, *J* = 7.5 Hz, 1H), 7.39 (d, *J* = 6.9 Hz, 2H), 7.33 - 7.18 (m, 5H), 6.90 (m, 2H), 5.22 (m, 2H), 4.35 (dd, *J* = 9.5, 4.9 Hz, 1H), 4.19 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.05 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.69 - 3.54 (m, 4H), 2.75 (m, 2H), 2.53 - 2.38 (m, 5H), 2.26 (m, 2H). MS (ESI) *m/z* 632 (MH^+).

5.1.5.5.35 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-5-(4-(2-fluorobenzyl)-piperazin-1-yl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (12a). Yield 54%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, *J* = 7.5 Hz, 1H), 7.49 (td, *J* = 7.5, 1.9 Hz, 1H), 7.44 - 7.28 (m, 5H), 7.27 - 7.17 (m, 3H), 7.11 (td, *J* = 7.5, 1.3 Hz, 1H), 7.01 (ddd, *J* = 9.6, 8.1, 1.3 Hz, 1H), 5.40 (s, 2H), 4.37 (dd, *J* = 9.4, 4.8 Hz, 1H), 4.21 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.06 (dd, *J* = 12.9, 4.9 Hz, 1H), 3.71 - 3.47 (m, 4H), 2.79 (d, *J* = 10.5 Hz, 2H), 2.48 (t, *J* = 13.8 Hz, 2H), 2.32 (s, 3H), 2.22 (t, *J* = 10.7 Hz, 2H). MS (ESI) *m/z* 614 (MH^+).

5.1.5.5.36 (*R*)-2-((4-(3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)benzonitrile (12b). Yield 41%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (m, 1H), 7.61 (m, 1H), 7.56 (m, 2H), 7.45 - 7.28 (m, 6H), 7.27 - 7.18 (m, 2H), 5.41 (s, 2H), 4.37 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.21 (dd, *J* = 13.0, 9.5 Hz, 1H), 4.06 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.74 (s, 2H), 3.60 (q, *J* = 12.3 Hz, 2H), 2.76 (d, *J* = 10.5 Hz, 2H), 2.49 (t, *J* = 13.6 Hz, 2H), 2.37 - 2.21 (m, 5H). MS (ESI) *m/z* 621 (MH^+).

5.1.5.5.37 (*R*)-Methyl 2-((4-(3-(2-amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)benzoate (12c). Yield 68%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, *J* = 7.8Hz, 1H), 7.56 (m, *2*H), 7.44 - 7.38 (m, 4H), 7.34 - 7.18 (m, 5H), 5.40 (s, 2H), 4.36 (dd, *J* = 9.4, 5.1 Hz, 1H), 4.20 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.06 (dd, *J* = 12.9, 4.9 Hz, 1H), 3.92 (s, 3H), 3.79 (s, 2H), 3.55 (m, 2H), 2.70(m, 2H), 2.44 (m, 2H), 2.32 (s, 3H), 2.20 (m, 2H). MS (ESI) *m/z* 654 (MH^+).

5.1.5.5.38 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(3-nitrobenzyl) piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (12d). Yield 50%, yellowish solid. ^{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^+).

5.1.5.5.39 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(3-(trifluoromethoxy) benzyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (12e). Yield 35%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, *J* = 7.9 Hz, 1H), 7.45 - 7.18 (m, 10H), 7.10 (m, 1H), 5.41 (s, 2H), 4.37 (dd, *J* = 9.5, 4.8 Hz, 1H), 4.21 (m, 1H), 4.07 (m, 1H), 3.70 - 3.47 (m, 4H), 2.75 (d, *J* = 10.5 Hz, 2H), 2.47 (m, 2H), 2.33 (s, 3H), 2.17 (m, 2H). MS (ESI) *m/z* 680 (MH^+).

5.1.5.5.40 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-5-(4-(3-fluorobenzyl)-piperazin-1-yl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (12f). Yield 21%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.0 Hz, 1H), 7.45 - 7.29 (m, 5H), 7.28 - 7.18 (m, 3H), 7.17 - 7.07 (m, 2H), 6.93 (t, *J* = 8.5 Hz, 1H), 5.41 (s, 2H), 4.38 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.22 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.07 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.69 - 3.45 (m, 4H), 2.76 (m, 2H), 2.49 (m, 2H), 2.33 (s, 3H), 2.18 (m, 2H). MS (ESI) *m/z* 614 (MH^+).

5.1.5.5.41 (*R*)-3-((4-(3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)benzonitrile (12g).

Yield 46%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (dt, *J* = 6.1, 1.6 Hz, 2H), 7.54 (m, 2H), 7.45 - 7.36 (m, 4H), 7.36 - 7.29 (m, 2H), 7.28 - 7.18 (m, 2H), 5.48 - 5.33 (m, 2H), 4.37 (dd, *J* = 9.5, 4.9 Hz, 1H), 4.21 (dd, *J* = 13.0, 9.5 Hz, 1H), 4.06 (dd, *J* = 12.9, 4.9 Hz, 1H), 3.70 - 3.48 (m, 4H), 2.72 (d, *J* = 10.6 Hz, 2H), 2.49 (t, *J* = 13.7 Hz, 2H), 2.34 (s, 3H), 2.16 - 2.13 (m, 2H). MS (ESI) *m/z* 621 (MH^+).

5.1.5.5.42 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(3-(trifluoromethyl)benzyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (12h). Yield 60%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 - 7.47 (m, 4H), 7.46 - 7.37 (m, 4H), 7.36 - 7.29 (m, 2H), 7.26 - 7.18 (m, 2H), 5.41 (s, 2H), 4.36 (dd, *J* = 9.4, 5.1 Hz, 1H), 4.21 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.06 (dd, *J* = 12.9, 4.9 Hz, 1H), 3.70 - 3.51 (m, 4H), 2.75 (m, 2H), 2.48 (m, 2H), 2.33 (s, 3H), 2.18 (m, 2H). MS (ESI) *m/z* 664 (MH^+).

5.1.5.5.43 (*R*)-Methyl 3-((4-(3-(2-amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)benzoate (12i). Yield 87%, white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.92 (d, *J* = 8.0 Hz, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.54 (d, *J* = 8.0 Hz,

1H), 7.41 - 7.37 (m, 3H), 7.33 - 7.30 (m, 2H), 7.25 - 7.19 (m, 2H), 5.40 (s, 2H), 4.36 (dd, *J* = 9.5, 4.5 Hz, 1H), 4.20 (dd, *J* = 13.0, 9.5 Hz, 1H), 4.06 (dd, *J* = 13.0, 5.0 Hz, 1H), 3.92 (s, 3H), 3.55 (m, 4H), 2.74 (m, 2H), 2.47 (m, 2H), 2.17 (m, 2H). MS (ESI) *m/z* 654 (MH^+).

5.1.5.5.44 (*R*)-3-((4-(3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)benzamide (12j). Yield 52%, white foam. ^1H NMR (300 MHz, $CDCl_3$) δ 7.85 (t, *J* = 1.8 Hz, 1H), 7.77 (dt, *J* = 7.6, 1.5 Hz, 1H), 7.58 - 7.48 (m, 2H), 7.45 - 7.27 (m, 6H), 7.27 - 7.17 (m, 2H), 6.57 (bs, 1H), 5.90 (bs, 1H), 5.40 (s, 2H), 4.37 (dd, *J* = 9.3, 4.9 Hz, 1H), 4.22 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.06 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.69 - 3.46 (m, 4H), 2.77 (d, *J* = 10.6 Hz, 2H), 2.47 (t, *J* = 14.4 Hz, 2H), 2.34 (s, 3H), 2.17 (m, 2H). MS (ESI) *m/z* 639 (MH^+).

5.1.5.5.45 (*R*)-3-((4-(3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)-*N*-methylbenzamide (12k). Yield 49%, white foam. ^1H NMR (300 MHz, $CDCl_3$) δ 7.74 (s, 1H), 7.70 (dt, *J* = 7.6, 1.5 Hz, 1H), 7.54 (d, 1H), 7.47 (dt, *J* = 7.6, 1.5 Hz, 1H), 7.44 - 7.28 (m, 6H), 7.26 - 7.18 (m, 2H), 6.36

- 6.27 (m, 1H), 5.40 (s, 2H), 4.37 (dd, *J* = 9.3, 5.0 Hz, 1H), 4.21 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.06 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.67 - 3.56 (m, 2H), 3.55 (s, 2H), 3.04 (d, *J* = 4.8 Hz, 2H), 2.75 (d, *J* = 10.5 Hz, 2H), 2.54 - 2.41 (m, 2H), 2.33 (s, 3H), 2.24 - 2.11 (m, 2H). MS (ESI) *m/z* 653 (MH^+).

5.1.5.5.46 (*R*)-3-((4-(3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)-*N*-ethylbenzamide (12l). Yield 17%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.72 - 7.67 (m, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.50 - 7.46 (m, 1H), 7.43 - 7.28 (m, 6H), 7.26 - 7.17 (m, 2H), 6.32 - 6.22 (m, 1H), 5.40 (s, 2H), 4.37 (dd, *J* = 9.3, 5.0 Hz, 1H), 4.21 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.06 (dd, *J* = 13.0, 5.0 Hz, 1H), 3.69 - 3.47 (m, 6H), 2.82 - 2.71 (m, 2H), 2.57 - 2.41 (m, 2H), 2.33 (s, 3H), 2.25 - 2.12 (m, 2H), 1.28 (t, *J* = 7.3 Hz, 3H). MS (ESI) *m/z* 667 (MH^+).

5.1.5.5.47 (*R*)-5-(4-(3-Acetylbenzyl)piperazin-1-yl)-3-(2-amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (12m). Yield 31%, yellowish powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (t, *J* = 1.7 Hz, 1H), 7.84 (dt, *J* = 7.8, 1.5 Hz, 1H), 7.62 (dt, *J* = 7.7, 1.5 Hz, 1H), 7.54 (d, *J* = 7.8

Hz, 1H), 7.45 - 7.28 (m, 6H), 7.27 - 7.17 (m, 2H), 5.40 (s, 2H), 4.37 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.21 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.06 (dd, *J* = 13.0, 4.9 Hz, 1H), 3.69 - 3.44 (m, 4H), 2.76 (d, *J* = 10.4 Hz, 2H), 2.62 (s, 3H), 2.44 (t, *J* = 10.2 Hz, 2H), 2.34 (s, 3H), 2.24 - 2.09 (m, 2H). MS (ESI) *m/z* 638 (MH^+).

5.1.5.5.48 3-((*R*)-2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(3-(methylsulfinyl) benzyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (12n). Yield 72 %, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (s, 1H), 7.58 - 7.29 (m, 9H), 7.27 - 7.18 (m, 2H), 5.41 (s, 2H), 4.37 (dd, *J* = 9.5, 5.0 Hz, 1H), 4.21 (dd, *J* = 13.0, 9.5 Hz, 1H), 4.06 (dd, *J* = 12.9, 4.9 Hz, 1H), 3.69 - 3.50 (m, 4H), 2.83 - 2.68 (m, 5H), 2.48 (m, 2H), 2.34 (s, 3H), 2.26 - 2.10 (m, 2H). MS (ESI) m/z 658 (MH^+).

5.1.5.5.49 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(3-(methylsulfonyl)benzyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (12o). Yield 45%, white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (t, *J* = 1.7 Hz, 1H), 7.83 (ddd, *J* = 7.8, 2.0, 1.2 Hz, 1H), 7.74 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.57 - 7.48 (m, 2H), 7.45 - 7.28 (m, 5H), 7.26 - 7.18 (m, 2H), 5.41 (s, 2H), 4.37 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.21 (dd, *J* = 13.0,

9.5 Hz, 1H), 4.06 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.69 - 3.47 (m, 4H), 3.07 (d, *J* = 1.1 Hz, 3H), 2.74 (d, *J* = 10.5 Hz, 2H), 2.49 (t, *J* = 13.5 Hz, 2H), 2.34 (s, 3H), 2.20 (m, 2H). MS (ESI) *m/z* 658 (MH^+).

5.1.5.5.50 (*R*)-3-((4-(3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)benzoic acid (12p). Yield 63%, yellowish powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.90 (d, *J* = 7.5 Hz, 1H), 7.52 (m, 2H), 7.43 (m, 2H), 7.39 - 7.29 (m, 5H), 7.11 (m, 1H), 5.38 (s, 2H), 4.53 (m, 1H), 4.33 (m, 1H), 4.05 (m, 1H), 3.60 - 3.47 (m, 4H), 2.75(m, 2H), 2.60 - 2.40 (m, 2H), 2.39 - 2.23 (m, 5H). MS (ESI) *m/z* 640 (MH^+).

5.1.5.5.51 (*R*)-4-((4-(3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)benzonitrile (12q). Yield 37%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (d, *J* = 8.2 Hz, 2H), 7.57 - 7.52 (d, *J* = 7.8 Hz, 1H), 7.49 (d, *J* = 8.2 Hz, 2H), 7.44 - 7.29 (m, 5H), 7.28 - 7.18 (m, 2H), 5.41 (s, 2H), 4.37 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.21 (dd, *J* = 13.0, 9.4 Hz, 1H), 4.06 (dd, *J* = 12.9, 4.9 Hz, 1H), 3.70 - 3.53 (m, 4H), 2.72 (d, *J* = 10.5 Hz, 2H), 2.48 (t, *J* = 13.6

Hz, 2H), 2.34 (s, 3H), 2.20 (t, *J* = 10.9 Hz, 2H). MS (ESI) *m/z* 621 (MH^+).

5.1.5.5.52 (*R*)-3-(2-Amino-2-phenylethyl)-5-(4-(2,3-difluorobenzyl)piperazin-1-yl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (12r). Yield 48%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.1 Hz, 1H), 7.44 - 7.17 (m, 8H), 7.07 - 7.00 (m, 2H), 5.40 (s, 2H), 4.37 (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 - 3.46 (m, 4H), 2.77 (d, *J* = 10.5 Hz, 2H), 2.48 (t, *J* = 13.5 Hz, 2H), 2.33 (s, 3H), 2.23 (t, *J* = 10.7 Hz, 2H). MS (ESI) *m/z* 632 (MH^+).

5.1.5.5.53 (*R*)-3-(2-Amino-2-phenylethyl)-5-(4-(2-fluoro-3-nitrobenzyl)piperazin-1-yl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (12s). Yield 80%, yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (m, 2H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.43 - 7.36 (m, 3H), 7.36 - 7.28 (m, 2H), 7.25 - 7.18 (m, 3H), 5.41 (s, 4H), 4.35 (d, *J* = 4.9 Hz, 1H), 4.28 - 4.15 (m, 1H), 4.12 - 4.01 (m, 1H), 3.66 (m, 4H), 2.74 (m, 4H), 2.34 (m, 5H). MS (ESI) *m/z* 659 (MH^+).

5.1.5.5.54 (*R*)-3-((4-(3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)-2-fluorobenzonitrile (12t). Yield 80%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (m, 1H), 7.72 (t, *J* = 6.8 Hz, 1H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.49 - 7.27 (m, 7H), 7.24 - 7.15 (m, 1H), 5.57 (d, *J* = 17.4 Hz, 1H), 5.15 (d, *J* = 17.4 Hz, 1H), 4.77 (d, *J* = 10.7 Hz, 1H), 4.68 - 4.54 (m, 1H), 4.31 (s, 2H), 3.98 (d, *J* = 12.9 Hz, 1H), 3.88 - 3.64 (m, 2H), 3.47(m, 2H), 2.99 - 2.59 (m, 4H), 2.28 (s, 3H). MS (ESI) *m/z* 639 (MH^+).

5.1.5.5.55 (*R*)-2-((4-(3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)-6-fluorobenzonitrile (12u). Yield 83%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 - 7.62 (m, 2H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.50 - 7.27 (m, 7H), 7.24 - 7.15 (m, 1H), 5.57 (d, *J* = 17.4 Hz, 1H), 5.17 (d, *J* = 17.3 Hz, 1H), 4.79 (d, *J* = 10.3 Hz, 1H), 4.70 - 4.56 (m, 1H), 4.37 (q, *J* = 13.5 Hz, 2H), 4.00 (d, *J* = 11.9 Hz, 1H), 3.76 (m, 2H), 3.43 (m, 2H), 3.00 - 2.62 (m, 4H), 2.29 (s, 3H). MS (ESI) *m/z* 639 (MH^+).

5.1.5.5.56 (*R*)-3-((4-(3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)-2-fluorobenzamide (12v). Yield 25%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (m, 1H), 7.68 (m, 1H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.46 (m, 2H), 7.39 (m, 1H), 7.37 - 7.13 (m, 5H), 6.93 (m, 1H), 6.05 (m, 1H), 5.46 and 5.31 (d, *J* = 17.1 Hz, 2H), 4.56 (m, 1H), 4.40 (m, 1H), 4.09 (m, 1H), 3.75 (m, 2H), 3.63 (m, 2H), 2.79 (m, 2H), 2.64 (m, 2H), 2.45 (m, 2H), 2.38 (m, 3H). MS (ESI) *m/z* 657 (MH^+).

5.1.5.5.57 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(3-(oxazol-2-yl)benzyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (12w). Yield 15%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (s, 1H), 8.00 (d, *J* = 7.7 Hz, 1H), 7.78 -7.66 (m, 2H), 7.55 - 7.44 (m, 4H), 7.38 (m, 1H), 7.33 - 7.13 (m, 5H), 5.49 and 5.25 (d, *J* = 17.1 Hz, 2H), 4.65 (m, 1H), 4.48 (m, 1H), 4.16 - 3.92 (m, 3H), 3.71 (m, 2H), 3.24 - 3.00 (m, 2H), 2.54 - 2.41 (m, 2H), 2.24 - 2.11 (m, 2H), 2.30 (s, 3H). MS (ESI) *m/z* 663 (MH^+).

5.1.5.5.58 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(pyridin-3-

ylmethyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (13a). Yield 25%, white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 - 8.48 (m, 2H), 7.76 (d, *J* = 7.7 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.45 - 7.36 (m, 3H), 7.36 - 7.29 (m, 2H), 7.29 - 7.18 (m, 2H), 5.41 (s, 2H), 4.37 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.21 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.06 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.66 - 3.48 (m, 4H), 2.75 (m, 2H), 2.48 (m, 2H), 2.33 (s, 3H), 2.19 (m, 2H). MS (ESI) *m/z* 597 (MH^+).

5.1.5.5.59 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-((2-methylpyridin-3-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (13b). Yield 25%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (dd, *J* = 5.0, 1.5 Hz, 1H), 7.64 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.54 (d, *J* = 7.5 Hz, 1H), 7.40 (m, 3H), 7.25 - 7.20 (m, 2H), 7.08 (dd, *J* = 7.5, 5.0 Hz, 1H), 5.40 (s, 2H), 4.36 (dd, *J* = 9.5, 5.0 Hz, 1H), 4.22 (dd, *J* = 13.0, 9.5 Hz, 1H), 4.07 (dd, *J* = 13.0, 5.0 Hz, 1H), 3.58 (m, 2H), 3.46 (s, 2H), 2.73 (m, 2H), 2.57 (s, 3H), 2.47 (m, 2H), 2.34 (s, 3H), 2.19 (m, 2H). MS (ESI) *m/z* 611 (MH^+).

5.1.5.5.60 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-((6-methylpyridin-3-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (13c). Yield

62%, white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (d, *J* = 2.0 Hz, 1H), 7.63 (dd, *J* = 8.0, 2.5 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.41 - 7.11 (m, 8H), 5.40 (s, 2H), 4.36 (dd, *J* = 9.5, 5.0 Hz, 1H), 4.20 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.06 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.58 (m, 2H), 3.48 (s, 2H), 2.73 (m, 2H), 2.53 (s, 3H), 2.45 (m, 2H), 2.32 (s, 3H), 2.19 (m, 2H), 2.16 (m, 2H). MS (ESI) *m/z* 611 (MH^+).

5.1.5.5.61 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-((5-methylpyridin-3-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (13d). Yield 27%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (m, 2H), 7.56 (s, 1H), 7.53 (d, *J* = 8.0 Hz, 1H), 7.39 (m, 3H), 7.32 (m, 2H), 7.25 - 7.20 (m, 2H), 5.40 (s, 2H), 4.36 (dd, *J* = 9.5, 5.0 Hz, 1H), 4.20 (dd, *J* = 13.0, 9.5 Hz, 1H), 4.06 (dd, *J* = 13.0, 5.0 Hz, 1H), 3.58 (m, 2H), 3.48 (s, 2H), 2.74 (m, 2H), 2.53 (s, 3H), 2.47 (m, 2H), 2.32 (s, 3H), 2.19 (m, 2H). MS (ESI) *m/z* 611 (MH^+).

5.1.5.5.62 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-5-(4-((2-fluoropyridin-3-yl)methyl)piperazin-1-yl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (13e). Yield 52%, white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 (d, *J* = 5.2 Hz, 1H), 7.98 (ddd, *J* = 9.5, 7.4, 2.0 Hz, 1H), 7.54 (d, *J* = 7.8

Hz, 1H), 7.44 - 7.28 (m, 5H), 7.25 - 7.14 (m, 3H), 5.40 (s, 2H), 4.37 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.21 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.07 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.70 - 3.50 (m, 4H), 2.49 (m, 2H), 2.35 - 2.19 (m, 5H). MS (ESI) *m/z* 615 (MH^+).

5.1.5.5.63 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-5-(4-((5-fluoropyridin-3-yl)methyl)piperazin-1-yl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (13f). Yield 15%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 - 8.31 (m, 2H), 7.53 (m, 2H), 7.46 -7.28 (m, 5H), 7.28 - 7.17 (m, 2H), 5.41 (s, 2H), 4.37 (dd, *J* = 9.3, 4.9 Hz, 1H), 4.21 (dd, *J* = 13.0, 9.3 Hz, 1H), 4.07 (dd, *J* = 13.0, 4.8 Hz, 1H), 3.68 - 3.50 (m, 4H), 2.73 (d, *J* = 10.4 Hz, 1H), 2.46 (m, 2H), 2.33 (s, 3H), 2.21 (m, 2H). MS (ESI) *m/z* 615 (MH^+).

5.1.5.5.64 (*R*)-3-(2-Amino-2-phenylethyl)-5-(4-((2-chloropyridin-3-yl)methyl)piperazin-1-yl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (13g). Yield 7%, yellowish foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (dd, *J* = 4.5, 2.0 Hz, 1H), 7.99 (dd, *J* = 7.5, 2.0 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.41 (m, 3H), 7.25 - 7.32 (m, 2H), 7.25 - 7.22 (m, 3H), 5.41 (s, 2H), 4.37 (dd, *J* = 9.5, 5.0 Hz, 1H), 4.22 (dd, *J* = 13.0, 9.5 Hz, 1H), 4.07 (dd, *J* = 13.0,

5.0 Hz, 1H), 3.70 - 3.58 (m, 4H), 2.76 (m, 2H), 2.50 (m, 2H), 2.35 - 2.29 (m, 5H). MS (ESI) *m/z* 631 (MH^+).

5.1.5.5.65 (*R*)-3-(2-Amino-2-phenylethyl)-5-(4-((6-chloropyridin-3-yl)methyl)piperazin-1-yl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (13h). Yield 8%, yellowish foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.28 (d, *J* = 2.0 Hz, 1H), 7.35 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.40 (m, 3H), 7.25 - 7.20 (m, 2H), 7.08 (dd, *J* = 7.5, 5.0 Hz, 1H), 5.40 (s, 2H), 4.36 (dd, *J* = 9.5, 5.0 Hz, 1H), 4.22 (dd, *J* = 13.0, 9.5 Hz, 1H), 4.07 (dd, *J* = 13.0, 5.0 Hz, 1H), 3.58 (m, 2H), 3.46 (s, 2H), 2.73 (m, 2H), 2.57 (s, 3H), 2.47 (m, 2H), 2.34 (s, 3H), 2.19 (m, 2H). MS (ESI) *m/z* 631 (MH^+).

5.1.5.5.66 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-((2-(trifluoromethyl) pyridin-3-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (13i). Yield 59%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, *J* = 5.1 Hz, 1H), 8.34 (d, *J* = 7.9 Hz, 1H), 7.55 (d, *J* = 7.8 Hz, 1H), 7.48 (dd, *J* = 7.9, 4.5 Hz, 1H), 7.45 - 7.36 (m, 3H), 7.36 - 7.29 (m, 2H), 7.27 - 7.19 (m, 2H), 5.41 (s, 2H), 4.38 (dd, *J* = 9.4, 4.8 Hz, 1H), 4.22 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.07 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.70 (s,

2H), 3.62 (m, 2H), 2.72 (m, 2H), 2.51 (m, 2H), 2.35 - 2.26 (m, 5H). MS (ESI) *m/z* 665 (MH^+).

5.1.5.5.67 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-((6-(trifluoromethyl) pyridin-3-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (13j). Yield 57%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.63 (s, 1H), 7.96 (d, *J* = 8.4 Hz, 1H), 7.65 (d, *J* = 9.0 Hz, 1H), 7.55 (d, *J* = 8.0 Hz, 1H), 7.45 - 7.36 (m, 3H), 7.36 - 7.28 (m, 2H), 7.26 - 7.19 (m, 2H), 5.41 (s, 2H), 4.37 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.21 (dd, *J* = 13.0, 9.4 Hz, 1H), 4.06 (dd, *J* = 13.0, 5.0 Hz, 1H), 3.68 - 3.50 (m, 4H), 2.73 (m, 2H), 2.49 (m, 2H), 2.34 (s, 3H), 2.22 (m, 2H). MS (ESI) *m/z* 665 (MH^+).

5.1.5.5.68 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-((6-(trifluoromethyl) pyridin-2-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (13k). Yield 42%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (m, 2H), 7.55 (d, *J* = 7.5 Hz, 2H), 7.42 (m, 3H), 7.33 (t, *J* = 7.4 Hz, 2H), 7.25 - 7.18 (m, 2H), 5.42 (s, 2H), 4.37 (m, 1H), 4.22 (m, 1H), 4.10 (m, 1H), 3.76 (s, 2H), 3.71 - 3.56 (m, 2H), 2.76 (m, 2H), 2.58 - 2.42 (m, 2H), 2.39 - 2.26 (m, 5H). MS (ESI) *m/z* 665 (MH^+).

5.1.5.5.69 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-((2-(trifluoromethyl) pyridin-4-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (13l). Yield 73%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.65 (d, *J* = 4.9 Hz, 1H), 7.70 (s, 1H), 7.58 -7.51 (m, 2H), 7.45 - 7.29 (m, 5H), 7.28 - 7.18 (m, 2H), 5.41 (s, 2H), 4.38 (dd, *J* = 9.3, 5.0 Hz, 1H), 4.22 (dd, *J* = 12.8, 9.5 Hz, 1H), 4.07 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.74 - 3.51 (m, 4H), 2.73 (d, *J* = 10.1 Hz, 1H), 2.50 (m, 2H), 2.34 (s, 3H), 2.24 (m, 2H). MS (ESI) *m/z* 665 (MH^+).

5.1.5.5.70 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(pyrazin-2-ylmethyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (13m). Yield 74%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.79 (d, *J* = 1.5 Hz, 1H), 8.49 (m, 1H), 8.46 (d, *J* = 2.7 Hz, 1H), 7.54 (d, *J* = 7.7 Hz, 1H), 7.45 - 7.35 (m, 3H), 7.35 - 7.28 (m, 2H), 7.26 - 7.17 (m, 2H), 5.41 (s, 2H), 4.37 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.21 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.07 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.73 (s, 2H), 3.70 - 3.52 (m, 2H), 2.79 (d, *J* = 10.4 Hz, 2H), 2.50 (m, 2H), 2.39 - 2.23 (m, 5H). MS (ESI) *m/z* 670 (MH^+).

5.1.5.5.71 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(pyrimidin-4-ylmethyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (13n). Yield 61%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.11 (d, *J* = 1.4 Hz, 1H), 8.67 (d, *J* = 5.1 Hz, 1H), 7.65 (dd, *J* = 5.2, 1.3 Hz, 1H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.43 - 7.37 (m, 3H), 7.36 - 7.28 (m, 2H), 7.25 - 7.16 (m, 2H), 5.40 (s, 2H), 4.37 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.21 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.06 (dd, *J* = 13.0, 5.0 Hz, 1H), 3.74 - 3.56 (m, 2H), 3.65 (s, 2H), 2.81 - 2.69 (m, 2H), 2.58 - 2.42 (m, 2H), 2.39 - 2.26 (m, 2H), 2.34 (s, 3H). MS (ESI) *m/z* 598 (MH^+).

5.1.5.5.72 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-((2-oxo-2H-pyran-6-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (13o). Yield 42%, yellow foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, *J* = 7.9 Hz, 1H), 7.46 - 7.29 (m, 6H), 7.28 - 7.20 (m, 2H), 6.38 (d, *J* = 6.6 Hz, 1H), 6.19 (d, *J* = 9.3 Hz, 1H), 5.41 (s, 2H), 4.42 - 4.33 (m, 1H), 4.27 - 4.16 (m, 1H), 4.07 (dd, *J* = 12.9, 4.9 Hz, 1H), 3.60 (m, 2H), 3.39 (s, 2H), 2.82 (m, 2H), 2.50 (m, 2H), 2.41 - 2.27 (m, 5H). MS (ESI) *m/z* 614 (MH^+).

5.1.5.5.73 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-((2-oxo-1,2-dihydropyridin-3-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (13p). Yield 62%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (m, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.45 - 7.36 (m, 3H), 7.36 - 7.28 (m, 3H), 7.28 - 7.18 (m, 2H), 6.32 (t, *J* = 6.7 Hz, 1H), 5.41 (s, 2H), 4.39 (dd, *J* = 9.4, 4.8 Hz, 1H), 4.23 (dd, *J* = 13.0, 9.5 Hz, 1H), 4.08 (dd, *J* = 13.0, 4.9 Hz, 1H), 3.64 (q, *J* = 12.3 Hz, 2H), 3.50 (s, 2H), 2.82 (d, *J* = 10.5 Hz, 2H), 2.51 (t, *J* = 13.8 Hz, 2H), 2.39 - 2.23 (m, 5H). MS (ESI) *m/z* 613 (MH^+).

5.1.5.5.74 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-((6-oxo-1,6-dihydropyridin-3-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (13q). Yield 25%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (m, 2H), 7.35 (m, 3H), 7.34 - 7.20 (m, 5H), 6.60 (d, *J* = 10.9 Hz, 1H), 5.40 (s, 2H), 4.36 (dd, *J* = 9.5, 5.0 Hz, 1H), 4.22 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.07 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.58 (m, 2H), 3.29 (s, 2H), 2.73 (m, 2H), 2.47 (m, 2H), 2.34 (s, 3H), 2.19 (m, 2H). MS (ESI) *m/z* 613 (MH^+).

5.1.5.5.75 (*R*)-3-(2-Amino-2-phenylethyl)-5-(4-(benzo[*b*]thiophen-7-ylmethyl)piperazin-1-yl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (13r). Yield 20%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (m, 1H), 7.54 (m, 1H), 7.47 - 7.16 (m, 11H), 5.41 (s, 2H), 4.37 (dd, *J* = 9.5, 4.9 Hz, 1H), 4.23 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.06 (dd, *J* = 12.9, 4.9 Hz, 1H), 3.79 (s, 2H), 3.69 (m, 2H), 2.80 (m, 2H), 2.47 (m, 2H), 2.34 (s, 3H), 2.26 (m, 2H). MS (ESI) *m/z* 652 (MH^+).

5.1.5.5.76 (*R*)-3-(2-Amino-2-phenylethyl)-5-(4-(benzo[*c*][1,2,5]oxadiazol-4-ylmethyl)piperazin-1-yl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (13s). Yield 17%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (dd, *J* = 9.0, 0.9 Hz, 1H), 7.58 - 7.51 (m, 2H), 7.46 - 7.29 (m, 6H), 7.28 - 7.17 (m, 2H), 5.41 (s, 2H), 4.38 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.22 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.07 (dd, *J* = 12.9, 4.9 Hz,, 1H), 3.97 (d, *J* = 1.1 Hz, 2H), 3.66 (m, 2H), 2.87 (d, *J* = 10.5 Hz, 2H), 2.52 (t, *J* = 13.6 Hz, 1H), 2.42 - 2.25 (m, 5H). MS (ESI) *m/z* 638 (MH^+).

5.1.5.5.77 (*R*)-3-(2-Amino-2-phenylethyl)-5-(4-(benzo[*d*]isoxazol-7-ylmethyl)piperazin-1-yl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (13t). Yield 64%, yellowish

foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, *J* = 7.7 Hz, 1H), 7.46 - 7.12 (m, 10H), 6.80 (t, *J* = 7.6 Hz, 1H), 5.40 (s, 2H), 4.37 (dd, *J* = 9.5, 4.8 Hz, 1H), 4.20 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.05 (dd, *J* = 12.9, 4.7 Hz, 1H), 3.77 (s, 2H), 3.67 (m, 2H), 2.91 (d, *J* = 10.0 Hz, 2H), 2.58 (m, 2H), 2.44 - 2.25 (m, 5H). MS (ESI) *m/z* 637 (MH^+).

5.1.5.5.78 (*R*)-3-(2-Amino-2-phenylethyl)-5-(4-(benzofuran-4-ylmethyl)piperazin-1-yl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (13u). Yield 50%, yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (d, *J* = 2.2 Hz, 1H), 7.55 (d, *J* = 7.8 Hz, 1H), 7.45 - 7.37 (m, 4H), 7.37 - 7.28 (m, 2H), 7.26 - 7.16 (m, 4H), 7.09 (dd, *J* = 2.2, 1.0 Hz, 1H), 5.41 (s, 2H), 4.37 (dd, *J* = 9.5, 4.9 Hz, 1H), 4.22 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.06 (dd, *J* = 13.0, 4.9 Hz, 1H), 3.76 (s, 2H), 3.68 - 3.47 (m, 2H), 2.86 - 2.72 (m, 2H), 2.55 - 2.39 (m, 2H), 2.34 (s, 3H), 2.27 - 2.10 (m, 2H). MS (ESI) *m/z* 636 (MH^+).

5.1.5.5.79 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(quinolin-8-ylmethyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (13v). Yield 23%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.92 (m, 1H), 8.17 (m, 1H), 7.72 (m, 1H), 7.60 - 7.14 (m, 11H), 5.42 (s, 2H), 4.37 (dd, *J* = 9.5, 4.9 Hz, 1H), 4.23 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.06 (dd, *J* = 12.9,

4.9 Hz, 1H), 3.79 - 3.60 (m, 4H), 2.95 (m, 2H), 2.47 (m, 2H), 2.34 (s, 3H), 2.26 (m, 2H). MS (ESI) *m/z* 647 (MH^+).

5.1.5.5.80 (*R*)-3-(2-Amino-2-phenylethyl)-5-(4-(benzo[*d*]thiazol-2-ylmethyl)piperazin-1-yl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (13w). Yield 28%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (ddd, *J* = 8.1, 1.4, 0.7 Hz, 1H), 7.88 (ddd, *J* = 7.8, 1.4, 0.7 Hz, 1H), 7.55 (m, 1H), 7.48 - 7.30 (m, 7H), 7.28 - 7.18 (m, 2H), 5.41 (s, 2H), 4.39 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.23 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.08 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.97 (s, 2H), 3.69 (m, 1H), 2.93 (d, *J* = 10.4 Hz, 2H), 2.62 - 2.36 (m, 4H), 2.35 (s, 3H). MS (ESI) *m/z* 653 (MH^+).

5.1.5.5.81 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(thiazol-2-ylmethyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (14a). Yield 65%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (d, *J* = 3.3 Hz, 1H), 7.57 - 7.50 (m, 1H), 7.45 - 7.27 (m, 6H), 7.27 - 7.18 (m, 2H), 5.41 (s, 2H), 4.38 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.22 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.07 (dd, *J* = 12.9, 4.9 Hz, 1H), 3.88 (s, 2H), 3.64 (q, *J* = 12.2 Hz, 2H), 2.88 (d, *J* = 10.4 Hz, 2H), 2.50 (t, *J* = 13.5 Hz, 2H), 2.41 - 2.25 (m, 5H). MS (ESI) *m/z* 603 (MH^+).

5.1.5.5.82 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(thiazol-4-ylmethyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (14b). Yield 53%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.76 (d, *J* = 2.1 Hz, 1H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.45 - 7.35 (m, 3H), 7.35 - 7.17 (m, 5H), 5.40 (s, 2H), 4.37 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.21 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.06 (dd, *J* = 12.9, 4.9 Hz, 1H), 3.78 (d, *J* = 0.8 Hz, 2H), 3.60 (m, 2H), 2.84 (m, 2H), 2.49 (m, 2H), 2.37 - 2.19 (m, 5H). MS (ESI) *m/z* 603 (MH^+).

5.1.5.5.83 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(thiazol-5-ylmethyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (14c). Yield 73%, pink foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.75 (d, *J* = 0.7 Hz, 1H), 7.69 (q, *J* = 0.8 Hz, 1H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.44 - 7.28 (m, 5H), 7.25 - 7.17 (m, 2H), 5.40 (s, 2H), 4.37 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.21 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.06 (dd, *J* = 12.9, 4.9 Hz, 1H), 3.76 (d, *J* = 1.0 Hz, 2H), 3.58 (m, 2H), 2.80 (d, *J* = 10.3 Hz, 2H), 2.48 (t, *J* = 13.6 Hz, 2H), 2.32 (s, 3H), 2.20 (t, *J* = 10.4 Hz, 2H). MS (ESI) *m/z* 603 (MH^+).

5.1.5.5.84 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-5-(4-(isoxazol-3-ylmethyl)piperazin-1-yl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (14d). Yield 63%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (d, *J* = 1.7 Hz, 1H), 7.55 (d, *J* = 7.7 Hz, 1H), 7.50 - 7.16 (m, 7H), 6.44 (d, *J* = 1.7 Hz, 1H), 5.41 (s, 2H), 4.37 (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), 3.73 - 3.48 (m, 4H), 2.75 (m, 2H), 2.49 (t, *J* = 13.9 Hz, 2H), 2.33 (s, 3H), 2.24 (t, *J* = 10.8 Hz, 2H). MS (ESI) *m/z* 587 (MH^+).

5.1.5.5.85 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(oxazol-4-ylmethyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (14e). Yield 34%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (d, *J* = 1.0 Hz, 1H), 7.60 (q, *J* = 1.0 Hz, 1H), 7.54 (d, *J* = 7.6 Hz, 1H), 7.45 - 7.28 (m, 5H), 7.28 - 7.18 (m, 2H), 5.40 (s, 2H), 4.37 (dd, *J* = 9.4, 4.8 Hz, 1H), 4.22 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.05 (dd, *J* = 13.0, 4.9 Hz, 1H), 3.71 - 3.47 (m, 4H), 2.86 (d, *J* = 10.5 Hz, 2H), 2.50 (t, *J* = 14.6 Hz, 2H), 2.36 - 2.21 (m, 5H). MS (ESI) *m/z* 587 (MH^+).

5.1.5.5.86 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-5-(4-(furan-2-ylmethyl)piperazin-1-yl)-6-

methylpyrimidine-2,4(1*H*,3*H*)-dione (14f). Yield 45%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, *J* = 7.7 Hz, 1H), 7.44 - 7.17 (m, 8H), 6.32 (dd, *J* = 3.2, 1.9 Hz, 1H), 6.21 (d, *J* = 3.2 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), 4.05 (dd, *J* = 12.9, 4.9 Hz, 1H), 3.70 - 3.50 (m, 4H), 2.79 (m, 2H), 2.48 (m, 2H), 2.30 (s, 3H), 2.22 (m, 2H). MS (ESI) *m/z* 586 (MH^+).

5.1.5.5.87 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-5-(4-(furan-3-ylmethyl)piperazin-1-yl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (14g). Yield 53%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.0 Hz, 1H), 7.45 - 7.28 (m, 7H), 7.27 - 7.14 (m, 2H), 6.41 (d, *J* = 1.8 Hz, 1H), 5.40 (s, 2H), 4.36 (dd, *J* = 9.6, 5.0 Hz, 1H), 4.21 (dd, *J* = 12.9, 9.6 Hz, 1H), 4.06 (dd, *J* = 12.9, 4.8 Hz, 1H), 3.69 - 3.47 (m, 2H), 3.38 (s, 2H), 2.80 (m, 2H), 2.48 (m, 3H), 2.32 (s, 3H), 2.11 (m, 2H). MS (ESI) *m/z* 586 (MH^+).

5.1.5.5.88 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-(thiophen-2-ylmethyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (14h). Yield 34%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, *J* = 7.8 Hz, 1H), 7.45 - 7.28 (m, 5H), 7.28 - 7.17 (m, 3H), 6.96 - 6.88 (m, 2H), 5.40

(s, 2H), 4.37 (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), 3.74 (s, 2H), 3.60 (m, 2H), 2.84 (d, *J* = 10.5 Hz, 2H), 2.48 (t, *J* = 13.8 Hz, 2H), 2.32 (s, 3H), 2.23 (m, 2H). MS (ESI) *m/z* 602 (MH^+).

5.1.5.5.89 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-((5-methylfuran-2-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (14i). Yield 57%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, *J* = 7.8 Hz, 1H), 7.44 - 7.37 (m, 3H), 7.37 - 7.28 (m, 2H), 7.26 - 7.18 (m, 2H), 6.07 (d, *J* = 3.0 Hz, 1H), 5.89 (dd, *J* = 3.0, 1.2 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, 2H), 3.51 (s, 2H), 2.88 - 2.72 (m, 2H), 2.58 - 2.40 (m, 2H), 2.31 (s, 3H), 2.28 (d, *J* = 1.0 Hz, 3H), 2.26 - 2.12 (m, 2H). MS (ESI) *m/z* 600 (MH^+).

5.1.5.5.90 (*R*)-3-(2-Amino-2-phenylethyl)-5-(4-((5-(*tert*-butyl)furan-2-yl)methyl)piperazin-1-yl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (14j). Yield 28%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, *J* = 7.9 Hz, 1H), 7.44 - 7.27 (m, 5H), 7.27 - 7.17 (m, 2H), 6.06 (d, *J* = 3.0 Hz, 1H), 5.87 (d, *J* = 3.1 Hz, 1H), 5.40 (s, 2H), 4.36 (dd, *J* = 9.4, 4.8 Hz, 1H), 4.21 (dd, *J* = 12.9,

9.5 Hz, 1H), 4.05 (dd, *J* = 12.9, 4.9 Hz, 1H), 3.71 - 3.44 (m, 4H), 2.79 (m, 2H), 2.48 (m, 2H), 2.30 (s, 3H), 2.22 (m, 2H), 1.26 (s, 9H). MS (ESI) *m/z* 642 (MH^+).

5.1.5.5.91 (*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(1*H*,3*H*)-dione (14k). 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^+).

5.1.5.5.92 (*R*)-3-(2-Amino-2-phenylethyl)-5-(4-((5-chlorofuran-2-yl)methyl)piperazin-1-yl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methylpyrimidine-2,4(1*H*,3*H*)-dione (14l). Yield 21%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, *J* = 7.9 Hz, 1H), 7.45 - 7.28 (m, 5H), 7.28 - 7.17 (m, 2H), 6.21 (d, *J* = 3.0 Hz,1H), 6.09 (dd, *J* = 3.2, 0.8 Hz, 1H), 5.48 - 5.33 (m, 2H), 4.36 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.18 (dd, *J* = 12.9, 9.4Hz, 1H), 4.05 (dd, *J* = 12.9, 4.9Hz,, 1H), 3.70 -

3.46 (m, 4H), 2.79 (m, 2H), 2.48 (m, 2H), 2.31 (d, *J* = 0.8 Hz, 3H), 2.22 (m, 2H). MS (ESI) *m/z* 620 (MH^+).

5.1.5.5.93 (*R*)-Methyl 5-((4-(3-(2-amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)furan-2-carboxylate (14m). Yield 53%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.1 Hz, 1H), 7.45 - 7.28 (m, 5H), 7.27 - 7.18 (m, 2H), 7.14 (dd, *J* = 3.4, 1.0 Hz, 1H), 6.37 (dd, *J* = 3.4, 0.8 Hz, 1H), 5.40 (s, 2H), 4.36 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.21 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.06 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.88 (d, *J* = 1.3 Hz, 3H), 3.69 - 3.49 (m, 4H), 2.81 (d, *J* = 10.4 Hz, 2H), 2.49 (t, *J* = 13.7 Hz, 2H), 2.34 - 2.19 (m, 5H). MS (ESI) *m/z* 644 (MH^+).

5.1.5.5.94 (*R*)-5-((4-(3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)furan-2-carbonitrile (14n). Yield 13%, off-white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, *J* = 7.8 Hz, 1H), 7.44 - 7.35 (m, 3H), 7.35 - 7.28 (m, 2H), 7.26 - 7.17 (m, 2H), 7.05 (d, *J* = 3.5 Hz, 1H), 6.36 (d, *J* = 3.5 Hz, 1H), 5.40 (s, 2H), 4.36 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.21 (dd, *J* = 12.9,

9.4 Hz, 1H), 4.06 (dd, *J* = 12.9, 4.9 Hz, 1H), 3.68 - 3.49 (m, 4H), 2.78 (m, 2H), 2.49 (m, 2H), 2.34 - 2.18 (m, 5H). MS (ESI) *m/z* 611 (MH^+).

5.1.5.5.95 (*R*)-5-((4-(3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)furan-2-carboxamide (14o). Yield 67%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.0 Hz, 1H), 7.44 - 7.35 (m, 3H), 7.35 - 7.28 (m, 2H), 7.26 - 7.17 (m, 2H), 7.09 (d, *J* = 3.4 Hz, 1H), 6.66 (bs, 1H), 6.33 (d, *J* = 3.4 Hz, 1H), 6.51 (bs, 1H), 5.40 (s, 2H), 4.36 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.21 (dd, *J* = 12.9, 9.4 Hz, 1H), 4.06 (dd, *J* = 12.9, 5.0 Hz, 1H), 3.69 - 3.47 (m, 4H), 2.79 (d, *J* = 10.4 Hz, 2H), 2.49 (m, 2H), 2.32 (m, 3H), 2.22 (m, 2H). MS (ESI) *m/z* 629 (MH^+).

5.1.5.5.96 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-((4-(trifluoromethyl) furan-2-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (14p). Yield 21%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.11 (d, *J* = 1.5 Hz, 1H), 8.67 (d, *J* = 5.2 Hz, 1H), 7.67 - 7.62 (m, 1H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.44 - 7.27 (m, 5H), 7.24 - 7.17 (m, 1H), 5.40 (s, 2H), 4.37 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.21 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.06 (dd, *J* = 13.0, 5.0 Hz, 1H), 3.65 (s, 2H), 3.73 - 3.56 (m, 2H), 2.82 - 2.69 (m,

2H), 2.60 - 2.43 (m, 2H), 2.34 (s, 3H), 2.38 - 2.25 (m, 2H). MS (ESI) *m/z* 654 (MH^+).

5.1.5.5.97 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-((5-methyl-2-(trifluoromethyl)furan-3-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (14q). Yield 68%, white solid, mp = 117 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.0 Hz, 1H), 7.44 -7.28 (m, 5H), 7.27 - 7.17 (m, 2H), 6.19 (s, 1H), 5.40 (s, 2H), 4.37 (dd, *J* = 9.3, 4.9 Hz, 1H), 4.21 (dd, *J* = 13.0, 9.5 Hz, 1H), 4.06 (dd, *J* = 12.9, 4.9 Hz, 1H), 3.56 (m, 2H), 2.42 (d, *J* = 1.8 Hz, 2H), 2.74 (m, 2H), 2.48 (m, 2H), 2.32 (s, 3H), 2.30 (t, *J* = 1.1 Hz, 3H), 2.15(m, 2H). MS (ESI) *m/z* 668 (MH^+).

5.1.5.5.98 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-((5-(trifluoromethyl) thiophen-2-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (14r). Yield 55%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, *J* = 9.0 Hz, 1H), 7.44 - 7.37 (m, 3H), 7.37 - 7.29 (m, 2H), 7.28 - 7.17 (m, 2H), 6.86 and 6.65 (m, 2H), 5.40 (s, 2H), 4.38 (dd, *J* = 9.4, 4.8 Hz, 1H), 4.22 (dd, *J* = 13.0, 9.6 Hz, 1H), 4.10 - 4.02 (dd, *J* = 13.0, 9.4Hz,

1H), 3.72 (s, 2H), 3.69 - 3.50 (m, 2H), 2.84 (d, *J* = 10.3 Hz, 2H), 2.49 (m, 2H), 2.33 (s, 3H), 2.22 (m, 2H). MS (ESI) *m/z* 670 (MH^+).

5.1.5.5.99 (*R*)-3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-5-(4-((5-(trifluoromethyl)-1,3,4-oxadiazol-2-yl)methyl)piperazin-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (14s). Yield 41%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, *J* = 7.9 Hz, 1H), 7.45 - 7.36 (m, 3H), 7.32 (t, *J* = 7.4 Hz, 2H), 7.25 - 7.17 (m, 2H), 5.40 (s, 1H), 4.36 (dd, *J* = 9.5, 5.0 Hz, 1H), 4.20 (dd, *J* = 12.9, 9.5 Hz, 1H), 4.06 (dd, *J* = 12.9, 5.1 Hz, 1H), 3.95 (s, 2H), 3.59 (m, 2H), 2.82 (m, 2H), 2.59 - 2.33 (m, 4H), 2.31 (s, 3H). MS (ESI) *m/z* 656 (MH^+).

5.1.5.6 (*R*)-3-((4-(3-(2-((*tert*-Butoxycarbonyl)amino)-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)-benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)benzoic acid (15). To a solution of compound **9** (363 mg, 0.586 mmol) in dichloromethane (3 mL) were added triethylamine (0.18 ml, 1.29 mmol) and 3-(bromomethyl)benzoic acid methyl ester (152 mg, 0.644 mmol), followed by stirring at room temperature for 1 h. The reaction mixture was diluted with dichloromethane and washed

with saturated sodium bicarbonate solution. The organic layer was dried over sodium sulfate, filtered and concentrated. The residue was purified by column chromatography on silica gel using hexane/EtOAc (2:1) as eluent to yield the corresponding methyl benzoate (394 mg, 89%) as a yellowish solid. The ester (394 mg, 0.522 mmol) dissolved in methanol (5 mL) was treated with 1 *N* NaOH (3.1 mL, 3.14 mmol), followed by stirring at 60 °C for 1.5 h. The mixture was cooled to room temperature, concentrated *in vacuo*, and then acidified to pH 3 with aqueous 0.2 *N* HCl. After extraction with ethyl acetate (5 mL), the organic layer was dried over sodium sulfate and filtered to obtain crude compound **15** (443 mg) as a yellowish solid, which was used for the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (s, 1H), 8.03 (d, *J* = 7.7 Hz, 1H), 7.54 (t, *J* = 8.4 Hz, 2H), 7.44 - 7.28 (m, 6H), 7.26 - 7.16 (m, 2H), 5.74 and 5.67 (m, 1H), 5.52 - 5.31 (m, 2H), 5.08 - 4.79 (m, 1H), 4.27 (m, 1H), 4.12 (q, *J* = 7.2 Hz, 2H), 4.09 - 3.98 (m, 1H), 3.87 - 3.73 (m, 4H), 3.02 (m, 2H), 2.66 (m, 4H), 2.53 (m, 2H), 2.35 (s, 3H), 1.97 (m, 2H), 1.35 - 1.21 (m, 9H), 1.26 (t, *J* = 7.2 Hz, 3H).

5.1.6 General Procedure for Amide Coupling (16). To a solution of compound **15** (1 mmol) and amino ethyl ester hydrochloride (2 mmol) in DMF (10 mL) were added *N,N*-diisopropylethylamine (4 mmol) and HBTU (3 mmol). The reaction mixture was stirred at room temperature for 6 h. The mixture was diluted in EtOAc and washed with saturated NH_4Cl and saturated sodium bicarbonate solution, respectively. The organic layer was dried over sodium sulfate, filtered and concentrated. The residue was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (30:1) as eluent.

5.1.6.1 (*R*)-Methyl 2-(3-((4-(3-(2-((*tert*-butoxycarbonyl)amino)-2-phenylethyl)-1-(2-fluoro-6-(trifluoro-methyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)-benzamido)acetate (16a). Yield 95%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.74 (dt, *J* = 7.7, 1.5 Hz, 1H), 7.55 (d, *J* = 7.8 Hz, 2H), 7.45 - 7.28 (m, 6H), 7.26 - 7.15 (m, 2H), 6.88 (m, 1H), 5.78 and 5.67 (m, 1H), 5.55 - 5.27 (m, 2H), 5.02 and 4.88 (m, 1H), 4.27 (m, 2H), 4.21 (m, 1H), 4.04 (d, *J* = 13.3, 3.6 Hz, 1H), 3.81 (s, 3H), 3.70 - 3.57 (m, 4H), 2.83 (m, 2H), 2.51 (m, 2H), 2.34 (s, 3H), 2.25 (m, 2H), 1.34 and 1.19 (s, 9H).

5.1.6.2 (*R*)-Ethyl 3-(3-((4-(3-(2-((tert-butoxycarbonyl)amino)-2-phenylethyl)-1-(2-fluoro-6-(trifluoro-methyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)-benzamido)propanoate (16b). Yield 99%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.66 (d, *J* = 7.7 Hz, 1H), 7.55 (d, *J* = 7.7 Hz, 2H), 7.45 - 7.28 (m, 6H), 7.26 - 7.16 (m, 2H), 6.91 (t, *J* = 6.0 Hz, 1H), 5.80 (m, 1H), 5.62 (m, 1H), 5.55 - 5.25 (m, 2H), 5.02 (m, 1H), 4.87 (m, 1H), 4.27 (t, *J* = 12.3 Hz, 1H), 4.18 (q, *J* = 6.9 Hz, 2H), 4.04 (dd, *J* = 13.4, 3.6Hz, 1H), 3.74 (q, *J* = 6.0 Hz, 2H), 3.69 - 3.55 (m, 4H), 2.80 (m, 2H), 2.67 (t, *J* = 6.0 Hz, 2H), 2.51 (m, 2H), 2.35 (s, 3H), 2.23 (m, 2H), 1.35 (s, 9H), 1.20 (s, 9H), 1.28 (td, *J* = 7.0, 0.5 Hz, 3H).

5.1.6.3 (*R*)-Ethyl 4-(3-((4-(3-(2-((tert-butoxycarbonyl)amino)-2-phenylethyl)-1-(2-fluoro-6-(trifluoro-methyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)-benzamido)butanoate (16c). Yield 99%, yellowish foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 1.7 Hz, 1H), 7.72 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.58 - 7.49 (m, 2H), 7.45 - 7.29 (m, 6H), 7.27 - 7.16 (m, 2H), 6.76 (m, 1H), 5.79 (m, 1H), 5.67 (m, 1H), 5.54 - 5.27 (m, 2H), 5.02 (m, 1H), 4.87 (m, 1H), 4.27 (t, *J* = 12.2 Hz, 1H), 4.13 (q, *J* = 7.2 Hz, 2H),

4.09 - 3.98 (m, 1H), 3.71 - 3.56 (m, 4H), 3.52 (q, *J* = 6.6 Hz, 2H), 3.28 (td, *J* = 7.0, 5.4 Hz, 1H), 2.85 (m, 2H), 2.53 (m, 2H), 2.45 (t, *J* = 6.9 Hz, 2H), 2.38 - 2.25 (m, 5H), 1.97 (m, 2H), 1.35 (m, 9H), 1.19 (m, 9H), 1.25 (t, *J* = 7.2 Hz, 3H).

5.1.7 General Procedure for Hydrolysis and *N*-Boc Deprotection (17) (for Compounds 17a-c). A mixture of compound **16** (1 mmol) and 1 *N* NaOH (6 mmol) in ethanol (10 mL) was stirred at room temperature for 30 min. The solution was diluted by water and acidified by 0.2 *N* HCl. The mixture was extracted with mixed solvent of dichloromethane and methanol, followed by drying over sodium sulfate and filtration. After concentration, the residue was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (20:1) as eluent to give an acid intermediate. This acid dissolved in dichloromethane (10 mL) was treated with TFA (1.5 ml) at 0 °C and then stirred at room temperature for 30 min. The mixture was neutralized with saturated sodium bicarbonate solution before extraction with mixed solvent of EtOAc and methanol, which was then concentrated. The residue was purified by preparative LC (MeOH:distilled water containing 0.1% formic acid).

5.1.7.1 (*R*)-2-(3-((4-(3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)benzamido)acetic acid (17a). Yield 35%, white powder. ^{1}H NMR (300 MHz, CD_3OD) δ 8.34 (s, 1H), 7.97 (t, *J* = 1.8 Hz, 1H), 7.88 (dt, *J* = 7.6, 1.4 Hz, 1H), 7.61 (m, 2H), 7.51 (m, 2H), 7.42 - 7.31 (m, 6H), 5.41 (m, 2H), 4.64 (t, *J* = 7.0 Hz, 1H), 4.36 (d, *J* = 7.0 Hz, 2H), 4.03 (m, 4H), 3.64 (m, 2H), 3.10 (m, 2H), 2.81 - 2.51 (m, 4H), 2.42 (s, 3H). MS (ESI) *m/z* 697 (MH^+).

5.1.7.2 (*R*)-3-(3-((4-(3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)benzamido)propanoic acid (17b). Yield 21%, off-white powder. ^{1}H NMR (300 MHz, CD_3OD) δ 8.37 (s, 1H), 7.91 (s, 1H), 7.82 (dt, *J* = 7.7, 1.5 Hz, 1H), 7.62 (m, 2H), 7.51 (m, 2H), 7.42 - 7.30 (m, 6H), 5.37 (m, 2H), 4.65 (t, *J* = 7.0 Hz, 1H), 4.36 (m, 2H), 4.05 (s, 2H), 3.72-57 (m, 4H), 3.13 (m, 2H), 2.84 - 2.54 (m, 6H), 2.41 (s, 3H). MS (ESI) *m/z* 711 (MH^+).

5.1.7.3 (*R*)-4-(3-((4-(3-(2-Amino-2-phenylethyl)-1-(2-fluoro-6-(trifluoromethyl)benzyl)-6-methyl-2,4-dioxo-1,2,3,4-

tetrahydropyrimidin-5-yl)piperazin-1-yl)methyl)benzamido)butanoic acid (17c). Yield 26%, white powder. ^{1}H NMR (300 MHz, CD_3OD) δ 8.38 (s, 1H), 7.89 (s, 1H), 7.83 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.62 (d, *J* = 8.1Hz, 2H), 7.52 (q, *J* = 8.1Hz, 2H), 7.40 - 7.32 (m, 6H), 5.37 (m, 2H), 4.65 (m, 1H), 4.36 (m, 2H), 4.01 (m, 2H), 3.62 (m, 2H), 3.44 (t, *J* = 6.8 Hz, 2H), 3.11 (m, 2H), 2.77 - 2.56 (m, 4H), 2.75 - 2.61 (m, 2H), 2.42 - 2.37 (m, 5H), 1.93 (p, *J* = 7.0 Hz, 2H). MS (ESI) m/z 725 (MH^+).

5.1.8 General Procedure for Alkylation (18, 19, 20).

5.1.8.1 Method A (for Compounds 18a, 18b, 18c, 19a, 19b). To a solution of compound **12** or **14** (1 mmol) in acetonitrile (2 mL) were added *N,N*-diisopropylethylamine (3 mmol) and 4-bromobutyric acid ethyl ester or diethyl (3-bromopropyl)phosphonate (1 mmol). After the reaction mixture was stirred at 95 °C for 12 h, it was cooled to ambient temperature, diluted with dichloromethane and washed with saturated sodium bicarbonate solution. After the organic layer was concentrated, the residue was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (30:1) as eluent to obtain the ester compound. To a solution of the ester (1 mmol) in ethanol (3.5 mL)/water (2.5 mL) was

slowly added 1 *N* NaOH (10 mmol). The mixture was heated to 60 °C and stirred 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 column chromatography on silica gel using CH_2Cl_2/MeOH (10:1) as eluent. For compound **19b**, to a solution of the above intermediate ester compound (1 mmol) in dichloroethane (100 mL) was added trimethylsilyl bromide (6 mmol). The mixture was heated to 45 °C and stirred for 5 h. It was cooled to ambient temperature and concentrated under reduced pressure. The residue was diluted with ethylacetate/water (1:1) and stirred for 30 min. The organic layer was separated and filtered. The resulting solution was lyophilized and purified by preparative LC using MeOH/ H_2O containing 0.1% formic acid (50:50).

5.1.8.1.1 (*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(6*H*)-yl)-1-phenylethyl)amino)-butanoic acid (18a). Compound **18a** was prepared by following the procedure described above (Method A), and was solidified by trituration with isopropanol-heptane.

Yield 46%, white solid, mp = 90 °C, purity 99%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, *J* = 8.1 Hz, 1H), 7.42 - 7.27 (m, 6H), 7.20 (m, 1H), 6.72 (d, *J* = 3.2 Hz, 1H), 6.30 (m, 1H), 5.39 (s, 2H), 4.43 (m, 2H), 4.29 (m, 1H), 4.04 (m, 1H), 3.66 - 3.49 (m, 4H), 2.80 (m, 1H), 2.73 - 2.37 (m, 6H), 2.35 (s, 3H), 2.25 (m, 2H), 1.64 (m, 2H). HRMS (FAB) calcd for $C_{35}H_{36}F_7N_5O_5$ (M + H^+): 740.2605 Found: 740.2682. The hydrochloride salt of compound **18a** was prepared as follows: a mixture of compound **18a** (1 g, 1.35 mmol) in dichloromethane was slowly added 5-6 *N* HCl in isopropanol (595 μL, 2.97 mmol) and then stirred for 30 min. The resultant solution was added dropwise to methyl *t*-butyl ether (30 mL) and stirred for 30 min. The suspension was filtered and washed with methyl *t*-butyl ether. The filtered solid was dried *in vacuo* at 40 °C. Yield 84%, white solid, mp = 159 °C, purity 99.8%. ^{1}H NMR (300MHz, $CDCl_3$) δ 7.63 (m, 2H), 7.50 (d, *J* = 7.9 Hz, 1H), 7.44 - 7.31 (m, 4H), 7.17 (m, 1H), 7.05 (m, 1H), 6.83 (m, 1H), 5.47 (m, 1H), 5.23 (m, 1H), 4.77 (m, 1H), 4.60 (m, 1H), 4.46 (m, 2H), 4.24 (m, 1H), 4.03 (m, 2H), 3.57 (m, 1H), 3.43 (m, 1H), 3.24 - 2.71 (m, 6H), 2.60 (m, 1H), 2.42 (m, 1H), 2.32 (s, 3H), 1.97 (m, 2H). AQF-IC (chloride) 7.2%.

5.1.8.1.2 (*R*)-4-((2-(3-(2-Fluoro-6-(trifluoromethyl)benzyl)-4-methyl-5-(4-(3-nitrobenzyl)piperazin-1-yl)-2,6-dioxo-2,3-dihydropyrimidin-1(6*H*)-yl)-1-phenylethyl)amino)butanoic acid (18b). Yield 30%, white powder, mp = 89 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (t, *J* = 2.0 Hz, 1H), 8.10 (ddd, *J* = 8.2, 2.4, 1.1 Hz, 1H), 7.74 (dt, *J* = 7.6, 1.3 Hz, 1H), 7.53 (d, *J* = 7.9 Hz, 1H), 7.47 (t, *J* = 7.9 Hz, 1H), 7.43 - 7.27 (m, 6H), 7.25 - 7.16 (m, 1H), 5.40 (s, 2H), 4.41 (dd, *J* = 13.1, 10.3 Hz, 1H), 4.29 (dd, *J* = 10.2, 4.1 Hz, 1H), 4.04 (dd, *J* = 13.1, 4.1 Hz, 1H), 3.61 - 3.55(m, 4H), 2.77 - 2.34 (m, 11H), 2.23 (t, *J* = 10.8 Hz, 2H), 1.66 (m, 2H). MS (ESI) m/z 724 (MH^+).

5.1.8.1.3 (*R*)-4-((2-(3-(2-Fluoro-6-(trifluoromethyl)benzyl)-4-methyl-2,6-dioxo-5-(4-(3-(trifluoromethyl)-benzyl)piperazin-1-yl)-2,3-dihydropyrimidin-1(6*H*)-yl)-1-phenylethyl)amino)butanoic acid (18c). Yield 35%, white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.65 (s, 1H), 7.60 (d, *J* = 7.7 Hz, 1H), 7.55 - 7.48 (m, 2H), 7.47 - 7.39 (m, 3H), 7.39 - 7.27(m, 4H), 7.18 (m, 1H), 5.33 (m, 2H), 4.50 (m, 2H), 4.22 (m, 1H), 3.83 (q, *J* = 13.3 Hz, 2H), 3.63 (t, *J* = 11.8 Hz, 2H), 3.02 (m, 2H), 2.87 - 2.76 (m, 1H), 2.75 - 2.61 (m, 2H), 2.66 - 2.34 (m, 5H), 2.32 (s, 3H) 1.76 (m, 2H). MS (ESI) m/z 750 (MH^+).

5.1.8.1.4 Ethyl hydrogen (3-(((R)-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(6*H*)-yl)-1-phenylethyl)amino) propyl)phosphonate (19a). Yield 13%, white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 - 7.44 (m, 3H), 7.37 (td, *J* = 8.1, 5.0 Hz, 1H), 7.30 - 7.12 (m, 4H), 6.72 (dd, *J* = 3.3, 1.3 Hz, 1H), 6.29 (d, *J* = 3.3 Hz, 1H), 5.26 (s, 2H), 4.78 - 4.39 (m, 3H), 4.02 - 3.85 (m, 2H), 3.58 (s, 2H), 3.53 - 3.30 (m, 2H), 3.10 (q, *J* = 7.3 Hz, 2H), 2.87 - 2.64 (m, 4H), 2.51 - 2.37 (m, 1H), 2.33 - 1.93 (m, 4H), 2.22 (s, 3H), 1.74 - 1.45 (m, 2H), 1.39 (t, *J* = 7.3 Hz, 3H). MS (ESI) *m/z* 804 (MH^+).

5.1.8.1.5 (*R*)-(3-((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(6*H*)-yl)-1-phenylethyl)amino)propyl)phosphonic acid (19b). Yield 30%, white solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 7.54 (s, 1H), 6.80 (d, *J* = 8.2 Hz, 1H), 6.70 (td, *J* = 8.0, 4.9 Hz, 1H), 6.58 (s, 5H), 6.56 - 6.48 (m, 1H), 6.14 (dt, *J* = 3.4, 1.3 Hz, 1H), 5.72 (d, *J* = 3.4 Hz, 1H), 4.54 (s, 2H), 3.81 (t, *J* = 7.1 Hz, 1H), 3.70 (dd, *J* = 13.3, 6.9 Hz, 1H), 3.57 (dd, *J* = 13.3, 7.3 Hz, 1H), 2.92 (s, 2H), 2.85 - 2.58 (m, 2H), 2.19 - 1.94 (m,

4H), 1.78 (d, *J* = 11.2 Hz, 1H), 1.66 - 1.46 (m, 2H), 1.55 (s, 3H), 1.19 - 0.98 (m, 2H), 0.91 - 0.72 (m, 2H). MS (ESI) m/z 776 (MH^+).

5.1.8.2 Method B (for Compound 20). A mixture of compound **14k** (1 mmol) and 1,3-propanone (2 mmol) in acetonitrile (20 mL) were stirred at 90 °C for 12 h. After the reaction mixture was cooled to room temperature and concentrated under reduced pressure, the residue was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (8:1) as eluent.

5.1.8.2.1 (*R*)-3-((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(6*H*)-yl)-1-phenylethyl)amino)-propane-1-sulfonic acid (20). Yield 93%, white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 - 7.47 (m, 3H), 7.46 - 7.30 (m, 4H), 7.18 (dd, *J* = 11.6, 8.2 Hz, 1H), 6.72 (dd, *J* = 3.0, 1.4 Hz, 1H), 6.25 (d, *J* = 3.4 Hz, 1H), 5.39 (m, 2H), 4.79 (m, 2H), 4.09 (d, *J* = 11.1 Hz, 1H), 3.61 - 3.36 (m, 4H), 3.21 (m, 1H), 3.04 (m, 2H), 2.82 (m, 1H), 2.74 - 2.37 (m, 4H), 2.33 (s, 3H), 2.23 - 2.03 (m, 4H). MS (ESI) *m/z* 776 (MH^+).

5.1.8.2.2 1-(2-Fluoro-6-trifluoromethyl-benzyl)-6-methyl-5-piperazin-1-yl-1*H*-pyrimidine-2,4-dione (22). Compound **21** (1.50 g, 3.94 mmol), 1-benzylpiperazine (5.5 mL, 31.5 mmol) and acetonitrile (1 mL) were combined in a microwave vessel. The mixture was placed under microwave irradiation at 120 °C for 1.5 h. After concentration, the residue was purified by 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). To a solution of the above compound (1.48 g, 3.11 mmol) in 10 mL of methanol/dichloromethane (1:1) was hydrogenated over 10% Pd/C (280 mg) under atmospheric pressure at room temperature for 5 h. After celite filtration, the filtrate was concentrated. The residue was purified using amine silica gel chromatography (CH_2Cl_2/MeOH, 10:1) to give the title product **22** as a white solid (934 mg, 78%). ^{1}H 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).

5.1.8.3 1-(2-Fluoro-6-trifluoromethyl-benzyl)-6-methyl-5-[4-(3-nitro-benzyl)-piperazin-1-yl-1*H*-pyrimidine-2,4-dione (23). A solution of compound **22** (1.93 g, 5.00 mmol), *N,N*-diisopropylethylamine (4.5 mL, 25.0 mmol), and 3-nitro-benzyl bromide (1.60 g, 7.50 mmol) in 1,2-dichloroethane (50 mL) was stirred at 50 °C under a nitrogen atmosphere for 2 h, and then cooled to room temperature. The mixture was washed with saturated ammonium chloride solution, and concentrated *in vacuo*. The residue was purified using silica gel chromatography (CH_2Cl_2/MeOH, 30:1) to afford the title product **23** as a yellowish foam (2.30 g, 77%). ^{1}H 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).

5.1.8.4 (*R*)-*tert*-Butyl (2-(3-(2-fluoro-6-(trifluoromethyl)benzyl)-4-methyl-5-(4-(3-nitrobenzyl)piperaz-in-1-yl)-2,6-dioxo-2,3-dihydropyrimidin-1(6*H*)-yl)-1-(2-methoxyphenyl)ethyl)carbamate (25). To a solution of [(*R*)-2-hydroxy-1-(2-methoxy-phenyl)-ethyl]-carbamic acid *tert*-butyl ester (493 mg, 1.84 mmol) in dichloromethane

(5 mL) were added triethylamine (310 μL, 2.23 mmol) and methanesulfonyl chloride (155 μL, 1.99 mmol). After stirring at room temperature for 20 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 **24**. To a solution of compound **24** in DMF (5 mL) was added **23** (385 mg, 0.738 mmol) and potassium carbonate (510 mg, 3.69 mmol) and stirred at 70 °C under a nitrogen atmosphere for 12 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate, and washed with saturated ammonium chloride solution. The organic layer was separated and concentrated, and the residue was purified using silica gel chromatography (Hexane/EtOAc, 2:1) to afford the title compound **25** as an off-white foam (351 mg, 62%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (t, *J* = 1.9 Hz, 1H), 8.15 - 8.07 (m, 1H), 7.78 (d, *J* = 7.8 Hz, 1H), 7.51 (m, 2H), 7.44 - 7.33 (m, 1H), 7.26 - 7.15 (m, 3H), 6.93 - 6.82 (m, 2H), 5.93 (d, *J* = 9.2 Hz, 1H), 5.40 (m, 2H), 5.25 (td, *J* = 9.8, 4.4 Hz, 1H), 4.62 – 4.45 (m, 1H), 4.06 (dd, *J* = 12.8, 4.2 Hz, 1H), 3.91 (s, 3H), 3.72 - 3.54 (m, 4H), 2.75 (d, *J* = 10.4 Hz, 2H), 2.48 (d, *J* = 11.2 Hz, 2H), 2.31 (s, 3H), 2.22 (m, 2H), 1.35 (s, 9H).

5.1.8.5 (*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(1*H*,3*H*)-dione (26). A solution of compound **25** (250 mg, 0.32 mmol) in anhydrous dichloroethane (5 mL) was chilled to -78 °C and treated with 1 M boron tribromide in dichloromethane (1.6 mL, 1.6 mmol) slowly under a nitrogen atmosphere. The mixture was then slowly heated to 40 °C and stirred for 48 h. Methanol (5 mL) was added to the reaction mixture and then stirred at room temperature for 20 min. After concentration, the residue was diluted in dichloromethane and washed with saturated sodium bicarbonate 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). 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^+).

5.1.8.6 (*R*)-*tert*-Butyl (2-(3-(2-fluoro-6-(trifluoromethyl)benzyl)-4-methyl-5-(4-(3-nitrobenzyl)piperaz-in-1-yl)-2,6-dioxo-2,3-dihydropyrimidin-1(6*H*)-yl)-1-(2-hydroxyphenyl)ethyl)carbamate (27). A solution of compound **26** (168 mg, 0.26 mmol) in anhydrous dichloroethane (5 mL) was treated with triethylamine (62.5 µl, 0.48 mmol) and di-*tert*-butyl-dicarbonate (75 µl, 0.35 mmol) and stirred at 40 °C for 3 h. The mixture was cooled to room temperature, and washed with saturated ammonium chloride solution. After concentration of the organic layer, the residue was purified using silica gel chromatography (Hexane/EtOAc, 2:1) to afford the title compound **27** as an off-white foam (130 mg, 67%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (m, 1H), 8.12 (d, *J* = 8.2 Hz, 1H), 7.79 (d, *J* = 7.6 Hz, 1H), 7.58 - 7.46 (m, 2H), 7.41 (m, 1H), 7.25 - 7.14 (m, 3H), 6.96 - 6.83 (m, 2H), 5.68 (m, 1H), 5.43 (m, 1H), 5.11 (m, 1H), 4.53 (m, 1H), 3.97 (m, 1H), 3.72 - 3.55 (m, 4H), 2.76 (m, 2H), 2.50 (m, 2H), 2.37 (s, 3H), 2.30 (s, 3H), 2.22 (m, 2H), 1.35 (s, 9H).

5.1.9 General Procedure for O-Alkylation and Deprotection (for compounds 28a, 28b). A mixture of compound **27** (1 mmol), potassium carbonate (2 mmol), and bromo-alkyl *tert*-butyl ester (2 mmol) in DMF (10 mL) was stirred at 70 °C for 4 h, and then cooled to ambient temperature. The reaction mixture was diluted with dichloromethane and washed with saturated sodium bicarbonate solution. The organic layer was separated and concentrated, and the residue was purified using silica gel chromatography (hexane/EtOAc, 3:2) to give the ester. To a solution of the ester compound dissolved in dichloromethane (20 mL) was added trifluoroacetic acid (5 mL), followed by stirring at room temperature for 5 h. The reaction mixture was neutralized with saturated sodium bicarbonate solution and extracted with dichloromethane. After concentration of the organic layer, the residue was purified using silica gel chromatography (CH_2Cl_2/MeOH, 20:1- 10:1).

5.1.9.1 (*R*)-2-(2-(1-Amino-2-(3-(2-fluoro-6-(trifluoromethyl)benzyl)-4-methyl-5-(4-(3-nitrobenzyl)-piperazin-1-yl)-2,6-dioxo-2,3-dihydropyrimidin-1(6*H*)-yl)ethyl)phenoxy)acetic acid (28a). Yield 38%, white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (s, 1H), 8.08 (d, *J* = 8.1 Hz, 1H), 7.74 (d, *J* = 7.6 Hz, 1H), 7.51 - 7.43 (m, 2H), 7.39 -

7.30 (m, 1H), 7.23 - 7.05 (m, 3H), 6.82 - 6.71 (m, 2H), 5.38 - 5.28 (m, 2H), 4.70 - 4.55 (m, 1H), 4.47 - 4.26 (m, 4H), 3.53 (s, 2H), 3.50 - 3.42 (m, 2H), 2.78 - 2.66 (m, 2H), 2.51 - 2.38 (m, 2H), 2.30 (s, 3H), 2.23 - 2.14 (m, 2H). MS (ESI) m/z 715 (MH^+).

5.1.9.2 (*R*)-4-(2-(1-Amino-2-(3-(2-fluoro-6-(trifluoromethyl)benzyl)-4-methyl-5-(4-(3-nitrobenzyl)-piperazin-1-yl)-2,6-dioxo-2,3-dihydropyrimidin-1(6*H*)-yl)ethyl)phenoxy)butanoic acid (28b). Yield 39%, white solid. ^1H NMR (300 MHz, $CDCl_3$) δ 8.17 - 8.14 (m, 1H), 8.12 - 8.07 (m, 1H), 7.75 (d, *J* = 7.7 Hz, 1H), 7.49 (t, *J* = 8.0 Hz, 2H), 7.42 - 7.32 (m, 1H), 7.23 - 7.10 (m, 3H), 6.79 (t, *J* = 8.3 Hz, 2H), 5.40 - 5.21 (m, 2H), 4.65 - 4.48 (m, 2H), 4.28 (d, *J* = 10.9 Hz, 1H), 4.06 - 3.89 (m, 2H), 3.60 (s, 2H), 3.56 - 3.42 (m, 2H), 2.79 - 2.68 (m, 2H), 2.50 - 2.30 (m, 3H), 2.25 (s, 3H), 2.23 - 1.89 (m, 5H). MS (ESI) m/z 743 (MH^+).

5.2 Biological Study.

5.2.1 GnRH Receptor Competitive Binding Assay.

Competitive binding experiments were performed with [^{125}I]D-Trp^6-LHRH (0.2 nM) on membrane preparations from CHO-K1 cells stably transfected with human GnRH receptor (4 ng/μL, PerkinElmer) or

HEK293 cells transiently transfected with monkey or rat GnRH receptor (in house preparation) as described previously.[26] Human

$$\% \text{ Inhibition} = \left(1 - \frac{(\text{Compound-NSB})}{(\text{TB-NSB})}\right) \times 100$$

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 hour at 27 °C. Reaction mixtures were then filtered through FilterMate harvester (PerkinElmer cat #D961962) onto the filter paper (Filtermat A, PerkinElmer cat #1450-421). The filter was allowed to completely dry and solid scintillant (Meltilex A, PerkinElmer cat#1450-441) was added before counting radioactivity with Microbeta2 TriLux (4PM tubes/2 detector, PerkinElmer cat#2450-0020). Counts from each well were converted to % inhibition value according to the following formula:

Converted % inhibition values of each compound were analyzed using Prism (GraphPad Software, Inc.) fitting to a sigmoidal dose-response curve with variable slope.

5.2.2 Inhibition of NFAT reporter activity.

Antagonistic effect of test compounds on NFAT activation was assayed using luciferase assay according to the previously reported protocol.[26] HEK293 cells were stably transfected with pcDNA3.1-human-GnRHR and pGL4-NFAT promoter AP-1-luc, or transiently transfected with pcDNA3.1-monkey/rat-GnRHR and pGL4-NFAT promoter AP-1-luc. The transfected cells were pretreated with test compounds for an hour. Cells were subsequently stimulated with 20 nM GnRH (1 nM for Table 6) and incubated for 6 h at 37 °C. Luciferase activity as a result of NFAT promoter activation was determined afterwards from cell lysates. Inhibition of reporter gene activity was calculated as percentage of maximal, agonist-induced Luc activity. The experiments were performed in triplicate. Data were analyzed using Prism software (GraphPad Software, Inc., San Diego, CA, USA) fitting to a sigmoidal dose-response curve with variable slope.

5.2.3 Calcium flux assay.

In vitro calcium flux assay in human GnRH receptor-expressing Chem-1 cell line induced by LHRH was performed by Merck Millipore (Darmstadt, Germany). Compounds were plated in an eight-point, four-fold serial dilution series in duplicate with a top concentration of 10 μM.

Assay was read for 90 and 180 seconds using the FLIPR^{TETRA}. Maximum fluorescence values were exported, and percent activation and inhibition values were calculated.

5.2.4 Inhibition of ERK phosphorylation.

HEK293 cells transfected with pcDNA3.1-human GnRHR (PerkinElmer, cat #ES-600-M400UA) were serum starved for 1 hour, incubated for 5 min with various doses of test compounds. Cells were subsequently stimulated with 1 nM LHRH for 5 minutes at 37 ℃. Cells were washed once with PBS and harvested directly into 2X SDS sample buffer. Cell extracts were passed through 26 gauge needle 5 times, heated at 55℃ for 5 minutes, and subjected to SDS-PAGE. Resolved proteins were transferred onto PVDF membranes. The activated phosphorylated form of ERK1/2 was detected using an anti-phosphoMAPK p42/44 antibody (Cell Signaling Technology, Danvers, MA) diluted at 1:3000 in 1% nonfat dried milk in TBST (20mM Tris-HCl,pH 7.4, 137 mM NaCl, 0.1% Tween 20). Total ERK1/2 was detected with the anti-ERK2 antibody (K23; Santa Cruz Biotechnology, Santa Cruz, CA). Chemiluminescent detection was

performed with SuperSignal West Pico reagent (Pierce, Rockford, IL) and quantified on the LAS 3000 mini (Fujifilm) imaging system. Dose-response data were plotted and analyzed with GraphPad Prism software.

5.2.5 CYP3A4 enzyme inhibition.

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

5.2.6 Pharmacokinetics in SD rats.

Hydrochloride salt of compound **18a** (5 mg/kg as a base) dissolved in sterilized saline to a concentration of 5 mg/mL was injected in caudal vein of tail to fasted SD rats (male, 7 weeks old, n = 5). Blood samples were collected at 30 min and 1, 2, 4, 8,12 hr after intravenous administration (anticoagulant: heparin). Hydrochloride salt of

compound **18a** (10 mg/kg as a base) dissolved in sterilized saline to a concentration of 1mg/mL was orally administered to fasted SD rats (male, 7 weeks old, n = 5). Blood samples were collected at 30 min and 1, 2, 4, 8,12, 24 hr after oral administration (anticoagulant: heparin). The internal standard was added to 50 μL of plasma and then shaken for 10 seconds. 150 μL of acetonitrile was added and then vortexed for 30 seconds for protein precipitation. It was centrifuged for 5 minutes. 80 μL of the supernatant was transferred to analysis tube (Shiseido, 3207, 250 μL) for analysis. Compound **18a** and the internal standard were measured using API4000 triple quadrupole mass spectrometer (AB MD70496S Sciex, Toronto, Canada). The CAPCELL PAK MG C18 (5 μm, 2.0 mm x 50 mm) was used as a column for the analysis. For a mobile phase, 10 mM ammonium formate (adjusted to pH 4.0 with formic acid) /acetonitrile (40/60, v/v) was used. Flow rate was 0.25 mL/min and inject volume was 2 μL. After analyzing the concentrations of compound **18a**, AUC_{last} value was calculated from time-concentration curves in each animal using 'BA Calc 2002 (KFDA, Korea)'. The C_{max} was determined as the maximum plasma concentration, and T_{max} was the time to reach the maximum concentration.

5.2.7 Pharmacokinetics in Beagles.

Hydrochloride salt of compound **18a** (0.3 mg/kg as a base) dissolved in sterilized saline to a concentration of 1 mg/mL was slowly injected into the cephalic vein of fasted beagle dogs (male, 17~18 months old, n = 3). Blood samples were collected at predose, 1, 5, 10, 15, 30, 45 min, 1, 1.5, 2, 4, 8, 12hr after intravenous administration (anticoagulant: heparin). Hydrochloride salt of compound **18a** (10 mg/kg as a base) dissolved in sterilized saline to a concentation of 1mg/mL was administered into the stomach directly using a syringe and oral catheter to fasted beagle dogs (male, 17~18 months old, n = 3). Blood samples were collected at predose, 15, 30, 45 min, 1, 1.5, 2, 4, 8, 12, 24 hr after oral administration (anticoagulant: heparin). Plasma concentrations and pharmacokinetic parameters were determined by following the method described for SD rats.

5.2.8 Pharmacokinetics in castrated monkeys.

Hydrochloride salt of compound **18a** (0.5 mg/kg as a base) dissolved in sterilized saline to a concentration of 0.3 mg/mL was injected into the vein of fasted castrated cynomolgus monkeys (male, 5.5~6.5 years old,

n = 3, West China-Frontier Pharmatech, Co., China). Blood samples were collected at predose, 1, 15, 30 min, 1, 2, 4, 6, 8 and 12 hr after intravenous administration (anticoagulant: heparin). Hydrochloride salt of compound **18a** (10 mg/kg as a base) dissolved in sterilized saline to a concentration of 1mg/mL was administered with a nasogastric gavage to fasted castrated cynomolgus monkeys (male, 5.5-6.5 years old, n = 3). Blood samples were collected at predose, 1, 2, 4, 8, 12, 24, 36 and 48 hr after administration (anticoagulant: heparin). Plasma concentrations and pharmacokinetic parameters were determined by following the method described for SD rats.

5.2.9 *In Vivo* Efficacy in Cynomolgus Monkeys

Hydrochloride salt of compound **18a** (30 mg/kg as a base) and sodium salt of compound **2b** (30 mg/kg as a base) dissolved in distilled water were administrated to castrated cynomolgus monkeys (5.5~6.5 years old, n=3) by a nasogastric gavage. And distilled water was administrated to the control group by a nasogastric gavage to castrated cynomolgus monkeys (5.5~6.5 years old, n = 3). Blood samples were collected before and after each administration of drugs for analysis of serum LH and plasma compound concentration. Samples were

collected at 0, 1, 2, 4, 8, 12, 24, 36 and 48 h after oral administration. LH concentrations in serum samples were measured at Oregon National Primate Research Center using Radioimmunoassay (RIA).

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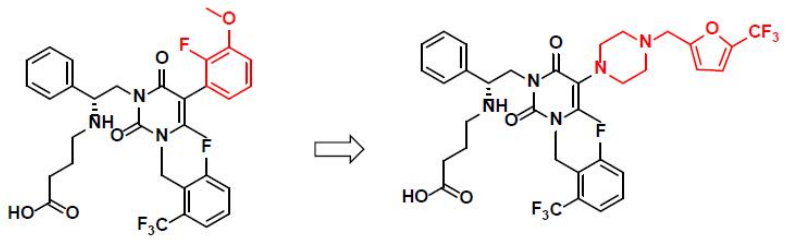
7. ABSTRACT IN KOREAN

경구용 고나도트로핀 방출 호르몬(GnRH) 수용체 길항제의 개발

우리는 uracil 모핵을 가진 경구용 GnRH 수용체 길항제 화합물 라이브러리를 합성하였다. In vitro 활성과 CYP 저해 프로필을 바탕으로 in vivo 시험용 물질 18a (SKI2496) 를 선정하였다. 18a 는 rat, monkey GnRH 수용체보다 human 에 대해 보다 선택적인 길항작용을 나타냈고, GnRH 매개 신호경로에서도 저해 효과를 보였다. 18a 의 체내동태 및 체내동력학 평가에서 임상적으로 가장앞선 물질인 Elagolix 보다 향상된 생체이용률과 우수한 고나도트로핀 억제효과가 입증되었다. 우수한 PK 프로필과 human GnRH 수용체에 보다 특이적인 길항작용을 가지므로, 18a 가 경구용 치료제로써 유망한 후보물질이 될 것으로 믿는다.



2b 18a

	Elagilix (2b)	SKI2496 (18a)
*h*GnRHR IC_{50}	0.58 nM	0.46 nM
max LH inhibit. (%, h)	70%, 8h	84%, 12h
LH inhibit. $_{24h}$	27%	76%
Oral availablity (BA)	5.8 (rat); 11% (monkey)	15.6% (rat); 13.2% (monkey)

[Part 2]

Synthesis and Biological Evaluation of 3-(2-Aminoethyl) uracil derivatives as Gonadotropin-releasing Hormone (GnRH) Receptor Antagonists

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. We believe that our SAR study offers useful insights to design GnRH antagonists as a potential treatment option for endometriosis.

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

Student Number : 2013-30502

1. INTRODUCTION

1.1 Endometriosis and its current treatments

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].

1.2 GnRH antagonist as an endometriosis treatment

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].

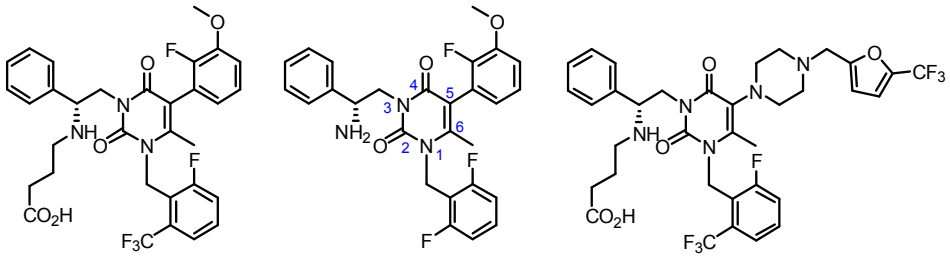


Figure 1. Examples of small-molecule GnRH antagonists

2. AIMS OF OUR STUDIES

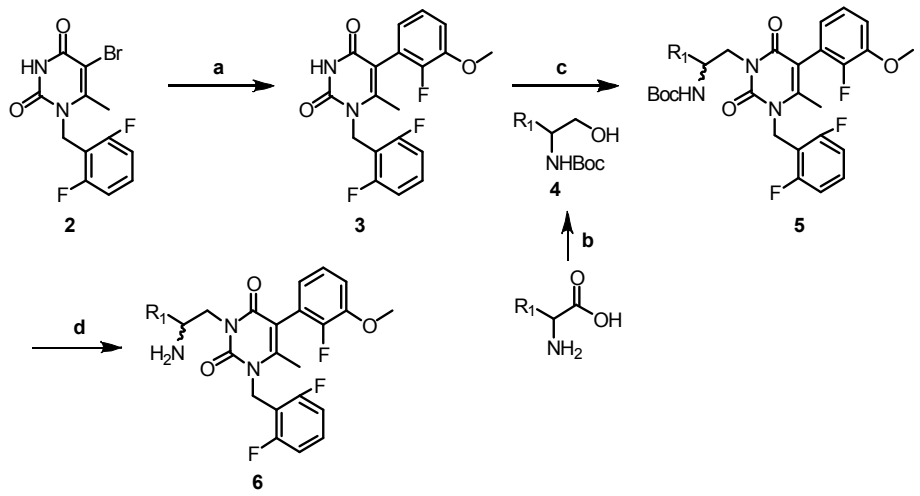
As a continued effort to diversify GnRH antagonists based on a uracil scaffold, we decided to shift our focus on the phenyl ring at the *N*-3 position of the uracil core. While previously reported SAR studies indicated that the 3-aminoethyl group is particularly important for metabolic stability [11, 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.

3. RESULTS AND DISCUSSION

3.1 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

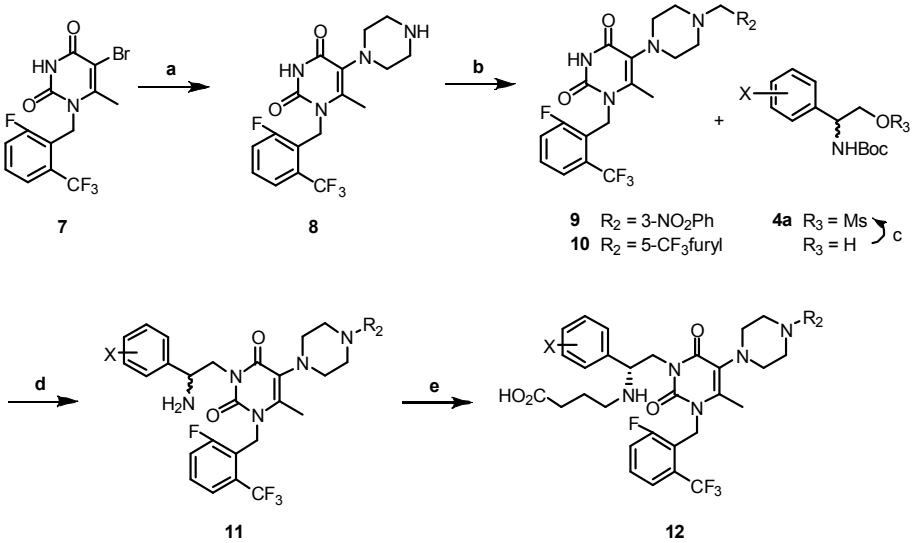


Reagents and coniditions: (a) i) p-methoxybenzyl chloride, K_2CO_3, DMF, 60 ^oC, 3 h, 56%, ii) 2-fluoro-3-methoxyphenylboronic acid, $Pd(PPh_3)_4$, saturated aq. $Ba(OH)_2$, benzene:EtOH:DME = 45:5:50, 85 ^oC, 2 days, 55%, iii) $AlCl_3$, anisole, r.t, 3 h, 93%; (b) i) $LiAlH_4$, THF, 70~80 ^oC, 3~4 h, 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



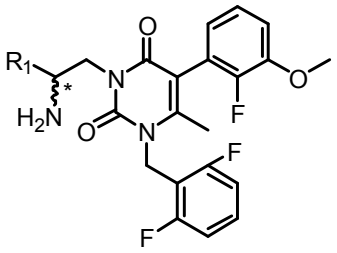
Reagents and conditions: (a) i) benzylpiperazine, MeCN, 120 ^{o}C, 2 h, microwave, 48%, ii) Pd/C, H_2, MeOH/CH_2Cl_2, r.t., 3 h, 87% (b) R_2CH_2Br, DIPEA, CH_2Cl_2, 2 h, r.t, 77~89%; (c) MsCl, TEA, CH_2Cl_2, 100%; (d) i) K_2CO_3, DMF, 70 ^{o}C, overnight, 65%, ii) TFA, CH_2Cl_2, r.t., 3 h, 65~85%; (e)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

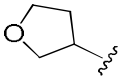
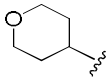
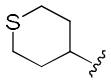
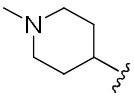
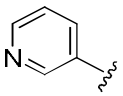
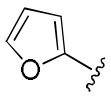
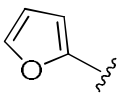
3.2 Structure-Activity Relationship

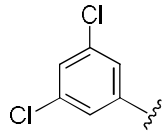
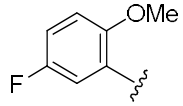
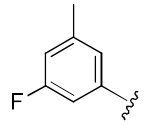
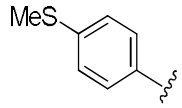
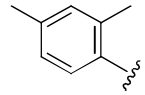
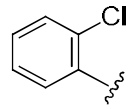
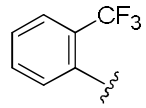
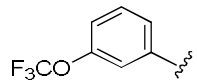
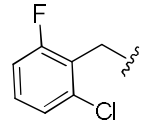
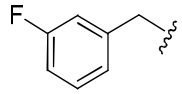
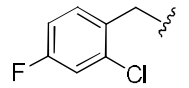
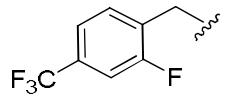
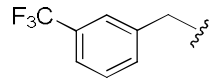
To evaluate the biological activity of the synthesized uracil analogues, we determined their in vitro binding affinities for the human GnRH receptors using the radiolabeled peptide, [^{125}I]D-Trp^{6}-LHRH. We first wanted to determine whether the modification of the phenyl ring at the *N*-3 position 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

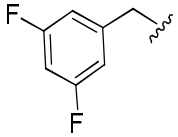
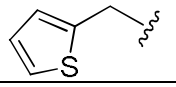
demonstrated weaker binding affinities, with IC_{50} values ranging from 9 to 62.3 nM.

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

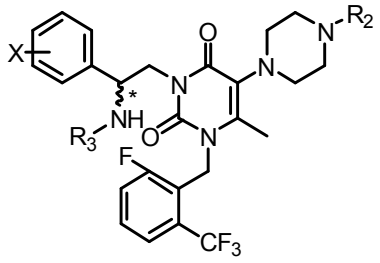
Because several analogues in this series demonstrated potent binding affinity that was comparable to **1a**, we next wanted to incorporate these modifications with the 5-piperazinyluracil moiety that showed superior potency and bioavailability in our previous study [10]. We selected 4-(3-nitrobenzyl)-piperazinyl (**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)

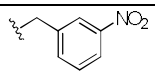
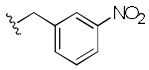
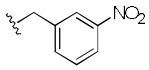
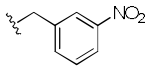
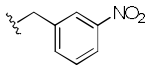
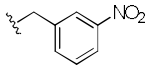
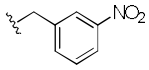
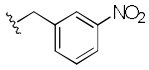
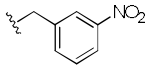
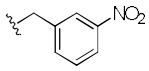
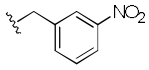
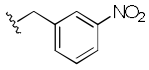
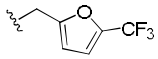
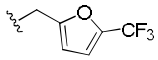
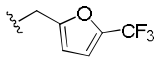
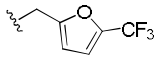
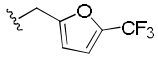
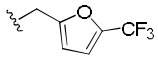
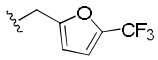
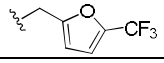
showed 5- to 7-fold higher activities than **11a** (X = H). It is interesting to note that compounds with these particular substituents in the previous series (**6i** and **6j**) also demonstrated potent in vitro binding affinities. While most of the compounds were tested as a racemic mixture, it appears 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	X	Stereo-chemistry	R_3	hGnRHR luciferase IC_{50} (nM)	CYP3A4 inh. % (1μM /10μM)
11a		-H	R	-H	165	ND
11b		2-Me	RS	-H	1066	ND
11c		2-OMe	R	-H	45	ND
11d		2-OH	R	-H	89	ND
11e		2-F	RS	-H	142	ND
11f		3-Me	RS	-H	160	ND
11g		4-F	R	-H	1682	ND
11h		3-F	RS	-H	144	ND
11i		2-OMe,5-F	R	-H	32	ND
11j		2-OMe,5-F	S	-H	26%*	ND
11k		3-Me,5-F	R	-H	22	ND
11l		3-Me,5-F	S	-H	35%*	ND
11m		H	R	-H	8.96	ND
11n		2-OMe	R	-H	1.54	88 / ND
11o		2-OMe,5-F	R	-H	3.03	89 / ND
11p		3-Me,5-F	R	-H	8.40	90 / ND
11q		2-OH	R	-H	15.2	ND
12a		2-OMe	R	-$(CH_2)_3CO_2H$	65.2	ND
12b		2-OMe,5-F	R	-$(CH_2)_3CO_2H$	38.5	ND / 28
12c		3-Me,5-F	R	-$(CH_2)_3CO_2H$	9.9	ND / 30

* assayed at 0.4 μM

3.3. 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

3.4 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**.

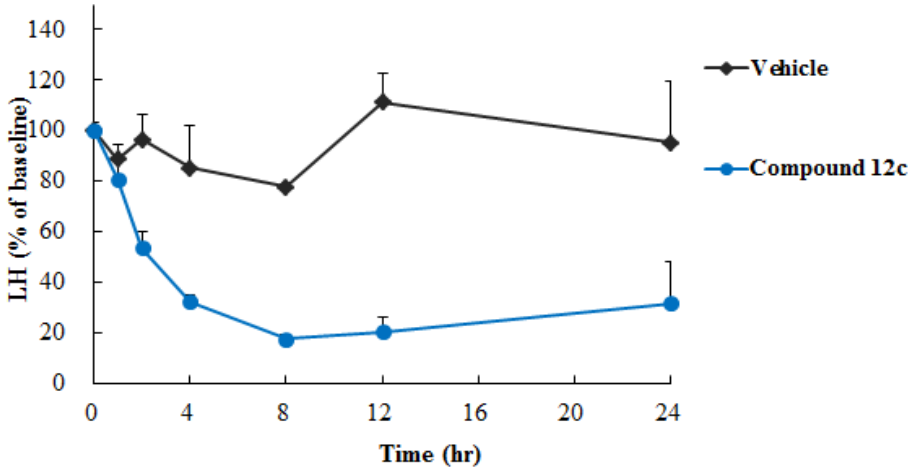


Figure 2. Suppression of plasma LH concentrations in castrated male cynomolgus monkeys after oral administration of compound **12c** at 30 mg/kg. Values shown are the mean ±SEM of bioactive LH levels expressed as a percentage of pretreatment LH levels for each of three individual animals.

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

4. 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. 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.

5. EXPERIMENTAL

5.1. Chemistry

5.1.1 General

^{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%.

5.1.2. Procedure for 5-phenyluracil preparation

*5.1.2.1. 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 (90mL) 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 (7.65g, 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 30min 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 (90mL) 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).

*5.1.3. 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 (1mmol) 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.

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

The suspension of compound **3** (1.1mmol) and compound **4** (1mmol) in DMF (9 mL) were treated with PBu_3 (1.5 mmol) and DEAD (1.5 mmol), then stirred at 80 °C for 3h. The reaction mixture was cooled to room temperature, diluted with CH_2Cl_2 (9mL) 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 (10ml) was added slowly to the solution of compound **5** (1mmol) in CH_2Cl_2 (100mL) 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.

*5.1.4.1. 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^+).

*5.1.4.2. (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^+).

*5.1.4.3. 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^+).

*5.1.4.4. 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^+).

*5.1.4.5. 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^+).

5.1.4.6. (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, ^1H 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^+).

5.1.4.7. (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, ^1H 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^+).

*5.1.4.8. 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^+).

*5.1.4.9. 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^+).

*5.1.4.10. 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^+).

*5.1.4.11. 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^+).

*5.1.4.12. 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^+).

*5.1.4.13. 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 ^{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^+).

*5.1.4.14. 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^+).

*5.1.4.15. 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^+).

*5.1.4.16. 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^+).

*5.1.4.17. 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^+).

*5.1.4.18. 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^+).

*5.1.4.19. 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 ^oC, ^1H 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^+).

*5.1.4.20. 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 ^oC, ^1H 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^+).

5.1.4.21. 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 °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^+).

*5.1.4.22. 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 = 6.6Hz, 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^+).

5.1.5. Procedure for 5-piperazinyluracil preparation

*5.1.5.1. 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, 10mL) 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 ^{o}C, ^{1}H 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).

*5.1.6. 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).

*5.1.6.1. 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 ^{o}C, ^{1}H 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).

*5.1.6.2. 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 ^{o}C, ^{1}H 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).

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

To a solution of compound **4** (1mmol) in CH_2Cl_2 (3mL) were added trimethylamine (1.3mmol) and methansulfonylchloride (1.1mmol). 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 (40mL) was treated with TFA (2mL), stirring at room temperature for 3h. 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).

*5.1.7.1. (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^+).

*5.1.7.2. 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, ^1H 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^+).

*5.1.7.3. (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^+).

*5.1.7.4. 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^+).

*5.1.7.5. 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^+).

*5.1.7.6. (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, ^1H 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^+).

*5.1.7.7. 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, ^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* 659 (MH^+).

*5.1.7.8. (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^+).

*5.1.7.9. (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, 3.0Hz, 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^+).

*5.1.7.10. (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^+).

*5.1.7.11. (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^+).

*5.1.7.12. (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^+).

*5.1.7.13. (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, 5.4Hz, 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^+).

*5.1.7.14. (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^+).

*5.1.7.15. (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^+).

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

The mixture of compound **9** or **10** (1 mmol), the mesylate (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). A solution of the alkylated compound (1mmol) 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 ($CH2Cl_2$/MeOH, 10:1).

5.1.8.1. (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+).

*5.1.8.2. (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^+).

*5.1.9. 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 (3mmol) 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**.

*5.1.9.1. (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 °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).

*5.1.9.2. (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 °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 99% (R_t = 16.45 min).

*5.1.9.3. (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 1M 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 ^{o}C, purity 99.8%. solubility (in H_2O) = 94.56 mg/mL. ^{1}H NMR (300 MHz, CD_3OD) 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).

5.2. 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.

5.3. In vitro Assays

5.3.1. Binding Assays

Receptor binding assays were performed as described previously.[13] CHO-K1 cells stably transfected with human GnRH receptor (4 ng/ µ L, 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 µ L/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

5.3.2. 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 1nM 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.

5.3.3. 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.

5.4. In vivo Assays

5.4.1. 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).

5.4.2. 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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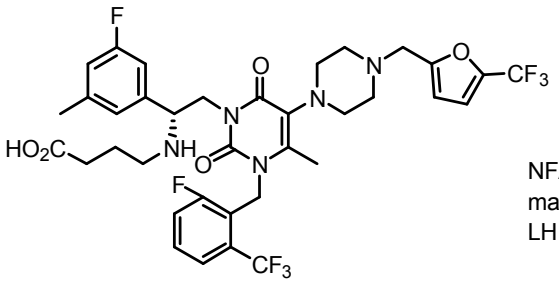
pyrimidin-1-yl]-1-phenylethylamino} butyrate (elagolix), a potent and orally available nonpeptide antagonist of the human gonadotropin-releasing hormone receptor. J. Med. Chem. 51 (23) (2008) 7478-7485.

7. ABSTRACT IN KOREAN

고나도트로핀 방출 호르몬 수용체 길항제로써 3-(2-Aminoethyl)uracil 유도체의 합성과 생물학적 평가

우리는 *N*-3 aminoethyl 측쇄의 phenyl ring에 다양한 치환기를 도입함으로써 일련의 우라실 유도체를 합성하고, 고나도트로핀 방출 호르몬 수용체에 대한 길항작용을 평가하였다. phenyl ring의 ortho 또는 meta 위치에 치환기를 가진 유도체가 강한 in vitro 길항작용을 나타냈다. 특히, 2-OMe 기를 도입했을 때, 치환되지 않은 경우 대비 6배 강한 nuclear factor of activated T-cells (NFAT) 저해효과를 보였다. 우리는 12c 화합물이 CYP 저해는 낮으면서 강한 GnRH 길항작용을 가짐을 확인하였다. In vivo 모델인 고환적출원숭이에 단회 경구투여시, 12c 는 강하면서 지속적인 LH 억제효과를 보였다. 본 연구에서 얻어진 구조활성관계는 자궁내막증 치료제로써의 GnRH 길항제를 설계하는 유용한 정보가 될 것으로 믿는다.



12c

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

+