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Synthesis and Structure–Activity Relationships of 1-Arylmethyl-3-(2-aminopropyl)-5-aryl-6-methyluracils as Potent GnRH Receptor Antagonists

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Abstract—The novel synthesis and SAR studies of 6-methyluracils as human GnRH receptor antagonists are discussed. Introduction of a small methyl substituent at the β-position from N3 of the uracil improved the GnRH binding potency by 5- to 10-fold. The best compound from the series had binding affinity of 5 nM (K_i) to the human GnRH receptor.

Gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone-releasing hormone (LHRH), is the decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2), which is produced in and secreted by the hypothalamus in a pulsatile manner.[1,2] It acts on the pituitary gland to stimulate the secretion of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These gonadotropines, in turn, act on the reproductive organs, where they participate in the regulation of gonadal steroid production, spermatogenesis in males and follicular development in females. Several reproductive disease conditions such as endometriosis, uterine fibroids and prostate cancer can be treated by suppression of the pituitary–gonadal axis. Currently, depot forms of peptidic GnRH agonists, represented by leuprorelin® are used to treat such conditions through a receptor down-regulation mechanism to suppress gonadal steroid production.[3] However, recent clinical evidence has shown that peptidic GnRH antagonists can act immediately at the receptor to lower steroid levels and therefore alleviate disease symptoms without the concomitant 'flare effect', which is exhibited by the peptide agonists due to their initial over-stimulation of the receptor.[4] Nevertheless, both peptide agonists and antagonists require parenteral administration, typically in depot form due to their poor oral bioavailability. Small molecule GnRH antagonists offer the potential for oral administration and therefore could gain wider acceptance from patients. In response to that need, intensive efforts have been initiated for the development of small molecule GnRH antagonists.[5,6]

In earlier papers,[7,8] we disclosed the SAR study of 7-phenylpyrrolo[1,2-*a*]pyrimid-4-ones **I** and **II**[7] (Fig. 1), and 2-phenylimidazolo[1,2-*a*]pyrimidines **III** and **IV**[8] as potent hGnRH receptor antagonists. These studies showed that the pyridyl-ethyl group in the basic side chain is very important for high receptor-binding activity and 3-methoxyphenyl is a good substitution on the right side of the molecules. Most recently,[9] we have discussed the initial SAR studies of a novel series of monocyclic uracils (**1** and **2**) as human GnRH receptor antagonists. The best compound (**2a**) from the initial SAR study has a K_i value of 34 nM, which is less potent than some of our bicyclic analogues. To enhance the potency, we rationalized that restriction of the flexible side chain from N3 may be beneficial, and furthermore, a small alkyl group such as a methyl at the β-position could serve such a purpose. In this letter, we report a novel and improved synthesis of the 6-methyl uracil core structure to give the 1-arylmethyl-3-(2-aminopropyl)-5-aryl-6-methyluracil structure. SAR of this series of uracils with an amino-isopropyl side chain at the N3 position is described.

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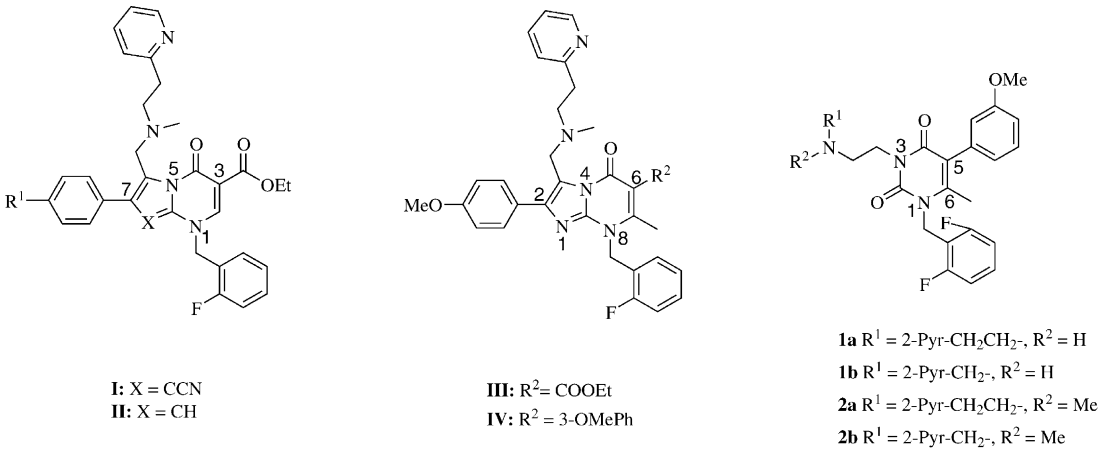
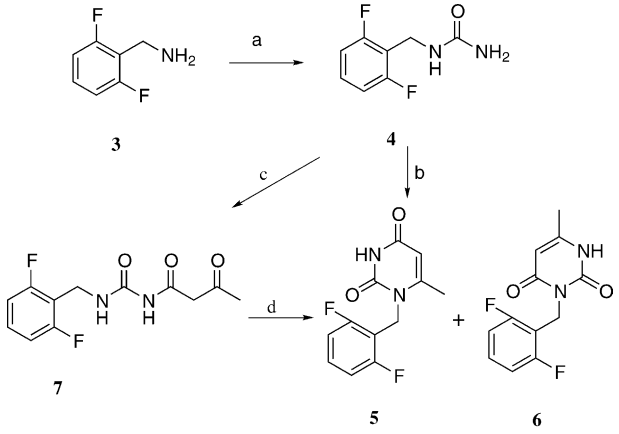


Figure 1. Small molecule GnRH antagonists.

The 6-methyluracil core structure was initially synthesized by condensation of allylurea and ethyl acetoacetate.[9,10] The yield was low and the substitution at N3 was limited by the availability of substituted ureas. To perform a more comprehensive SAR study at N3, a new synthetic route had to be developed. Thus, 2,6-difluorobenzyl amine and urea were refluxed in HCl/water solution to form the benzyl urea **4** (Scheme 1), and then crystallized from EtOAc in 79% yield. Urea **4** and diketene were then refluxed in acetic acid for 40 min to yield a mixture of N1- and N3-substituted 6-methyluracils (**5** and **6**) in 3:1 ratio. The structures of **5** and **6** were confirmed by NOE NMR experiments. In compound **5**, for instance, there was a clear NOE of the 6-methyl signal upon irradiation of the benzylic protons at N1; this effect was absent for **6**. 1-(2,6-difluorobenzyl)-6-methyluracil (**5**) was separated from the N3-isomer (**6**) by recrystallization in acetic acid. This two-step sequence afforded **5** in about 25% yield from **3**. After further experimentation, a more efficient route to uracil **5** was developed to afford the N1-substitued 6-methyluracil exclusively. Thus, compound **4** was first treated with diketene in pyridine at ambient temperature for 24 h, and the intermediate **7** was separated in 60% yield. Compound **7** was then refluxed in acetic acid for 1 h and the desired product **5** crystallized out from the reaction mixture in 95% yield (Scheme 1).



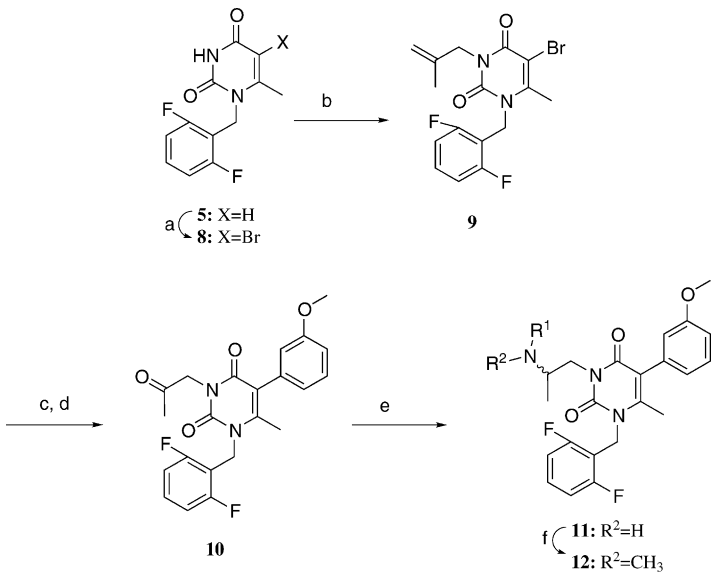
Scheme 1. Reagents and conditions: (a) urea, water, HCl, reflux, 79%; (b) diketene, HOAc, reflux, 40 min, 69%; (c) diketene, pyridine, rt, 24 h, 60%; (d) HOAc, reflux, 1 h, 95%.

Uracil **5** was brominated in acetic acid to yield **8** (Scheme 2), which was alkylated at the N3 position by treatment with 3-bromo-2-methylpropene and K_2CO_3 in DMF at ambient temperature. The resulting alkene (**9**) was then oxidized to the methyl ketone with $NaIO_4$/OsO_4 in 1:1 THF/water, followed by Suzuki coupling with 3-methoxyphenylboronic acid yielded the key intermediate **10**. The ketone (**10**) was treated under reductive amination conditions with a variety of amines (R_1R_2NH) in dichloroethane, followed by treatment with $NaBH(OAc)_3$ to afford the desired amines **11**. *N*-Methylation was achieved by reductive amination of **11** with formaldehyde to afford final products **12**.

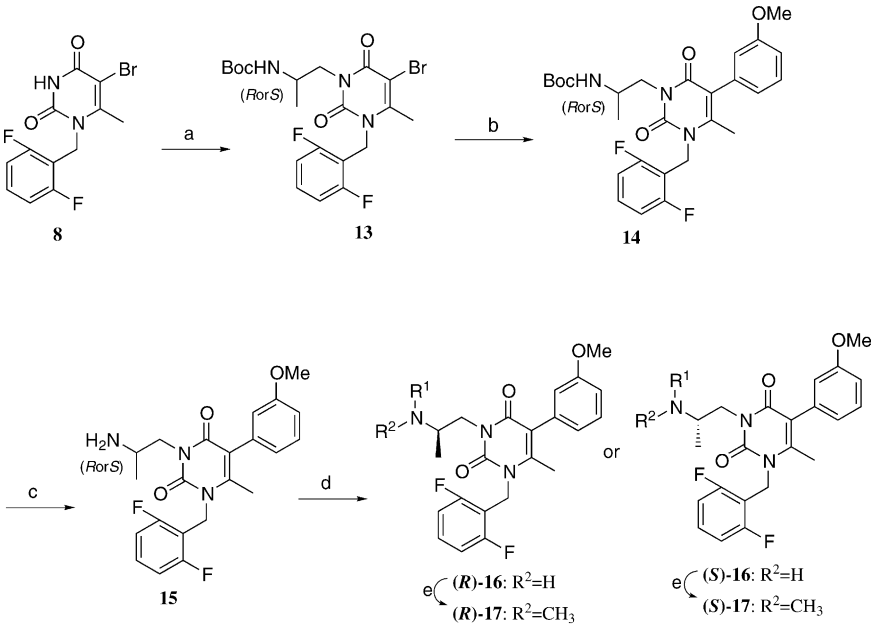
To prepare enantiomerically pure molecules, an alternative synthetic approach was used (Scheme 3). Compound **8** was treated with either *N*-*t*-Boc-*R*-alaninol or its corresponding *S*-isomer under Mitsunobu conditions in THF using di-*t*-butyl-azodicarboxylate as the coupling reagent to give **13**. Suzuki coupling of **13** with 3-methoxyphenylboronic acid under the catalysis of Pd(0) yielded **14**. Deprotection of the amino-Boc group with TFA followed by two successive reductive aminations, first with aldehydes to give **16** and then with formaldehyde generated the *N*-methylated final products **17**. Finally, cyclic analogues that link the *N*-methyl and the β-methyl group with a methylene moiety were prepared from **8** with either *N*-*t*-Boc-*R*-prolinol or its corresponding *S*-isomer to give **18** (Scheme 4). Suzuki coupling of **18** yielded **19**, deprotection of which afforded **20** and reductive amination of **20** with 2-pyridinecarboxaldehyde yielded the desired final products **21**.

All synthesized compounds were evaluated for their ability to inhibit des-Gly^{10}[^{125}I-Tyr^5,DLeu^6,NMeLeu^7, Pro^9-NEt]-GnRH radioligand binding to the cloned hGnRH receptor stably expressed in HEK293 cells using a 96-well filtration assay format.[11]

The binding affinities of **1**–**2** and **11**–**12** for the hGnRH receptor are summarized in Table 1. As previously observed with **1** and **2**, *N*-methylated compounds were



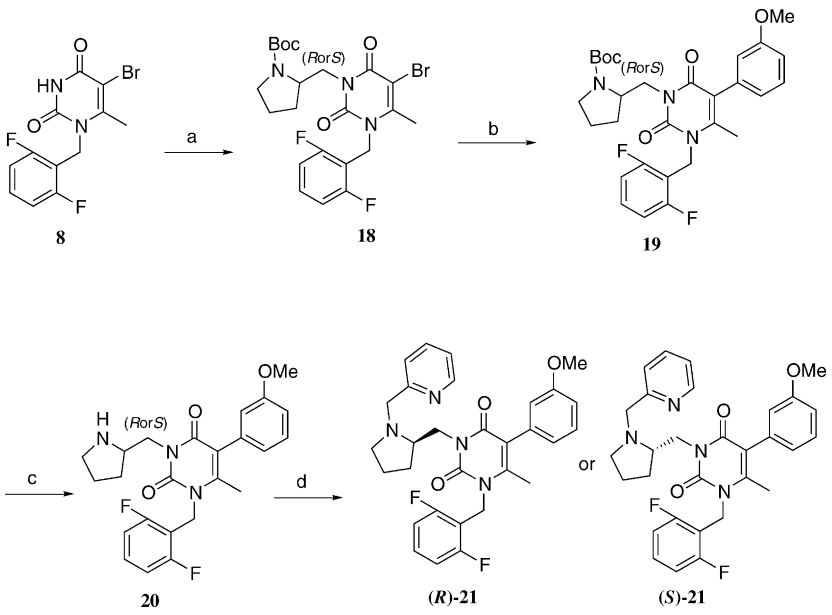
Scheme 2. Reagents and conditions: (a) Br_2, HOAc; (b) 3-bromo-2-methylpropene, K_2CO_3, DMF; (c) $NaIO_4$, OsO_4 (cat), THF/H_2O; (d) 3-methoxyphenyl boronic acid, $Pd(Ph_3P)_4$, Na_2CO_3, toluene/H_2O, reflux; (e) R^1NH_2, $NaBH(OAc)_3$, dichloroethane; (f) CH_2O, $NaBH(OAc)_3$, dichloroethane.



Scheme 3. Reagents and conditions: (a) *N*-*t*-Boc-*R*-alaninol or *N*-*t*-Boc-*S*-alaninol, PPh_3, di-*t*-butyl-azodicarboxylate, THF; (b) 3-methoxyphenyl boronic acid, $Pd(Ph_3P)_4$, Na_2CO_3, toluene/H_2O, reflux; (c) TFA/CH_2Cl_2 (1:1); (d) aldehyde, $NaBH(OAc)_3$, dichloroethane; (e) CH_2O, NaBH $(OAc)_3$, dichloroethane.

in general more potent than their NH analogues. Thus compound **12a** was 14 times; **12b** was 3 times more potent than **11a** and **11b**, respectively. Importantly, the potency of the earlier compounds (**1**–**2**) was greatly improved by the incorporation of a β-methyl group to the ethylene group. This trend was very clear, especially when there was a pyridine ring on the N3-branch substitution. **11a** was 6 times more potent than **1a** and **11b** was 10 times more potent than **1b**. Enhancement of binding affinity by the β-methyl group was also obvious in the *N*-methyl series compare **2a** and **2b** with **12a** and **12b**, respectively. **12a** and **12b** had comparable potency; the extra methylene did not make noticeable difference for activity. With the β-methyl group, *N*-alkyl substituents such as isobutoxypropyl (**11d**) also demonstrated good binding activity, which was even better than that of the benzyl analogue (**11c**).

The hGnRH receptor binding data for the enantiomeric compounds **15**–**17** and **21** are reported in Table 2. With the 2-pyridylmethyl- or 2-pyridylethyl-substituent, the *R*-isomers were obviously more active than the *S*-isomers by at least one order of magnitude. Interestingly, *N*-methylation improved GnRH binding specifically for the *R*-isomer, thereby enhancing the potency ratio to about 100-fold. Surprisingly, for the *N*-benzyl compounds, there was almost no difference between the *R* and *S* isomers in binding activity and *N*-methylation of



Scheme 4. Reagents and conditions: (a) *N*-*t*-Boc-*R*-prolinol or *N*-*t*-Boc-*S*-prolinol, PPh_3, di-*t*-butyl-azodicarboxylate, THF; (b) 3-methoxyphenyl boronic acid, $Pd(Ph_3P)_4$, Na_2CO_3, toluene/H_2O, reflux; (c) TFA/CH_2Cl_2 (1:1); (d) 2-pyridinecarboxaldehyde, $NaBH(OAc)_3$, dichloroethane.

Table 1. Binding affinities of 1-arylmethyl-3-(2-aminoethyl)-5-aryl-6-methyluracils (**1**–**2**) and 1-arylmethyl-3-(2-aminopropyl)-5-aryl-6-methyluracils (**11**–**12**) on the human GnRH receptor[12]

Compd	R_1	R_2	K_i (nM)
1a	H–	(2-Pyr)–CH_2CH_2–	1100
1b	H–	(2-Pyr)–CH_2–	550
2a	CH_3–	(2-Pyr)–CH_2CH_2–	34
2b	CH_3–	(2-Pyr)–CH_2–	96
11a	H–	(2-Pyr)–CH_2CH_2–	170
11b	H–	(2-Pyr)–CH_2–	53
11c	H–	Ph-CH_2–	280
11d	H–	*i*-Butyl-O–$CH_2CH_2CH_2$–	81
12a	CH_3–	(2-Pyr)-CH_2CH_2–	12
12b	CH_3–	(2-Pyr)-CH_2–	18
12c	CH_3–	Ph-CH_2–	310
12d	CH_3–	*i*-Butyl-O–$CH_2CH_2CH_2$–	240

Table 2. Binding affinities of 1-arylmethyl-3-(2-aminopropyl)-5-aryl-6-methyluracils (**15**–**17**) and 1-arylmethyl-3-[*N*-(2-pyridylmethyl)-2-pyrrolidinylmethyl]-5-aryl-6-methyluracils (**21**) on the human GnRH receptor[12]

Compd	R_1	R_2	K_i (nM) chirality	
			R	*S*
15	H–	H–	2500	18,000
16a	H–	(2-Pyr)–CH_2CH_2–	79	780
16b	H–	(2-Pyr)–CH_2–	15	460
16c	H–	Ph–CH_2–	130	140
17a	CH_3–	(2-Pyr)–CH_2CH_2–	5.2	890
17b	CH_3–	(2-Pyr)–CH_2–	5.5	470
17c	CH_3–	Ph–CH_2–	330	250
21			32	210

these compounds [(*R*)- and (*S*)-**17c**] did not improve their potency. Finally, the proline-based compound (*R*)-**21** was less potent than (*R*)-**17**(**a**–**b**), and the (*R*)-**21** was 6 times more potent than its *S*-isomer.

To ensure that these high-binding affinity molecules were functional antagonists, compound (*R*)-**17b** was selected for testing its ability to inhibit Ca^{2+} influx induced by GnRH.[13] The result shown in Figure 2 indicates that (*R*)-**17b** is a potent functional antagonist with $IC_{50} = 2.5$ nM. Similar to our previous results,[7a] these compounds demonstrated species differences in their binding to the GnRH receptors. Compound (*R*)-**17b** exhibited reduced binding affinity on the monkey GnRH receptor ($K_i = 102$ nM) and much lower affinity on the rat GnRH receptor ($K_i = 21$ μM).

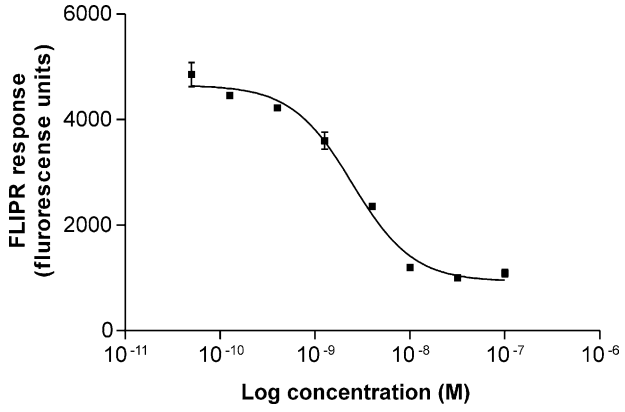


Figure 2. Inhibition of GnRH stimulated Ca^{2+} flux by compound (*R*)-**17b**.

In conclusion, we have discovered a novel, highly convergent cyclization procedure for assembly of the 6-methyl uracil core. Introduction of a small methyl substituent at the β-position from N3 of the uracil improved the GnRH binding potency by 5- to 10-fold. (*R*)-Enantiomers were much more potent than their (*S*)-counterparts when a 2-pyridyl group was attached. This phenomenon may be explained by a receptor model where the (*R*)-methyl group orients the pyridyl side chain in close proximity to the aspartic acid 302 on helix 7, which we believe is a critical recognition point on the receptor, thus allowing for optimal binding.

Further structure–activity relationships of this series of compounds as GnRH antagonists will be presented elsewhere.

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12. On each assay plate a standard antagonist of comparable affinity to those being tested was included as a control for plate-to-plate variability. Overall, K_i values were highly reproducible with an average standard deviation of 45% for replicate K_i determinations. Key compounds were assayed in 3–8 independent experiments.
13. Inhibition of GnRH stimulated Ca^{2+} flux: Functional activity of compounds for the human GnRH receptor was determined by inhibition of GnRH stimulated Ca^{2+} flux. RBL cells stably expressing the full-length human GnRH receptor were seeded into 96-well, black-wall clear-bottom plates (Corning) at a density of 50,000 cells/well and the cells allowed to attach overnight. Cells were then loaded with the Ca^{2+} sensitive dye, Fluo-4 (Molecular Probes), by incubation in loading medium [(DMEM with 20 mM Hepes, 10% FBS, 2 μM Fluo-4, 0.02% pluronic acid (Molecular Probes) and 2.5 mM probenecid (Sigma)] for 1 h at 37 °C. Cells were then washed three times with assay buffer (Hanks balanced salt, 20 mM Hepes, 2.5 mM probenecid). Compounds at varying concentrations in assay buffer were pre-incubated with cells for 1 min prior to stimulation with GnRH (5 nM). Measurement of fluorescence due to GnRH stimulated Ca^{2+} flux was performed according to manufacturer's instructions on a FLIPR system (Molecular Devices, FLIPR^{384} system). IC_{50} values for the inhibition of GnRH-stimulated Ca^{2+} flux were calculated using the Prism software package (GraphPad Software) with a 'sigmoidal dose–response (variable slope)' option for curve fitting.