Letters

Identification of 1-Arylmethyl-3-(2-aminoethyl)-5-aryluracil as Novel Gonadotropin-Releasing Hormone Receptor Antagonists

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Abstract: Based on SAR from bicyclic GnRH antagonists such as 6-aminomethyl-7-arylpyrrolo[1,2-*a*]pyrimid-4-ones (**1**) and 2-aryl-3-aminomethylimidazolo[1,2-*a*]pyrimid-5-ones (**2a**,**b**), a series of novel uracil compounds (**4**) were derived as the GnRH antagonists. Their syntheses and initial SAR are discussed herein. This is the first time that monocycle-based GnRH receptor antagonists are reported.

In previous papers[1,2] we disclosed the SAR study of 6-aminomethyl-7-arylpyrrolo[1,2-*a*]pyrimid-4-ones (**1**) and 2-aryl-3-aminomethylimidazolo[1,2-*a*]pyrimid-5-ones (**2a**,**b**) as potent human gonadotropin-releasing hormone (hGnRH) receptor antagonists. The SAR of **1** revealed that the binding affinity was enhanced by a 2-pyridylethyl group on the side chain of the 6-aminomethyl group and a para-substituent (R^3) on the 7-phenyl group. Furthermore, SAR studies of **2b** indicated that an aryl group, preferably a 3-methoxyphenyl group, could replace the 6-carboxylate of **2a**. The introduction of the 6-aryl group led to a different SAR profile in the two series represented by **2a** and **2b**. Thus, while the para-substituted (R^3) phenyl ring on the left side of the molecule **2a** was crucial for binding**,** it hardly affected the potency of **2b**. This observation led us to successfully design the 2-alkyl analogues **3**, which possess lower molecular weights but maintain equipotent GnRH antagonistic activity as analogues **1**.[3] On the basis of these SAR results, we further postulated that the whole left side of **3** including the alkyl group and the five-membered ring might not be necessary for binding, which would lead to much smaller monocyclic compounds. To test this hypothesis, the uracil was chosen as one of several possible monocyclic core structures due to its synthetic accessibility. The synthesis and initial SAR results of the uracil-based GnRH antagonists (**4**) are reported in this letter.

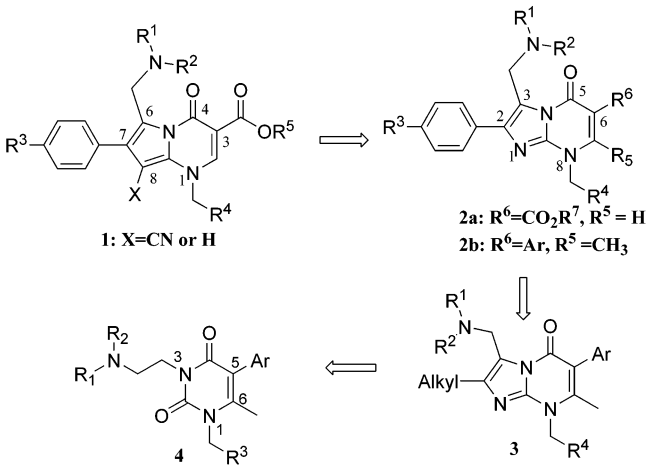

Figure 1. General structures of GnRH antagonists

Scheme 1 represents the initial approach to synthesize the fully substituted uracils. 3-Allyl-6-methyluracil (**5**) was obtained by condensation of *N*-allylurea with ethyl acetoacetate using the known procedure.[4] It was then treated with 2,6-difluorobenzyl bromide in the presence of n-tetrabutylammonium fluoride in THF to afford **6**, which underwent oxidative cleavage of the olefin, followed by bromination to yield **7**. As a versatile intermediate, **7** possesses the potential for two-point diversification at the bromo group and the aldehyde group, respectively. Thus, Suzuki reaction of **7** with 3-methoxyphenyboronic acid yielded **8**, which was subjected to reductive aminations with a set of amines (R^1R^2NH) to produce the final compounds **9a**−**g**. **7** was also coupled to 2-(*N*-methyl-2-aminoethyl)pyridine under reductive conditions to give **10**, which was then subjected to Suzuki coupling reactions with a variety of arylboronic acids to afford **11a**−**h**. Scheme 2 depicts the synthesis of the 6-desmethyl analogue **15**. 5-Bromouracil (**12**) was treated with *N*,*O*-bis(trimethylsily)-acetamide to form the intermediate bis-silyl ether, which was immediately quenched by 2,6-difluorobenzyl bromide to selectively produce the 1-benzylated **13**.[5] The structure of **13** was confirmed by NOE NMR experiments, and NOE effect was observed between the proton at the 6-position and the methylene group at the 1-position. Allylation at the 3-position was accomplished using allyl bromide and K_2CO_3, and oxidative cleavage of the double bond by OsO_4/$NaIO_4$ afforded the aldehyde **14**. Reductive amination of the aldehyde of **14** with 2-(2-methylaminoethyl)pyridine, then followed by a Suzuki reaction with 3-methoxyphenylboronic acid, yielded the desired product **15**. A 10-step synthesis of the 6-ethyl analogue **22** started from the allylurea **16** (Scheme 3), which reacted with diethyl malonate to form **17**. It was then heated in $POCl_3$ in the presence of water to yield **18**,[6] followed by benzylation at 1-position to afford **19**. An initial attempt to displace the 6-chloro group with diethyl methymalonate in one step to produce **20** failed. Therefore, the chloro group was first replaced by diethyl

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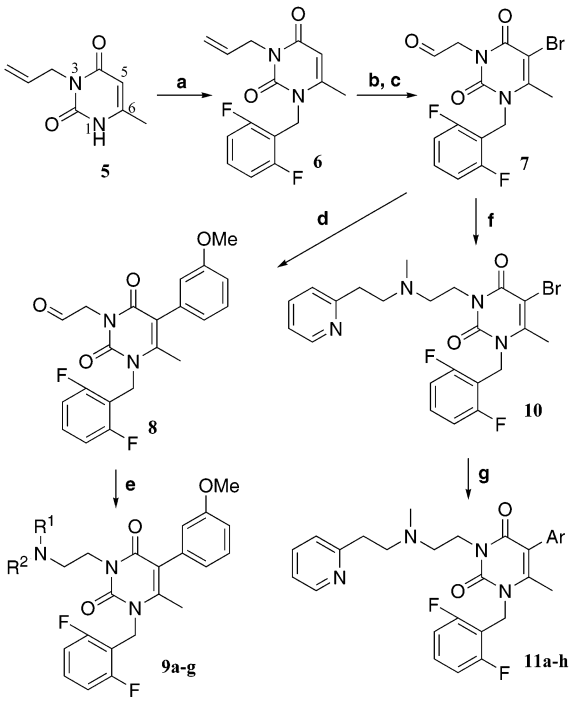
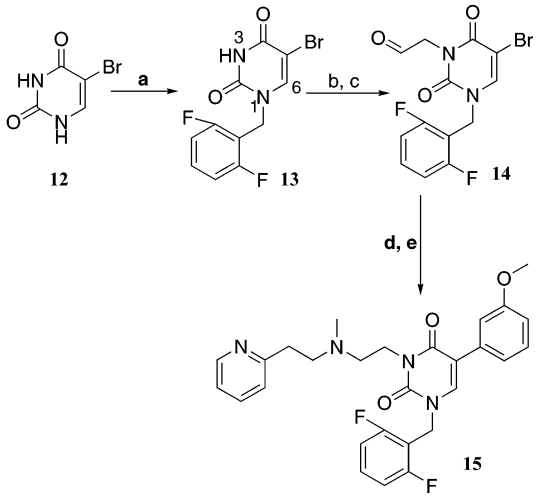
Scheme 1[a]



[a] (a) 2,6-Difluorobenzyl bromide, TBAF, THF, 59%; (b) $NaIO_4$, OsO_4 (cat), THF/H_2O, 99%; (c) Br_2, HOAc, 80%; (d) 3-methoxyphenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene/EtOH/H_2O, reflux, 80%; (e) R^1R^2NH, $NaBH(OAc)_3$, DCM, 25−80%; (f) 2-(*N*-methyaminoethyl)pyridine, $NaBH(OAc)_3$, DCM, 75%; (g) $ArB(OH)_3$, $Pd(PPh_3)_4$, Na_2CO_3, toluene/EtOH/H_2O, reflux, 50−90%.

Scheme 2[a]

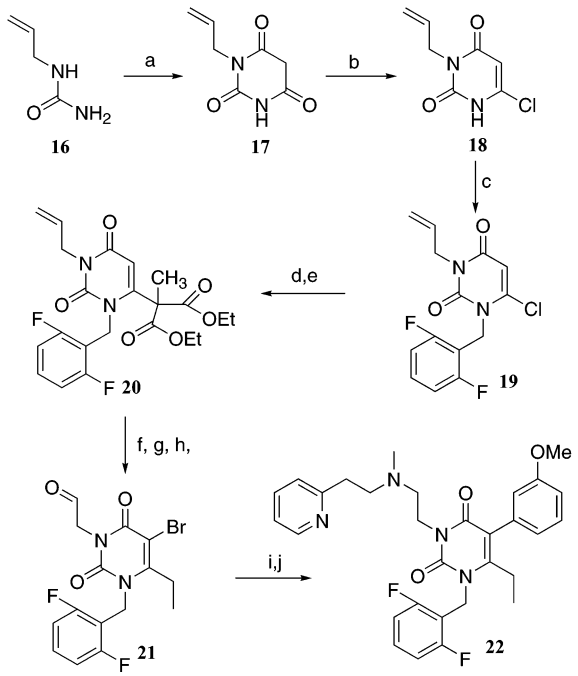


[a] (a) *N*,*O*−Bis(trimethylsily)acetamide, then 2,6-difluorobenzyl bromide, 99%; (b) allyl bromide, K_2CO_3, DMF, 49%; (c) $NaIO_4$, OsO_4 (cat), THF/H_2O, 85%; (d) 2-(*N*-methyl-2-aminoethyl)pyridine, $NaBH(OAc)_3$, DCM, 80%; (e) 3-methoxyphenylboronic acid, $Pd(Ph_3P)_4$, Na_2CO_3, toluene/H_2O, reflux, 90%.

malonate, and the product was methylated with methyl iodide to form **20**. Hydrolysis and decarboxylation of **20** was accomplished with potassium hydroxide in one step and followed by oxidative cleavage of the allylic double bond using OsO_4/$NaIO_4$ to yield **21**. Reductive amination and Suzuki coupling afforded the final product **22**.

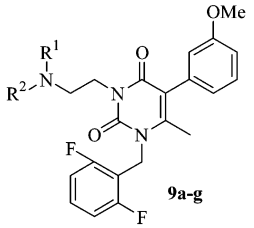
The binding affinities of **9a**−**g** to the cloned human GnRH receptor are summarized in Table 1.[7] The overall SAR was very similar to the early bicyclic series. The preferred side chain, 2-pyridylethyl for the bicyclic

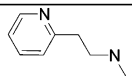
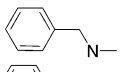
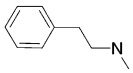
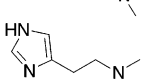
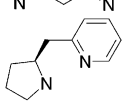
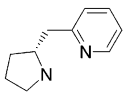
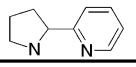
Scheme 3[a]



[a] (a) Diethyl malonate, NaOEt, EtOH, reflux, 89%; (b) $POCl_3$, H_2O, 60 °C, 63%; (c) 2,6-difluorobenzyl bromide, K_2CO_3, DMF, 95%; (d) diethyl malonate, NaH, THF; (e) NaH, MeI; (f) KOH, THF/H_2O, reflux, 19% for steps d, e, and f; (g) $NaIO_4$, OsO_4 (cat.), THF/H_2O; (h) Bromine, HOAc, 55% for steps g and h; (i) 2-(*N*-methyl-2-aminoethyl)pyridine, $NaBH(OAc)_3$, CH_2Cl_2; (j) 3-methoxyphenylboronic acid, $Pd(Ph_3P)_4$, Na_2CO_3, toluene, H_2O, 22% for steps i and j.

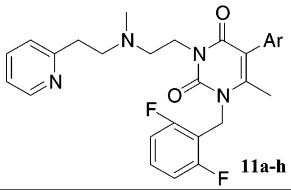
Table 1. Binding Affinities of Compounds **9a**−**g** to the Human GnRH Receptor[9]



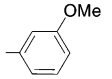
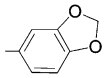
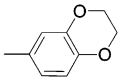
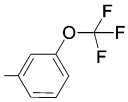
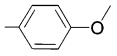
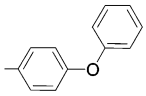
Compds	R^1R^2NH	K_i(nM)
9a		34
9b		240
9c		1100
9d		3800
9e		130
9f		4800
9g		530

GnRH antagonists, again produced the high affinity molecule **9a** (34 nM). However, the ring constrained analogues **9e**−**g** were much less potent, although one

Table 2. Binding Affinities of Compounds **11a**−**h**, **15**, **22** to the Human GnRH Receptor[9]



11a-h

Compds	Ar	K_i(nM)
11a		400
9a		34
11b		38
11c		24
11d		480
11e		4800
11f		220
11g		230
11h		30
15	------	1700
22	------	2200

enantiomer (**9e**) was much preferred than the other one (**9f**), and its affinity was within 5-fold of **9a**. In the meantime the impact of the substitution on the 5-phenyl group was examined, and the results are shown in Table 2. The unsubstituted compound, **11a,** was more than 10-fold less potent than **9a,** indicating that the 3-methoxy group may provide a productive H-bonding acceptor. Replacement of the methoxy group by 3,4-methylenedioxy (**11b**) or 3,4-ethylenedioxy (**11c**) generated equally potent molecules. However, replacement with the polar 3-hydroxyl group (**11d**, 480 nM) reduced the binding affinity significantly, as did the trifluoromethoxy group (**11e**, 4800 nM), again illustrating the need for a hydrogen bond acceptor. This requirement was further confirmed by **11f**, where deletion of the oxygen led to lower potency. Shifting the methoxy group from the 3- to the 4-position also resulted in a substantial reduction in potency (**11g**, 230 nM). Nevertheless, switch of the 4-methoxy to the 4-phenoxy fully regained the potency (**11h,** 30 nM), implying possible hydrophobic interaction of the extra phenyl group with the receptor. The enhancement of binding by the 6-methyl group on the uracil core was recognized by comparing the methyl analogue **9a** to hydrogen and ethyl analogues (**15**, **22)** on the 6-position, respectively; the latter two gave very poor binding affinities. Our speculation is that the 6-methyl group forces the 5-phenyl ring into a perpendicular conformation with respect to the uracil core, which in turn, may be preferred for $\pi-\pi$ interaction with the GnRH receptor. Deletion of the 6-methyl group obviously leads to unrestricted rotation of the 5-phenyl ring and thus reduces its binding capacity. By contrast, the 6-ethyl group of **22** could be too large and thus causes a steric clash with the binding pocket on the receptor.

While the principle goal of this study was to establish SAR of a simple monocyclic core with low molecular weight, we also want to take a first look at the basic pharmacokinetic profiles for possible orally active agents. **9a** was then selected for mouse and human liver microsome stability study and mouse pharmacokinetic experiments. In vitro, **9a** exhibited relatively poor metabolic stability with intrinsic clearance of 347 mL/min·kg and 84 mL/min·kg in the mouse and human liver microsomes, respectively. The predicted hepatic extraction was 79% for both mouse and human. In vivo, upon oral administration (10 mg/kg) in the mice, 25% of this compound made to the blood circulation, which suggested 75% hepatic extraction. This compound had high clearance (CL = 120 mL/min· kg) in the mice with and a V_d value of 4.2 l/kg and a half-life of 0.4 h. Oral bioavailability was 1.6%. The high lipophilicity[8] of this compound may be associated with the poor metabolic stability and thus low oral bioavailibility. Further optimization of this series of compounds is required to reduce lipophilicity and therefore increase metabolic stability.

In conclusion, on the basis of the SAR of the bicyclic analogues, we have successfully designed and synthesized a series of novel and potent monocyclic uracil GnRH antagonists. The initial work on this series provided us with new directions for obtaining orally active GnRH antagonists.

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Supporting Information Available: Description of the binding assay, stepwise syntheses, and characterization of compounds **9a** and **11c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) Human GnRH receptor was stably expressed in HEK293 cells and a 96-well filtration assay was used.
(8) Calculated logD was 5.30, based on ACD/Labs 6.00; Advanced ChemistryDevelopment Inc.
(9) 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. Compounds reported here were assayed two to eight times.

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