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Uracils as potent antagonists of the human gonadotropin-releasing hormone receptor without atropisomers

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Abstract—Uracil derivatives were designed and synthesized to avoid atropisomers observed in the 6-methyluracils as antagonists of the human GnRH receptor. Optimization at the 1- and 5-positions of the uracil resulted in potent compounds such as **24** (K_i = 0.45 nM).

Gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone-releasing hormone (LH-RH), is a linear decapeptide amide, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2. GnRH exerts its action by activating its cell surface receptor, a member of the class A G-protein-coupled receptor superfamily, in the pituitary to stimulate the secretion of the gonadotropins-luteinizing hormone (LH) and follicle-stimulating hormone (FSH).[1] These two hormones then act on the reproductive organs where they participate in the regulation of steroid production, gametogenesis, and ovulation.[2] Several disease conditions, such as endometriosis, uterine fibroids, and prostate cancer, can be treated by suppression of the pituitary–gonadal axis. GnRH peptide super-agonists, represented by leuprorelin,[3] based on a receptor down-regulation mechanism, are currently used clinically in the treatment of these conditions.[4] Recently, clinical evidence has shown that peptidic GnRH antagonists could directly lower gonadal sex hormone levels to alleviate these disease symptoms without the concomitant flare effect caused by super-agonists.[5] An orally bioavailable small molecule GnRH antagonist will have the advantage of flexible dosing and titration of drug concentrations, which may provide novel clinical management options.[6]

Previously, we reported that 6-methyluracils such as **1** (NBI 42902, Fig. 1) are potent and orally active antagonists of the human gonadotropin-releasing hormone receptor.[7] Compound **1** has been developed into clinical evaluation for potential treatment of GnRH-related diseases such as endometriosis and uterine fibroids. While the methyl group at the 6-position of uracil **1** provides the environment for an orthogonal relationship between the 5-aryl group and the uracil core, which is an important feature of pharmacophore for this class of compounds, it causes the carbon–carbon bond connecting the two aromatic rings to rotate slowly, as evidenced by both NMR and HPLC analyses for compound **1** and its close analogues. The interchange half-life for the two atropisomers (rotational diastereoisomers) of **1** is estimated to be 35 min at 25 °C.[8] While these inter-

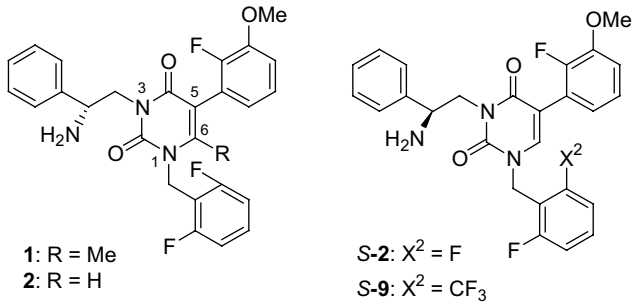

Figure 1. Structure of uracil analogues.

Keywords: GnRH; antagonist; Uracil; Atropisomer.
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changeable atropisomers with relative short half-lives equilibrate in solution and seem to be not an issue for development,[9] the atropisomerism of these compounds could cause some difficulties in term of manufacturing, such as batch to batch reproduciblility.

In our efforts to avoid the atropisomerism caused by 6-methyluracils, we synthesized a close analogue of **1** by deleting the 6-methyl group (Fig. 1). Thus, compound **2** exhibited a K_i value of 5.3 nM, which was about 10-fold less potent than **1** (K_i = 0.56 nM). However, **2** does not possess the atropisomeric property, as demonstrated by a single set of proton and carbon NMR signals. Compound **2** was also much easier to be crystallized than **1**. Interestingly, like the 6-methyluracil **1**, the crystal structure of **2** also displayed an orthogonal relationship between the 5-aryl group and the uracil core in solid state (Fig. 2). Since the 6-methyl group on the uracil also dictates the conformation of the 1-benzyl group, which is another important pharmacophoric element in this class of compounds, we conducted a study to modify the 1- and 5-substituents on the uracil ring in order to improve the potency. Here we report the results of this des-methyl series of compounds as GnRH receptor antagonists without atropisomers.

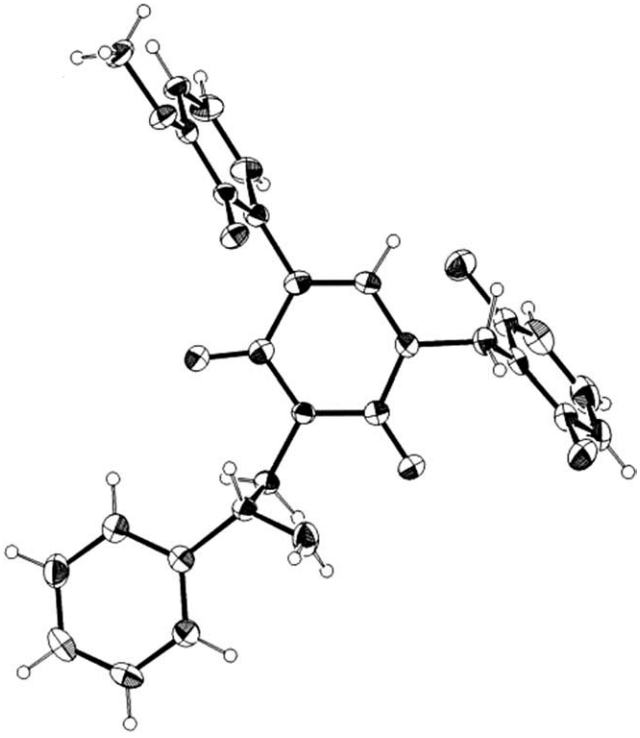


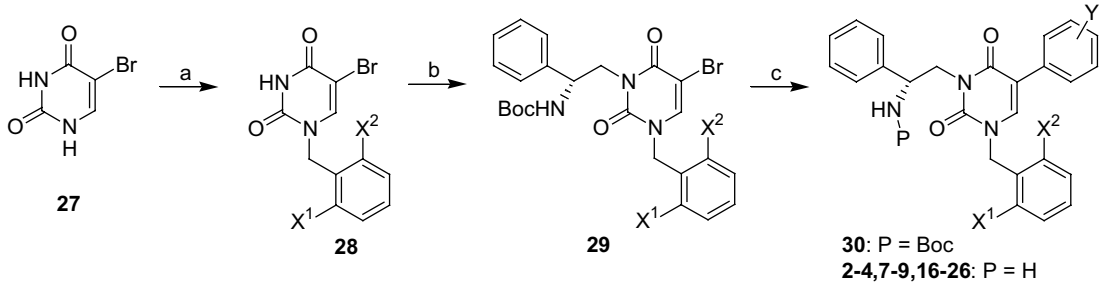
Figure 2. ORTEP drawing of the X-ray structure of compound **2**.[16]

The synthesis of these uracils started from the commercially available 5-bromouracil **27**, which was alkylated with different substituted benzyl bromides, promoted by *N*,*O*-bis(trimethylsilyl)acetamide (BSA), to provide intermediates **28**. A Mitsunobu coupling reaction of **28** with (*R*)-*N*-Boc-phenylglycinol (DEAD/PPh_3/THF) afforded the 1,3-dialkylated uracils **29**. Suzuki coupling reactions of **29** with various arylboronic acids ($Pd[PPh_3]_4/Na_2CO_3/H_2O$–dioxane/reflux) afforded the final products **2**–**4**, **7**–**9**, and **16**–**26**, after Boc-deprotection with trifluoroacetic acid (Scheme 1).[10]

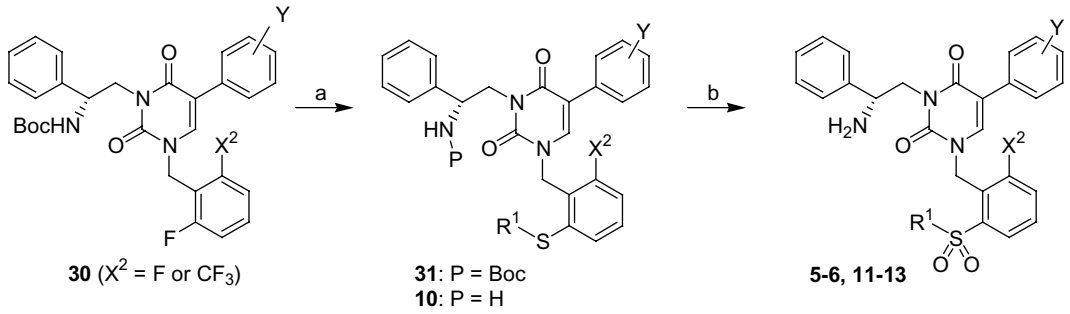
Nucleophilic displacement of the *ortho*-fluorine of intermediates **30** with alkylthiols promoted by a base such as sodium hydride afforded the sulfides **31**. The sulfide analogue **10** was obtained from Boc-deprotection with trifluoroacetic acid. Oxidation of **31** provided the corresponding sulfones **5**, **6**, and **11**–**13** in very good yields after deprotection (Scheme 2). Sulfonamides **14** and **15** were synthesized from **31** by oxidation with *N*-chlorosuccinamide in dichloromethane, followed by a coupling reaction with either dimethylamine or morpholine under basic conditions, and then TFA deprotection (Scheme 3).

The synthesized compounds were tested for their ability to displace a radiolabeled GnRH peptide in the GnRH receptor binding assay as previously reported,[7] and the results are listed in Table 1. Initial structure–activity relationship study of the non-methyl uracil analogues **2**–**4** and **7** suggests that substitutions on the 5-phenyl ring are very important for high affinity. Thus, in comparison with **2** (K_i = 5.3 nM), the 2-fluoro-3-hydroxyphenyl derivative **3** had a dramatic reduction in binding affinity (K_i = 660 nM). On the other hand, the 2-chloro-3-methoxyphenyl analogue **7** increased the potency about 5-fold (K_i = 1.0 nM). As expected, removal of the 3-methoxy group of **7** resulted in about 4-fold loss in affinity (**4**, K_i = 6.0 nM).

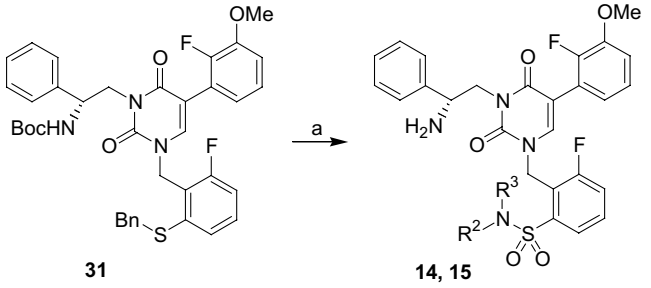
The substituted benzyl group at the 1-position of the uracil is also crucial for high affinity binding of these compounds to the GnRH receptor.[11] Based on our receptor



Scheme 1. Reagents and conditions: (a) BSA/$ArCH_2Br$/ACN/80 °C, 85%; (b) (*R*)-$PhCH(NHBoc)CH_2OH$/DEAD/Ph_3P/THF, 91%; (c) $ArB(OH)_2$/Pd(0)/Na_2CO_3; then TFA/CH_2Cl_2, 60–95%.



Scheme 2. Reagents and conditions: (a) R^1SNa/DMF/100 °C, 98% (R^1 = Me); (b) oxone/acetone/H_2O, 90%; then TFA/CH_2Cl_2, 90% (R^1 = Me).

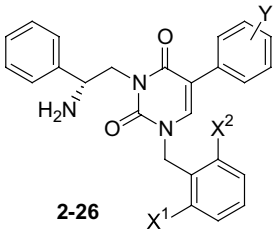


Scheme 3. Reagents and conditions: (a) i. NCS/DCM; ii. R^2R^3NH; iii. TFA/CH_2Cl_2, 40–50% overall yields.

modeling and mutagenesis studies, this group may bind in a pocket formed by three tyrosine residues (Tyr-283, 284, and 290) of the transmembrane domain six.[7,12] These results suggest an electron-deficient aromatic ring will be favored at this position for a strong interaction.[13] This is further supported by the affinity of **4** and **5**. The methylsulfone **5** had a K_i value of 1.3 nM, which is about 5-fold more potent than its fluorine analogue **4** (K_i = 6.0 nM). The increase in potency of **5** over **4** could be caused by a stronger electron-withdrawing effect of the methylsulfonyl group, and hence increasing the aromatic interaction of the substituted benzyl group with the tyrosine cluster. It is also possible that the large sized sulfone alters the benzyl group to a more favored position. Interestingly, the 2-trifluoromethyl-6-methylsulfonylbenzyl analogue **6** (K_i = 3,100 nM) exhibited a large reduction in binding affinity from **5**.

To further explore the role of the 1-benzyl group, a series of 2-fluorobenzyl with a different substituent at the 6-position of the phenyl ring were synthesized and tested (compounds **8**–**15**). The chlorinated derivative **8** (K_i = 1.4 nM) was about 4-fold better than the corresponding fluorinated analogue **2**. The trifluoromethyl **9** exhibited further improvement in potency (K_i = 0.64 nM). While **10** with a weakly electron-donating methylsulfide had a K_i of 19 nM, which is about 4-fold less active than the fluoro analogue **2**, the corresponding sulfone **11** exhibited over 20-fold increase in affinity from **10**. The binding affinity of the isopropylsulfone **12** decreased 33-fold from the methyl analogue **11**. This result demonstrates a size-limitation at this position, which is further supported by compounds **13**–**15**, which were all less potent than **11**.

Table 1. SAR of uracil analogues with different 1-benzyl and 5-aryl groups



Compd	X^1	X^2	Y	K_i (nM)[a]
1				0.56
2	F	F	2-F–3-OMe	5.3
S-**2**	F	F	2-F–3-OMe	570
3	F	F	2-F–3-OH	660
4	F	F	2-Cl	6.0
5	F	SO_2Me	2-Cl	1.3
6	CF_3	SO_2Me	2-Cl	3100
7	F	F	2-Cl–3-OMe	1.0
8	F	Cl	2-F–3-OMe	1.4
9	F	CF_3	2-F–3-OMe	0.64
S-**9**	F	CF_3	2-F–3-OMe	55
10	F	MeS	2-F–3-OMe	19
11	F	SO_2Me	2-F–3-OMe	0.90
12	F	SO_2Pr-*i*	2-F–3-OMe	30
13	F	$SO_2CH_2CH_2OH$	2-F–3-OMe	9.1
14	F	SO_2NMe_2	2-F–3-OMe	120
15	F	SO_2morphlinyl-1	2-F–3-OMe	1400
16	F	CF_3	H	7.8
17	F	CF_3	2-F	8.0
18	F	CF_3	2-Cl	1.2
19	F	CF_3	2-CF_3	4.0
20	F	CF_3	2-F–3-OH	18
21	F	CF_3	2-F–3-OEt	1.8
22	F	CF_3	2-F–3-OCH_2CF_3	8.2
23	F	CF_3	2-F–3-OPr-*i*	6.3
24	F	CF_3	2-Cl–3-OMe	0.45
25	F	CF_3	2-Cl–4-Me	2.6
26	F	CF_3	2-Cl–4-CF_3	200

[a] Key compounds were tested for two or more times in independent experiments. K_i values were highly reproducible with an average standard deviation of less than 45% for replicate K_i determinations.

Having identified the optimally substituted benzyl group at the 1-position, we next studied the 5-phenyl group with different substitutions (compounds **16**–**27**). The un-substituted phenyl compound **16** exhibited a K_i of 7.8 nM, while the 2-fluoro analogue **17** possessed a similar K_i value (8.0 nM). These results are quite interesting,

since in the 6-methyluracil series, the 2-fluorine group causes about 4-fold increase in binding affinity. This may indicate that the small fluorine atom, without the 6-methyl group at the uracil ring, has little effect on orientating the 5-phenyl ring. The larger chlorine atom (compound **18**, $K_i = 1.2$ nM), however, increased the binding affinity about 6-fold over **16**. On the other hand, the trifluoromethyl group (**19**, $K_i = 4.0$ nM) had minimal impact. The hydroxy analogue **20** ($K_i = 18$ nM) was slightly less active than its parent **17** ($K_i = 8.0$ nM), but much less potent than the methoxy compound **9** ($K_i = 0.64$ nM), suggesting that the methoxy group is strongly favored. Further increasing the bulk over the methoxy group led to progressive decrease in binding affinity (compound **21**–**23**). Although the 2-chloro-3-methoxylphenyl compound **24** ($K_i = 0.45$ nM) possessed similar binding affinity to **9**, **24** ($IC_{50} = 0.53$ nM) was much more potent than **9** ($IC_{50} = 7.2$ nM) in the functional IP_3 turnover assay.[14] While adding a methyl group at the 4-position on the 5-phenyl ring did not improve binding affinity (**25**, $K_i = 2.6$ nM), incorporating a trifluoromethyl group actually dramatically reduced its potency (**26**, $K_i = 200$ nM). These results may imply that an electron-deficient phenyl ring is unfavored at this position.

Similar to the 6-methyluracil series, compounds derived from *R*-phenylalaninol were much more potent than their *S*-isomers (*S*-**2** and *S*-**9**, $K_i = 570$ and 55 nM, respectively). This strong stereopreference suggests that both the amino and the phenyl groups of this side-chain have major contributions to the binding energy.

These non-methyl uracil GnRH antagonists do not possess atropisomers as evidenced by a single set of NMR signals in different solvents at room temperature. No such atropisomeric property was observed even for derivatives of the 5-phenyl group substituted with a larger *ortho* group such as chlorine.

On the bases of computational modeling of the 3-D human GnRH receptor,[15] the binding site for this 5-phenyl group could be located in the proximity at the top part between helices 4 and 5, where two residues, Tyr-211 and Asn-212, are identified to project into the binding pocket. While the tyrosine aromatic ring might interact with this 5-phenyl group through π–π stacking, the asparagine could form hydrogen-bond with the 3-methoxyl group on the phenyl ring. Asn-212 has been known to be important for the architecture of the ligand-binding pocket based on mutagenesis and computational modeling studies, and it is proposed that Asn-212 interacts with pGlu1 of GnRH via hydrogen-bonding.[1b] In addition, alanine replacement of Tyr-211 results in a receptor, which is neither capable of ligand binding nor signal transduction by GnRH peptide.[16]

In conclusion, a series of uracils with no 6-methyl group was synthesized to address the atropisomerism of the 6-methyl analogues such as **1**. While initial des-methylation of 6-methyluracil **1** ($K_i = 0.56$ nM) caused about 10-fold reduction in binding affinity (**2**, $K_i = 5.3$ nM), structure–activity relationship studies at the 1- and 5-positions of the uracil core resulted in re-optimized compounds with subnanomolar potency (i.e., **24**, $K_i = 0.45$ nM). Importantly, these compounds do not possess atropisomeric property.

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16. CCDC 265975 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.