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Synthesis and structure–activity relationships of (*R*)-1-alkyl-3-[2-(2-amino)phenethyl]-5-(2-fluorophenyl)-6-methyluracils as human GnRH receptor antagonists

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Abstract—The synthesis of a series of (*R*)-1-alkyl-3-[2-(2-amino)phenethyl]-5-(2-fluorophenyl)-6-methyluracils is discussed. SAR around N-1 of the uracil was explored, which led to the discovery that an electron-deficient 2,6-disubstituted benzyl group is required for optimal receptor binding. The best compound from the series had binding affinity of 0.7 nM (K_i) for the human GnRH receptor, which was 8-fold better than the 2,6-difluorobenzyl analog.

Gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone-releasing hormone (LHRH), is a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2), which is produced and secreted by the hypothalamus in a pulsitile manner.[1,2] Through interaction with specific GnRH receptors in the pituitary, both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are released from this site. These hormones, in turn, regulate the production of steroids and gametes.

A number of disease states can be controlled via the regulation of this pituitary–gonadal hormonal axis, in particular endometriosis, uterine fibroids, and prostate cancer. Suppression of both FSH and LH production can be achieved via the agonism or antagonism of the GnRH receptor. Continuous activation ultimately leads to down regulation of the receptor and a number of peptide agonists are commercially available, represented by Leuprorelin®.[3] However, treatment with GnRH agonists initially leads to overproduction of both FSH and LH with a concomitant 'flare effect', which tends to exacerbate symptoms in patients. In contrast GnRH antagonists act immediately at the receptor, quickly suppressing the release of FSH and LH. A number of peptide antagonists are currently available, represented by Cetrotide™.[1] Due to the very low oral bioavailability of these peptides, administration is normally via injection or depot formulation. In response to the need for a more convenient route of administration, intensive efforts have been initiated toward the development of orally bioavailable small-molecule GnRH antagonists.[4]

We had previously reported the discovery of a new class of uracils as orally bioavailable, human GnRH [hGnRH] receptor antagonists exemplified by 3-(2-aminoethyl)-5-aryl-6-methyluracils **3**,[5] which were evolved from 6-aminomethyl-7-aryl-pyrrolo[1,2-*a*]pyrimid-4-ones **1**[6] and 2-aryl-3-aminomethyl-imidazolo[1,2-*a*]pyrimid-5-ones **2a**,**b**[7] (Fig. 1). The synthetic route developed for **3** allowed for rapid SAR studies around the 3-position (of **3**) and potent analogs such as **4** were identified. We were also particularly interested in varying the substituent at N-1. Due to the synthetic route employed,[5a] in which this substituent was introduced early on (Fig. 2), only a very small number of variations at N-1 were screened. In this letter we report on an alternative synthesis of highly substituted 6-methyluracils, in which the N-1 substituent is introduced in the last step. This modification subsequently enabled us to efficiently

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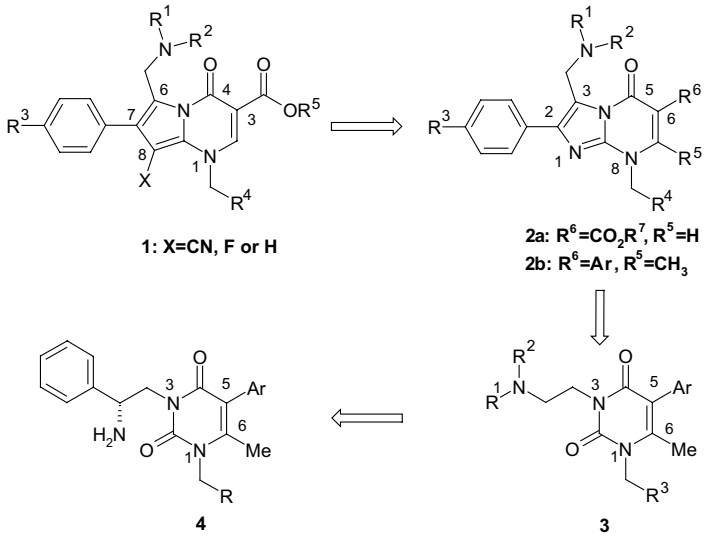


Figure 1. General structures of GnRH antagonists.

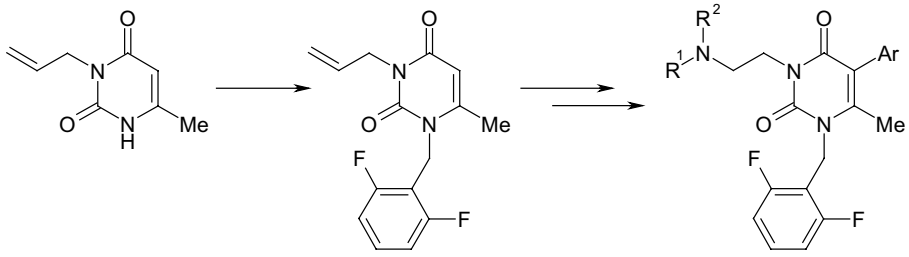


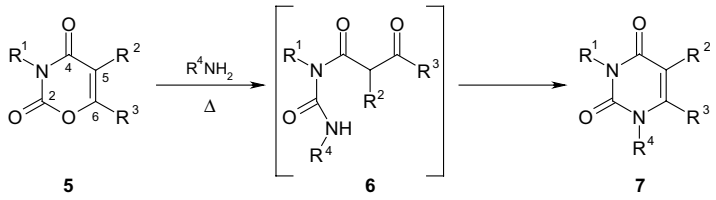
Figure 2. Previous synthetic route employed.

explore the SAR around N-1 and the results from this study are described.

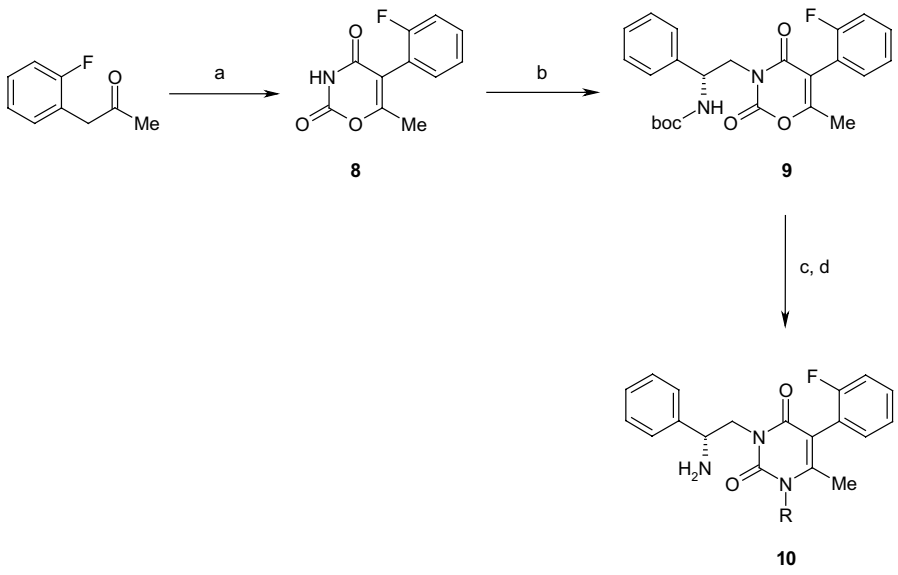
The synthesis of substituted uracils via the condensation of primary amines with 1,3-oxazine-2,4-diones had been reported previously by a number of groups.[8] Reaction of ammonia[8a,b,k] or primary alkyl amines[8b–i,k] with 1,3-oxazine-2,4-diones proceed efficiently under relatively mild conditions to yield the corresponding N-1(H) or N-1(alkyl) uracils, respectively. As outlined in Scheme 1 nucleophilic addition of the amine nitrogen to C-2 of the 1,3-oxazin-2,4-dione ring (**5**) with subsequent ring opening presumably leads to intermediate **6**,[8g] which upon loss of water results in formation of uracil ring **7**. Due to the much reduced nucleophilicity of primary aryl amines, acid catalysis and/or high temperatures are normally required to facilitate their reaction with 1,3-oxazine-2,4-diones.[8j]

We proposed that synthesis of the novel and highly substituted 1,3-oxazine-2,4-dione **9** (Scheme 2) and its subsequent condensation with a variety of primary alkyl amines to give the corresponding uracils, would enable us to rapidly screen a large number of substituents at N-1. Reaction of (2-fluorophenyl)acetone with chlorosulfonyl isocyanate (CSI), according to the general method previously described by Hassner and co-workers[9] gave 5-(2-fluorophenyl)-6-methyl-1,3-oxazine-2,4-(3*H*)-dione **8**, which was isolated in 33% yield.[10] Subsequent N-3 alkylation of **8** by (*R*)-*N*-Boc-2-phenylglycinol, via a Mitsunobu protocol, gave 1,3-oxazine-2,4-dione **9** in 81% yield. Reaction of 1,3-oxazine-2,4-dione **9** with alkyl amines at 100 °C for 4 h followed by *N*-Boc deprotection, gave the corresponding uracils (**10**), which were isolated in 5–60% yield as the trifluoroacetate salts.[11] Primary aryl amines and very sterically hindered primary alkyl amines failed to give the desired uracil products under these conditions.

Over 100 6-methyluracils (**10**) were prepared using the methodology described and a wide variety of substitution at N-1 explored. A selection of compounds (**11**–**36**) is listed in Tables 1 and 2. All 6-methyluracil derivatives **11**–**36**, were assayed for their ability to bind competitively to the cloned hGnRH receptor expressed in HEK293 cells, using a 96-well filtration apparatus[12,13] and des-Gly[10] [^{125}I-Tyr,[5] DLeu,[6] NMeLeu,[7] Pro[9]-NEt]GnRH as the radiolabeled ligand. Selected results are summarized in Tables 1 and 2. The functional antagonism of the described compounds was confirmed by their ability to inhibit GnRH stimulated Ca^{2+} flux in the transfected cells in a dose-dependent manner (Fig. 3).[7c]



Scheme 1. Mechanism of reaction for the synthesis of uracils from 1,3-oxazin-2,4-diones.

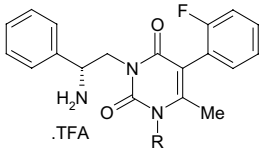


Scheme 2. Reagents and conditions: (a) chlorosulfonyl isocyanate, Et_2O, rt, 12 h, 33%; (b) (*R*)-*N*-Boc-2-phenylglycinol, DEAD, PPh_3, THF, rt, 12 h, 81%; (c) 1° Alkyl amine, neat, 100 °C, 4 h; (d) TFA, DCM, rt, 2 h, 5–60% over two steps.

From this study, it is clear that the N-1 substituent of 6-methyluracil **10**, participates in a key binding interaction with the hGnRH receptor. For instance, alkyl substituents containing polar groups as illustrated by **11**–**13** (Table 1), resulted in compounds with poor affinity for the receptor. In particular those compounds with a basic amine (**11** and **12)** and/or hydrogen bond donating group in this region (**12** and **13**) were not well tolerated. The introduction of lipophilic functionality, such as small alkyl and cycloalkyl groups, improved potency (as indicated by compounds **15**–**19**). Uracil **19** (which contains cyclohexyl methyl at N-1) had a K_i value of 200 nM and was approximately 5-fold more potent than the corresponding cyclopropyl methyl compound (**15**). This suggested that not only was lipophilicty important, but both the size and shape presented by cyclohexyl methyl to the binding pocket were also key factors. The introduction of pyridylethyl (**20**) or phenethyl (**24**) gave compounds with low affinity for the receptor (>10,000 and 1000 nM, respectively). However, moving the aromatic group toward the uracil core (by one carbon atom) resulted in markedly enhanced binding affinities (**21**–**23**, **25**, and **26**). This data suggests that an important aromatic π–π interaction between a certain residue in this region of the receptor and ligand must be taking place. In order to fully determine the nature of this interaction, a number of compounds containing a substituted benzyl ring at N-1 (**27**–**36**, Table 2) were prepared. It was clear that 2-substituted benzyls were preferred,[14] 2-fluorobenzyl (**29**) being approximately 3-fold more potent ($K_i = 20$ nM) than the corresponding 3- and 4-substituted isomers (**28** and **27**, respectively). The results also indicated that an electron-deficient ring was preferred (compare compounds **30**–**33**). Compound **30** (2-methoxybenzyl) was approximately 60-fold less potent than that containing 2-(trifluoromethyl)benzyl (**33**, $K_i = 4$ nM). This may suggest that an important π–π charge-transfer complex is being formed between the benzyl ring and an electron-rich aromatic, such as a tyrosine residue located in the binding pocket.[15] The addition of a second electron-withdrawing substituent at position 6 of the benzyl ring, further improved binding affinity (**34** and **35**). Compound **35** proved to be the most potent 6-methyluracil discovered from this study ($K_i = 0.7$ nM). Moving the 6-fluoro substituent of compound **35** to the 4-position of the benzyl ring (**36**) led to significant loss in potency, up to 50-fold ($K_i = 33$ nM), thus highlighting the importance of the 2,6-disubstitution pattern.

In summary, we have prepared a series of novel *N*-1-alkyl-*N*-3-[2-(2-amino)phenethyl]-5-(2-fluorophenyl)-6-

Table 1. Binding affinities of *N*-1-alkyl-6-methyluracils **11**–**26** toward the hGnRH receptor



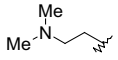
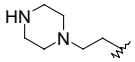
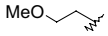
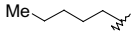
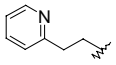
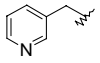
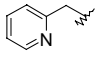
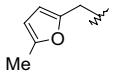
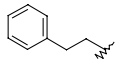
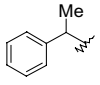
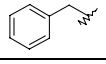
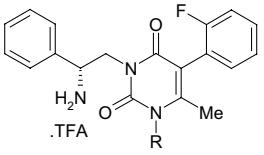
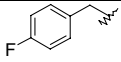
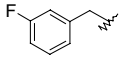
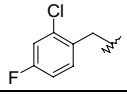
Compd	R	K_i (nM)
11		>10,000
12		>10,000
13		>10,000
14		1700
15		1100
16		500
17		260
18		230
19		200
20		>10,000
21		700
22		77
23		430
24		1000
25		140
26		57

Table 2. Binding affinities of *N*-1-benzyl-6-methyluracils **27**–**36** toward the hGnRH receptor



Compd	R	K_i (nM)
27		75
28		67
29		20
30		230
31		35
32		27
33		4
34		6
35		**0.7**
36		33

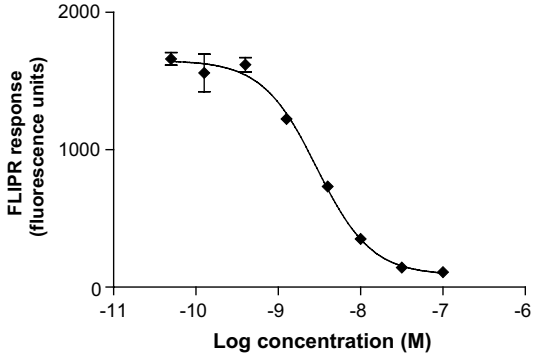


Figure 3. Inhibition of GnRH (5 nM) stimulated Ca^{2+} release, by compound **35**. Compound **35** has a measured IC_{50} of 2.8 nM.

methyluracils, via the condensation of a variety of alkyl amines with a novel and highly substituted 1,3-oxazin-2,4-dione. A number of these compounds have been shown to be potent antagonists of the hGnRH receptor. The SAR around N-1 of the uracil ring was explored, which led to the discovery that an electron-deficient 2,6-disubstituted benzyl group was preferred for optimal binding with the receptor. In addition the 2-chloro-6-fluorobenzyl group was found to be 8-fold more potent than the previously favored 2,6-difluorobenzyl group. We believe that the benzyl group is sitting in a lipophilic binding pocket and is involved in an important π–π charge-transfer interaction with a tyrosine residue of the receptor.

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10. The remaining material contained unidentified decomposition products.
11. Typical experimental procedure for the preparation of 6-methyluracils from 1,3-oxazin-2,4-dione **9**: Synthesis of (*R*)-*N*-3-[2-(2-amino)phenethyl]-*N*-1-(2-chloro-6-fluorobenzyl)-5-(2-fluorophenyl)-6-methyluracil trifluoroacetate **35**; a mixture of 1,3-oxazin-2,4-dione **9** (40 mg, 0.091 mmol) in neat 2-chloro-6-fluorobenzylamine (160 mg, 1.0 mmol) was heated at 100 °C in a sealed vial for 4 h. The reaction was allowed to cool to rt and trifluoroacetic acid (1 mL) was slowly added. After stirring for a further 2 h, the mixture was concentrated in vacuo. Direct purification via preparative LC–MS afforded **35** (23 mg, 43%) as a colorless oil; ^{1}H NMR δ_H (300 MHz; $CDCl_3$) 6.91–7.37 (12H, m), 5.47 and 5.35 (1H, d, $J = 16.5$ Hz), 5.24 and 5.14 (1H, d, $J = 16.5$ Hz), 4.44–4.65 (2H, m), 4.07 (1H, m), 2.06 (3H, s); HRMS calcd for $C_{26}H_{22}ClF_2N_3O_2$ 482.14469 (M+H). Found: 482.14354 (M+H).
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13. 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.
14. One possible explanation is that the presence of the 6-methyl group on the uracil ring may (for steric reasons) help orient the 2-substituted benzyl ring into the preferred conformation, this being out of the plane of the uracil ring. This steric interaction between the 6-methyl (of the uracil) and the benzyl group is not as pronounced in compounds **27** and **28**.
15. Mutational studies performed on the human GnRH receptor suggest that tyrosine residues 283 and 284 (located on TM domain 6) are crucial for binding of both

peptide agonists and antagonists. See: Hövelmann, S.; Hoffmann, S. H.; Kühne, R.; Laak, T.; Reiländer, H.; Beckers, T. *Biochemistry* **2002**, *41*, 1129. Based on the docking study using a GnRH homology model obtained from the crystal structure of b-rhodopsin, a possible interaction was observed between this benzyl group and tyrosine 283 or tyrosine 284. Unpublished results.