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A Novel Synthesis of 7-Aryl-8-fluoro-pyrrolo[1,2-*a*]pyrimid-4-ones as Potent, Stable GnRH Receptor Antagonists

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Abstract—A new class of small molecule GnRH antagonists, the 7-aryl-8-fluoro-pyrrolo[1,2-*a*]pyrimid-4-ones, was designed and a novel synthesis for these compounds was developed. The synthesis utilizes a base-catalyzed intramolecular cyclization of fluoromethyl pyrimidone **5** to generate the bicyclic core. Amongst the compounds synthesized, we discovered some highly potent GnRH receptor antagonists (e.g., **12**, $K_i = 9$ nM), which showed enhanced stability towards acidic physiological conditions compared to the des-fluoro analogues.

Gonadotropin-releasing hormone (GnRH) is a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) produced in and secreted by the hypothalamus in a pulsatile manner.[1] Through interactions at specific GnRH receptors, it stimulates the gonadotrophes in the pituitary gland to release follicle stimulating hormone (FSH) and luteinizing hormone (LH). These gonadotropins, in turn, regulate the production of sex steroids and gametogenesis. Antagonism of the GnRH receptor in the pituitary gland, therefore, constitutes a valid and effective way to treat diseases that are reproductive in nature, such as endometriosis, uterine fibroids and prostate cancer. Currently, these conditions are treated with peptide agonists, represented by Leuprorelin®.[2] Even though these are effective, they have to be administered in depot form since such peptides are not orally bioavailable. In response to that need, intensive efforts have been initiated for the development of small molecule GnRH antagonists.[3,4]

Very recently, we described our initial efforts towards potent, orally bioavailable small molecule GnRH antagonists, as exemplified by the 7-aryl-8-cyano-pyrrolo[1,2-*a*]pyrimid-4-one **1**[5a] and 7-arylpyrrolo[1,2-*a*]pyrimid-4-one **2**[5b] (Fig. 1). In the case of **2**, despite its good potency, preliminary studies indicated that this class of compounds is relatively unstable under acidic conditions. Incubation of **2** with 0.2 N HCl at 37 °C for 2 h results in the fast disappearance of the parent compound and the formation of a major degradation product, which was identified as **3** (Fig. 2). This degradation results from an acid catalyzed retro-Mannich reaction on the basic side-chain. Compound **1**, on the other hand, was much more stable under the same conditions. We reasoned that the presence of the cyano group at the 8-position of the bicyclic system reduces its electron density, which is also felt by the basic side chain, resulting in a compound with increased stability towards acid. Introduction of the cyano group however, led to a decrease in potency in the order of 5–13-fold,[5a,b] possibly because of a steric encumberance with the

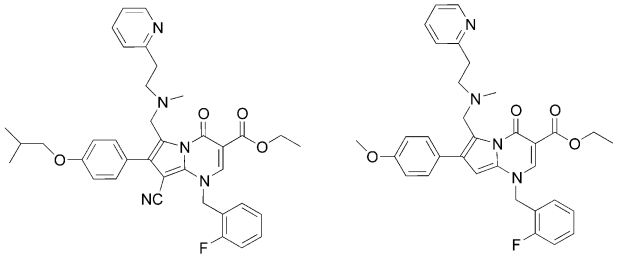

Figure 1.

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receptor and this moiety, when compared to the smaller hydrogen. A stable, more potent antagonist could be obtained if a smaller electron-withdrawing group, such as a fluoride is used. In this letter we report on the novel synthesis of 7-aryl-8-fluoro-pyrrolo[1,2-*a*]pyrimid-4-ones and their stability profile as potent GnRH receptor antagonists.

The synthetic approach is depicted in Scheme 1. α-Fluoroacetamidine hydrochloride was prepared in 32–43% by the action of HCl and EtOH on α-fluoroacetonitrile, according to a published procedure.[6] The acetamidine was then cyclized in the presence of diethyl ethoxymethylenemalonate, under basic conditions, to afford hydroxy pyrimidine **4**. This was then alkylated separately with α-bromo-3′-methoxyacetophenone or α-bromo-4′-isobutoxyacetophenone,[7] which were chosen based on previous knowledge from our work.[5] The reactions were performed in the presence of tetrabutylammonium fluoride (TBAF) as a base, to provide mixtures of the respective N^1-alkylated products **5a**–**b** and the *O*-alkylated side products **6a**–**b**, in 60 and 65% yield, respectively. After chromatographic separation, the ratios in both reactions were determined to be 2:1 in favor of the unwanted products **6a**–**b**. The structural assignment of regioisomers was performed by NOE experiments. **5a**–**b** were each then subjected to the key cyclization step in the presence of sodium ethoxide in ethanol, to give the desired core 7-aryl-8-fluoro-pyrrolo[1,2-*a*]pyrimidone **7a**–**b**. Each were alkylated with 2-

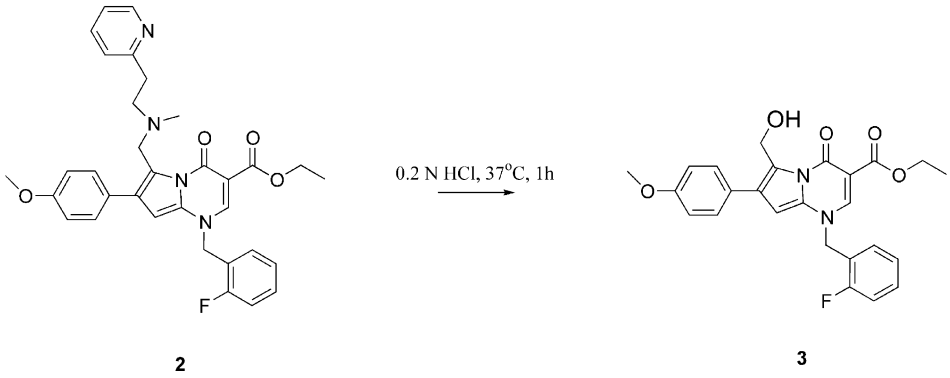
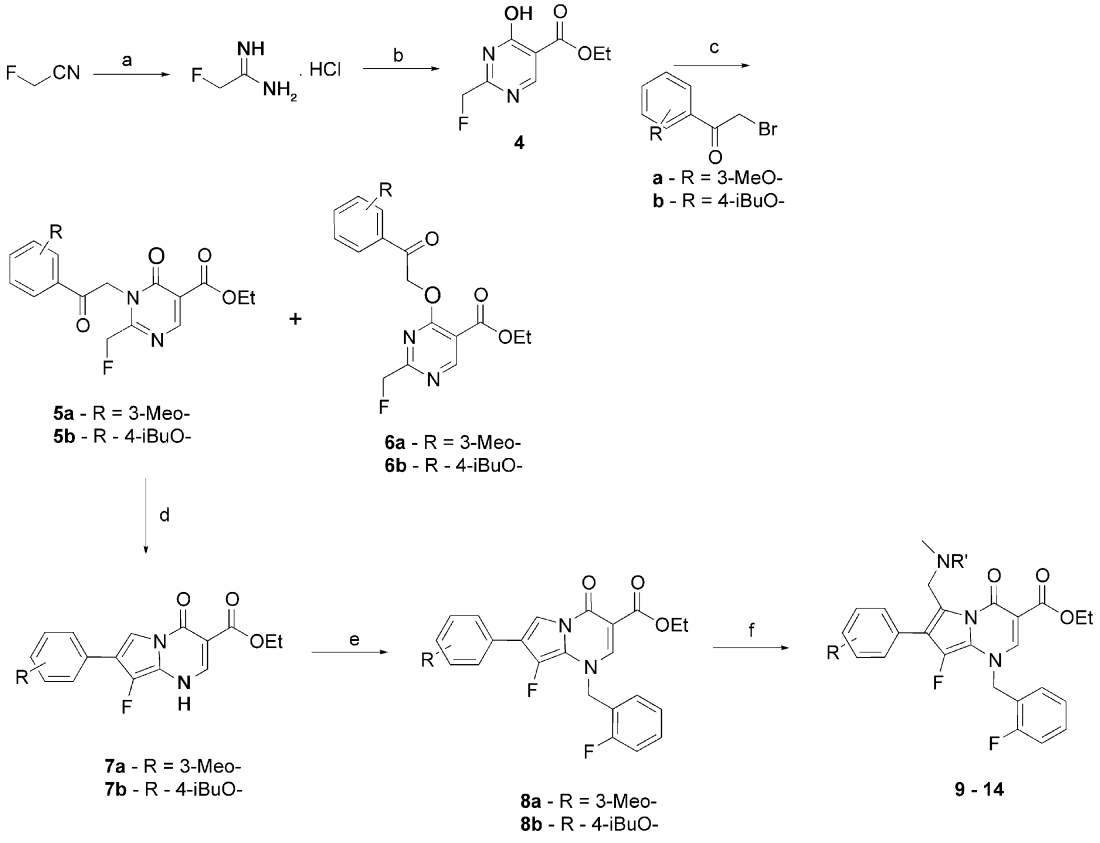


Figure 2. Degradation pathway of compound **2** in the presence of 0.2 N HCl at 37 °C.



Scheme 1. Reagents and conditions: (a) (i) HCl (g), 15 °C, 45 min; (ii) EtOH, −50 °C to rt, 16 h; (b) NaOH, EtOH, diethyl ethoxymethylene malonate, reflux, 7 h; (c) (i) TBAF, DME/THF, rt, 30 min; (ii) α-bromoacetophenones; (d) NaOEt, EtOH, rt, 4 h; (e) (i) TBAF, DME/THF, rt, 30 min; (ii) 2-fluorobenzyl bromide, 16 h; (f) R′NHMe, CH_2O aq, HOAc, rt, 1 h.

fluorobenzyl bromide, in the presence of TBAF, to give **8a–b** with combined yields over both steps of 25 and 30%. Finally, Mannich reactions with dialkylamines and formaldehyde in glacial acetic acid led to the target compounds **9–14**.

Compounds **9–14** were assayed for their ability to bind competitively to the cloned human GnRH receptor expressed in HEK293 cells using a 96-well filtration setup[8,9] and des-Gly^{10}[^{125}I-Tyr^{5}, DLeu^{6}, NMeLeu^{7}, Pro^{9}-NEt]GnRH as the radiolabel (Table 1). Their functional antagonism was confirmed by their ability to inhibit GnRH stimulated Ca^{2+} flux in a dose-dependent manner.[10] For instance, 1 μM of **12** completely blocked the influx of Ca^{2+} ions after stimulation with 10 nM of GnRH (Fig. 3).

The most potent compound in this series is **12**, which shows a K_i value of 9.0 nM. In general, compounds containing the 7-(4′-isobutoxy)aryl group were 20–260-fold more potent than their analogues with 7-(3′-methoxy)aryl, suggesting a possible lipophilic interaction with the receptor. In the 7-(4′-isobutoxy)aryl series, replacement of the (2-pyridyl)ethyl group on the basic nitrogen by either a benzyl (**13**) or a (2-furyl)methyl (**14**) led to a decrease of 5- and 10-fold in potency, respectively. This trend was also confirmed with compounds **9–11**, where the differences were even greater. When compared to the 8-cyano analogue **1**,[5a] the 8-fluoropyrrolopyrimidone **12** shows 10-fold increase in affinity for the GnRH receptor. This difference confirms our initial

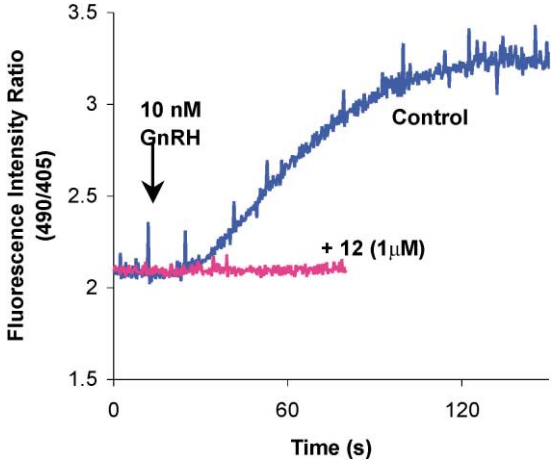
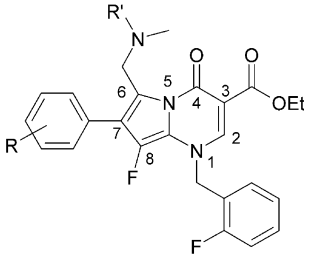
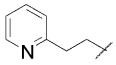
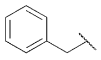
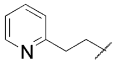
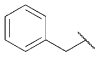


Figure 3. Inhibition of GnRH stimulated Ca^{2+} release by compound **12**.

Table 1. Binding affinities of 8-fluoro-pyrrolo[1,2-*a*]pyrimidones **9–14** towards the human GnRH receptor[9]



Compd	R	R′	K_i (nM)
9	3-MeO–		200
10	3-MeO–		2600
11	3-MeO–		24,000
12	4-iBuO–		9
13	4-iBuO–		42
14	4-iBuO–		92

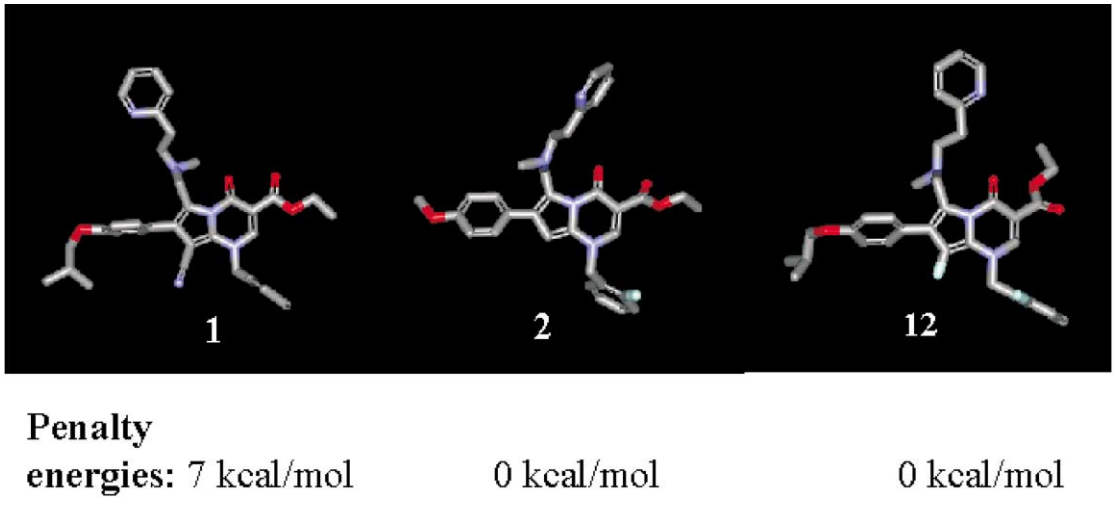


Figure 4. Alignment of minimum energy conformations of compounds **1**, **2** and **12**.

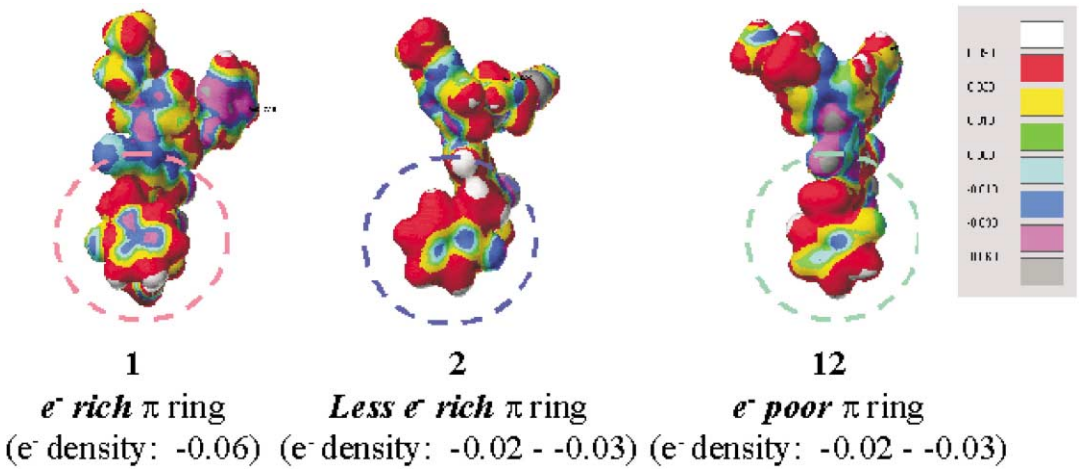


Figure 5. Electron density surface for compounds **1**, **2** and **12**. The 1-(2-fluorobenzyl) groups are highlighted in the circles. The surfaces are colored to reflect the electostatic potentials, with color changes indicating their magnitude and polarity. The gray and cyan represent the richest electron density, whereas the white means poorest electron density domain, according to the legend on the right.

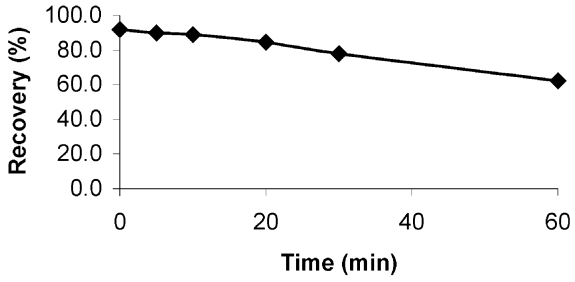


Figure 6. Stability curve of compound **12** after incubation with 0.2 N HCl for 2 h at 37 °C.

hypothesis that only a small substituent at the 8-position of the bicyclic system is tolerated so as to avoid an unfavorable steric interaction with the receptor. Another aspect of the presence of a small substituent at the 8-position is its impact on the orientation of the 1-(2-fluorobenzyl) group. The conformations of compounds **1**, **2** and **12** were compared using AM1/semi-empirical quantum mechanics. The most preferred conformation of **12** was used as a template to align with conformers of **1** and **2**, and the relative differences in energies between these conformers were then calculated (Fig. 4). The depicted conformations of **2** and **12** are energetically very similar (~0 kcal/mol difference), whereas the one for **1** is approximately 7 kcal/mol higher in energy. In the case of **2** and **12**, the 1-(2-fluorobenzyl) group can reside perpendicularly on either face of the plane established by the bicycle (Fig. 4), whereas in the case of **1**, the presence of the larger 8-cyano substituent forces the ring to adopt different orientations, which may not be the preferred mode of binding. This observation may help explain the differences in affinity of **1**,**2** and **12** for the GnRH receptor.

In addition to the conformational aspect, we conducted semi-empirical calculations using the MOPAC/AM1 method from 'Quantum CAChe 5.0',[11] in order to investigate other possible causes for the better affinity exhibited by **12** and **2** over **1**. The charge density surfaces of compounds **1**,**2** and **12** are presented in Figure 5,[12] and provide another useful qualitative tool to help rationalize the experimental data. The impact of the substituent at the 8-position on the electronics of the bicyclic core is not surprising—**1** and **12** are more electron-deficient than **2**. In fact, we deliberately used this notion to improve their stability towards acid, as alluded to earlier. Less obvious is the effect of the 8-substituent on the electronics of the 1-(2-fluorobenzyl) group. In the case of 8-cyano compound **1**, the charge density on the 1-(2-fluorobenzyl) group is the highest, followed by unsubstituted compound **2**. The 1-(2-fluorobenzyl) moiety of the 8-fluoro substituted pyrrolo-pyrimidone **12** has the lowest charge density among these compounds, which may help explain why it is a better binder. According to a 3-D model of the human GnRH receptor built based on the X-ray crystal structure of rhodopsin,[13] it is believed that in the binding pocket, the 1-(2-fluorobenzyl) group may interact with one of the two tyrosine residues on the transmembrane VI (Y283 and Y284) of the human GnRH receptor, either by π stacking or by edge-to-face interactions. Since the aromatic ring on the tyrosine is electron-rich, the binding of a ligand is more energetically favorable if it possesses an electron-deficient aromatic.

The stability of compound **12** under acidic conditions was then determined (Fig. 6). As expected, **12** showed decreased degradation when incubated with a 0.2 N HCl aqueous solution at 37 °C for up to 1 h—up to 63% of the parent compound remained after this time. This is in contrast to stability of **2**, which after 1 h showed more than 90% degradation.[14]

In conclusion, we have designed and achieved the novel synthesis of 7-aryl-8-fluoro-pyrrolo[1,2-*a*]pyrimid-4-ones, which behave as potent antagonists of the human GnRH receptor. In addition, we have demonstrated that introduction of a small electron-withdrawing group, such as a fluoride, is able to stabilize the pyrrolo[1,2-*a*]pyrimidone core, while maintaining the potency towards the human GnRH receptor.

Acknowledgements

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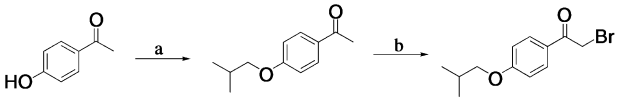
7. α-Bromo-3′-methoxyacetophenone was purchased from Aldrich Chemical Co. α-Bromo-4′-isobutoxyacetophenone was prepared in two steps from 4-hydroxyacetophenone:



Reagents and conditions: (a) K_2CO_3, EtOH, 1-bromo-2-methylpropane, reflux, 48 h (41%); (b) $CuBr_2$, EtOAc/$CHCl_3$, reflux, 2 h (89%).
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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. Key compounds were assayed in 3–8 independent experiments.
10. HEK293 cells stably expressing the hGnRH receptor were loaded with the calcium sensitive dye Indo-1, then pre-incubated with the compounds or vehicle control for 1 min prior to stimulation with 10 nM GnRH. Calcium mobilization was measured by the change in fluorescence intensity ratio (490 nm/405 nm) following excitation at 350 nm.
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12. The charge density surfaces of compounds **1**, **2** and **12**, represent the charge distribution on each molecule as the probability of electron availability. These were obtained directly from the conformations used in Figure 4, and may not represent the conformation of these compounds in the bound state. The charge density surfaces of compounds **1**, **2** and **12** are shown in colors according to the scale provided—the gray color at the bottom represents the highest possible electron density (−0.060, in arbitrary units) while the top white corresponds to the other end of the scale (0.090, in arbitrary units), with all the other colors in between.
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14. Unpublished results.