Design and Structure−Activity Relationships of 2-Alkyl-3-aminomethyl-6-(3-methoxyphenyl)-7-methyl-8-(2-fluorobenzyl)imidazolo[1,2-*a*]pyrimid-5-ones as Potent GnRH Receptor Antagonists

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SAR studies of 7-phenylpyrrolo[1,2-*a*]pyrimid-4-ones **1** and **2**, and 2-phenylimidazolo[1,2-*a*]-pyrimidines **3** and **4**, as nonpeptide human GnRH receptor antagonists, lead us to believe that the aromatic ring at position-2 of **4** is no longer crucial for the binding once an aryl group is incorporated at postion-6. We report here the use of a 2-alkyl group on the imidazolo[1,2-*a*]-pyrimidone core to generate potent GnRH receptor antagonists. This discovery enabled us to obtain smaller but equally potent GnRH receptor antagonists.

Introduction

Targeting gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone-releasing hormone (LHRH), receptor for the suppression of sex hormone levels has led to the successful clinical applications of peptide agonists and antagonists for a variety of diseases.[1,2] However, small molecule GnRH receptor antagonists could possess advantages over the existing peptidyl therapeutics due to their potential for oral administration.

To date, several classes of small molecule GnRH antagonists have been described in the literature.[3−6] Our group has discovered a series of 7-phenylpyrrolo-[1,2-*a*]pyrimid-4-ones **1** and **2**[5] and 2-phenylimidazolo-[1,2-*a*]pyrimidines **3** and **4**[6] (Figure 1) as nonpeptide human GnRH (hGnRH) receptor antagonists. In our efforts to optimize the biological activity of molecules, we found that the aromatic ring at position-2 of the imidazolo[1,2-*a*]pyrimidone **4** can be replaced with an alkyl group to generate potent GnRH antagonists. In this paper we report the synthesis and the SAR of 2-alkylimidazolo[1,2-*a*]pyrimidones as GnRH antagonists.

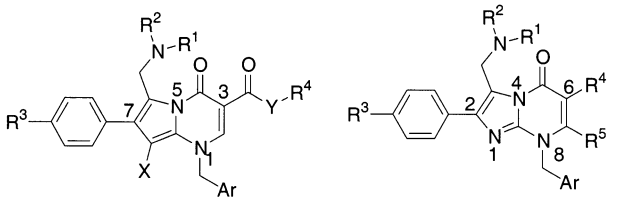

Figure 1. The general structures of **1**−**4**.

Chemistry

The synthesis of the 2-alkylimidazolo[1,2-*a*]pyrimid-5-one core structure was outlined in Scheme 1. 2-Amino-5-bromo-4-hydroxy-6-methylpyrimidine **5** was reacted with α-bromoketones (R^1COCH_2Br, **6**) in the presence of NaH in DMF to give the bicyclic products **7a**,**b** in good yield. This reaction required a bulky R^1 group, such as *tert*-butyl, to produce the sole desired products **7**. A small R^1 group, such as methyl, led to the formation of a mixture of unreacted **5** and overalkylated products **8** and **9** (based on LC-MS data). N-Benzylation of **7a** was accomplished by using 2-fluorobenzyl bromide in THF containing tetra-*n*-butylammonium fluoride (TBAF) to afford the desired product **10a** and a N^1-alkylation byproduct **11a**, which can be separated by flash column chromatography. However, in the case of **7b**, alkylation gave **10b** as the only isolated product (58% yield). The assignment of regioisomers **10a** and **11a** was confirmed by NOE NMR experiments. A clear NOE was observed between the 7-methyl group and the 8-benzylic protons for **10a**, while this effect was not seen for **11a**. Compounds **10a**,**b** were then subjected to Suzuki coupling conditions with 3-methoxylphenylboronic acid to give **12a**,**b**. Finally, Mannich reactions of **12a**,**b** with a variety of secondary amines (R^2R^3NH) yielded the desired products **13**−**31**. Alternatively, **12a** was formylated under Vilsmeier conditions ($POCl_3$/DMF) to afford the corresponding aldehyde **32** as described in Scheme 2. Reductive aminations of **32** with primarily amines (R^2NH_2) and $NaBH(OAc)_3$ gave **33**−**37** as the final products. Compound **12b**, in which R^1 was a 1-methyl-1-methoxycarbonylethyl group, was further modified as depicted in Scheme 3. Ester **12b** was hydrolyzed with KOH in a mixture of water and methanol at 50 ° C to give **38**, which was reduced with borane to yield the corresponding alcohol **39**. **39** was reacted with 2-(methylaminomethyl)pyridine and formaldehyde in acetic acid to give the amino product **40**. As shown in Scheme 4, **39** was also subjected to a Swern oxidation to give the

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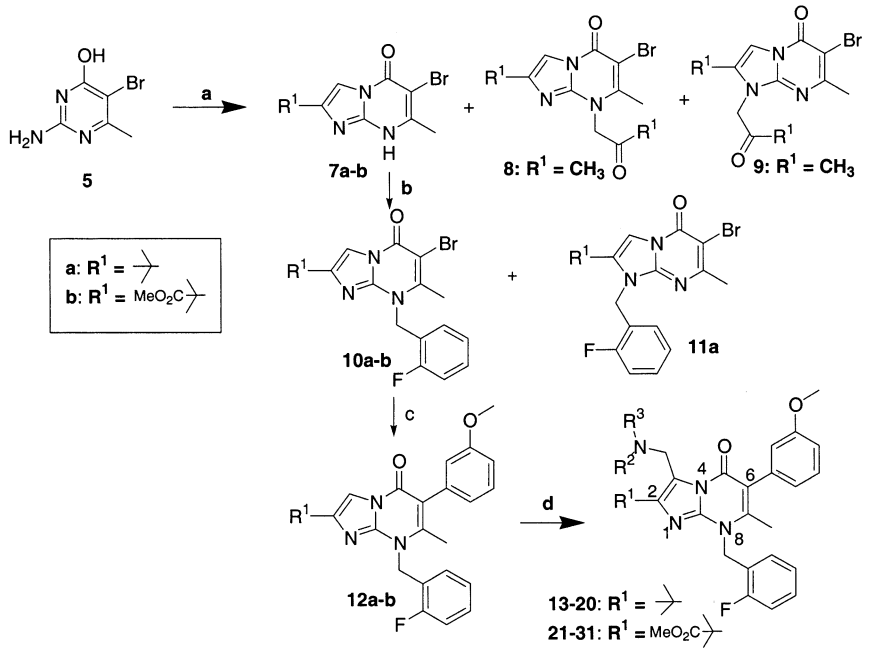
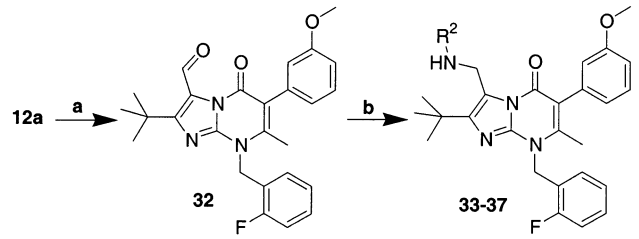
Scheme 1[a]



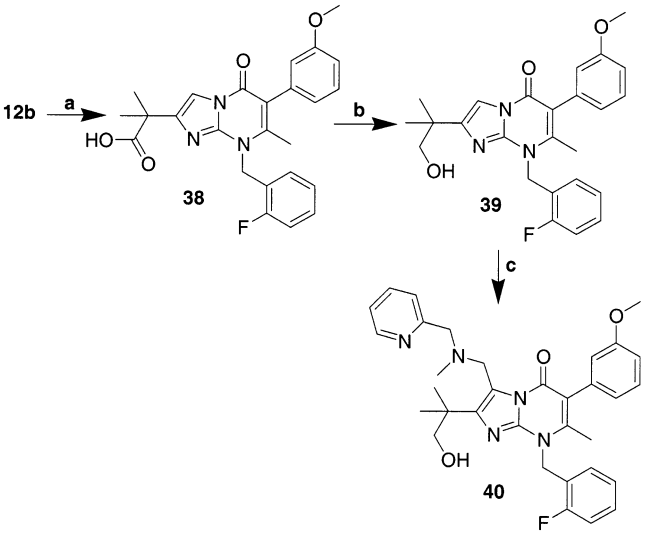
[a] (a): R^1COCH_2Br (**6**), NaH, DMF; (b): 2-fluorobenzyl bromide, TBAF, THF; (c): 3-methoxyphenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene, H_2O; (d): R^2R^3NH, CH_2O in water, HOAc.

Scheme 2[a]



[a] (a): $POCl_3$, DMF; (b): R^2NH_2, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$.

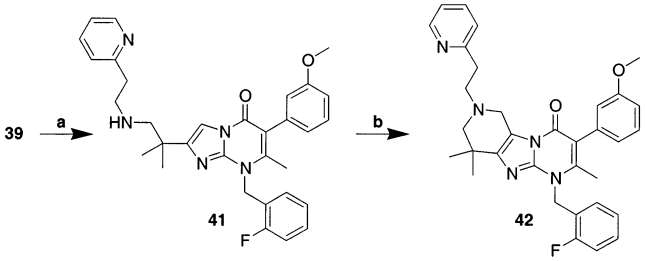
Scheme 3[a]



[a] (a): KOH, MeOH, H_2O, 50°C; (b): BH_3, THF; (c): CH_2O/H_2O, 2-(*N*-methylaminomethyl)pyridine, HOAc.

corresponding aldehyde which, without further purification, reacted with 2-(2-aminoethyl)pyridine in the presence of $NaBH(OAc)_3$ to give the amine compound **41**. An intramolecular Mannich reaction with formaldehyde yielded the tricyclic product **42**.

Scheme 4[a]

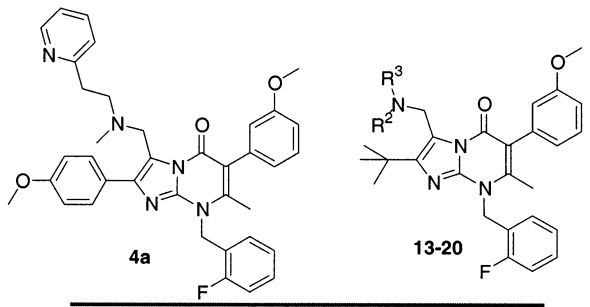


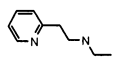
[a] (a): Swern oxidation, then 2-(2-aminoethyl)pyridine, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$; (b) CH_2O in water, HOAc.

Results and Discussions

Our earlier SAR studies had indicated that the para-substitution of a small lipophilic group with hydrogen bond acceptor such as isobutyramido (i-PrCONH) or isobutoxy group on the 7-phenyl of the pyrrolo[1,2-*a*]-pyrimidone or the 2-phenyl of the imidazolo[1,2-*a*]-pyrimidone (Figure 1) core was a crucial feature for obtaining highly potent GnRH antagonists.[5] However, the most recent SAR data[6] implied that when 3-methoxyphenyl was incorporated to position-6 of the imidazolo[1,2-*a*]pyrimidone core structure to replace the ester or amide group, the para-substituent at the 2-phenyl of imidazolo[1,2-*a*]pyrimidone played a much less important role in binding. We then further postulated that the phenyl may not be optimal for high potency since the 3-methoxyphenyl at position-6 may now have subtly reoriented the molecule so that further optimization may be beneficial. To test this hypothesis, a *tert*-butyl moiety was initially introduced at position-2 of the imidazolo[1, 2-*a*]pyrimidone core to replace the phenyl group. The binding affinities of such analogues are shown in Table 1. For easy comparison, the binding data of **4a** was also included in Table 1. Compound **13** having dimethylaminomethyl group at position-3 was only weakly active. As observed previously, replacement

Table 1. Binding Affinities of 2-*tert*-Butyl-6-(3-methoxyphenyl)-8-(2-fluorobenzyl)imidazolo[1,2-*a*]pyrimid-5-ones **13**−**20**



cmpd	R^2R^3N	K_i (nM)[a]
4a	---	4.6±0.2
13		1700± 81
14		5.2± 0.6
15		260± 23
16		430± 64
17		190± 21
18		72± 11
19		11± 1.9
20		7.9± 1.4

[a] K_i values for human GnRH receptor were the means ± SEM, determined from at least three independent experiments measured.

of one of the two *N*-methyl groups of **13** with a 2-(2-pyridyl)ethyl group resulted in a highly potent molecule (**14**, K_i = 5.2 nM), which is equally potent to **4a**, a 4-methoxyphenyl analogue. To determine whether the pyridyl nitrogen was responsible for higher binding affinity by acting as a second basic center, a simple diamine analogue **15** was prepared, but it was much less potent than **14** with a K_i value of 260 nM. Having a hydrogen bond acceptor in the same side chain (**16**) also failed to induce high potency. The binding data of **15** and **16** suggested the importance of the aromatic ring for binding. Introduction of a phenyl ring (**17**), a close analogue of **14** without the hydrogen bonding capability of the pyridine, also caused a loss in potency, although the benzyl substituent (**18**) was marginally more potent than **17** (K_i = 72 nM). Thus, the 2-pyridyl analogue **19** was prepared and proved to be a potent antagonist (K_i = 11 nM). Enhancement of the binding affinity by 2-pyridine in **14** and **19** may be explained by a hypothetical model shown in Figure 2. The nitrogen on the pyridine ring could enhance the interaction of the basic amine with an acidic residue of the GnRH receptor by forming a "five- or six-membered ring". At the same time

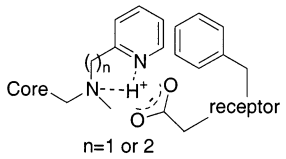
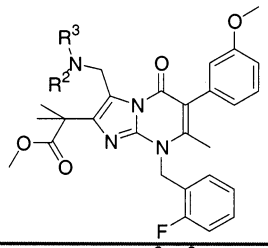
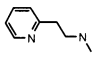
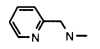
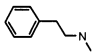
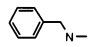
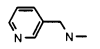
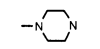
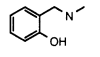
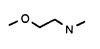
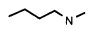
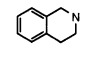
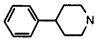


Figure 2. The hypothetical model for the interaction between compounds **14** or **19** with an acidic residue and an aromatic residue on the receptor.

Table 2. Binding Affinities of 2-(1-Methyl-1-methoxycarbonyl)ethyl-6-(3-methoxyphenyl)-8-(2-fluorobenzyl)imidazolo[1,2-*a*]pyrimid-5-ones **25**−**35**

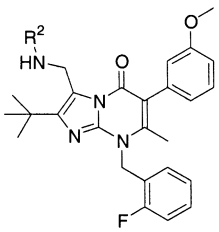


cmpd	R^2R^3N	K_i (nM)[a]
21		1.2 ± 0.2
22		4.2± 0.9
23		130± 15
24		59± 6.9
25		400± 22
26		7700± 660
27		490± 46
28		300± 29
29		320± 35
30		9200± 1000
31		5100± 380

[a] K_i values for human GnRH receptor were the means ± SEM, determined from at least 3 independent experiments.

the pyridine ring itself could provide aromatic $\pi-\pi$ interaction with a phenyl residue on the receptor.[7] Clearly none of the other less potent analogues could provide such dual interactions. Extension of the *N*-methyl group of **14** to *N*-ethyl (compound **20**) had no effect on binding affinity (K_i = 7.9 nM for **20** vs 5.2 nM for **14**). Table 2 summarizes the binding data for compounds **21**−**31** with the 1-methyl-1-methoxycarbonyl-ethyl group as the substituent at position-2 of the bicyclic core. SAR of these compounds is generally paralleled to the corresponding 2-*tert*-butyl series, although compounds in this series were more potent than their counterpart. As an interesting note, **25** was nearly

Table 3. Binding Affinities of 2-*tert*-butyl-6-(3-methoxyphenyl)-8-(2-fluorobenzyl)-imidazolo[1,2-*a*])-pyrimid-5-ones **33**−**37**



cmpd	R^2NH	K_i(nM)[a]
33		160± 23
34		240± 24
35		30± 9.3
36		430± 62
37		6400± 520

[a] K_i values for human GnRH receptor were the means ± SEM, determined from at least three independent experiments.

Table 4. Binding Affinities of 3-Aminomethyl-8-(2-fluorobenzyl)imidazolo[1,2-*a*]pyrimid-5-ones **40**−**43**

cmpd	K_i (nM)[a]
40	8.0 ± 0.5
41	350 ± 17
42	12000 ± 1600

[a] K_i values for human GnRH receptor were the means ±SEM, determined from at least 3 independent experiments.

100-fold less potent than its 2-pyridyl analogue **22**. Once again all these data again support the dual interaction model shown in Figure 2. Attempt to use 2-hydroxyphenyl as possible replacement of 2-pyridyl failed since compound **27** displayed only a modest binding affinity. Obviously the oxygen of **28** played no role in hydrogen bonding since compound **29** was equally potent to **28**. Attempts to constrain the phenyl ring caused a large reduction in potency (compounds **30** and **31**). Surprisingly, the *N*-desmethyl analogue of **14**, **33** (K_i = 160 nM) lost about 30-fold potency while the *N*-desmethyl analogues of **18** and **19**, **34** and **35** lost about 3-fold potency (Table 3). The reason for the decreased potencies of **33**, **34**, and **35** is not clear, but it could be attributed to the conformational difference in the side chains between the secondary amines and the corresponding tertiary amines. Furthermore, saturated cycloalkyl compounds **36** and **37** proved to be only weak binders. As shown in Table 4, the alcohol **40** was as potent as **19**. The cyclic derivative **42** lost binding affinity completely, presumably due to the relative orientation of the basic amine and pyridine side chain resulting in an undesired conformation for interaction with the receptor. These results offer us some hints about the required conformation for high potent antagonists.

To ensure that these potent molecules were functional antagonists, compound **14** was selected for testing its ability to inhibit Ca^{2+} influx induced by GnRH. The result indicates that **14** is a potent functional antagonist with IC_{50} = 27 nM (Figure 3, in Supporting Information).

Conclusion

We have discovered that once an optimal 3-methoxyphenyl was attached at position-6, position-2 of the imidazolo[1, 2-*a*]pyrimidone did not require a para-substituted aromatic ring such as *p*-butyramidophenyl or *p*-methoxyphenyl to achieve high binding affinity to the receptor. This modification led to potent compounds with reduced molecular weights. The SAR results also implied that even the alkyl group at this position might not be required since the modifications with several different alkyl groups produced similarly potent compounds. This hypothesis has been investigated, and the results will be published elsewhere.

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Supporting Information Available: Binding assay and functional assay; synthetic procedures and characterization of all compounds; molecular modeling of compounds **14** and **42**. This material is available free of charge via the Internet at http://pubs.acs.org.

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