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A Novel Synthesis of 2-Arylpyrrolo[1,2-*a*]pyrimid-7-ones and Their Structure–Activity Relationships as Potent GnRH Receptor Antagonists

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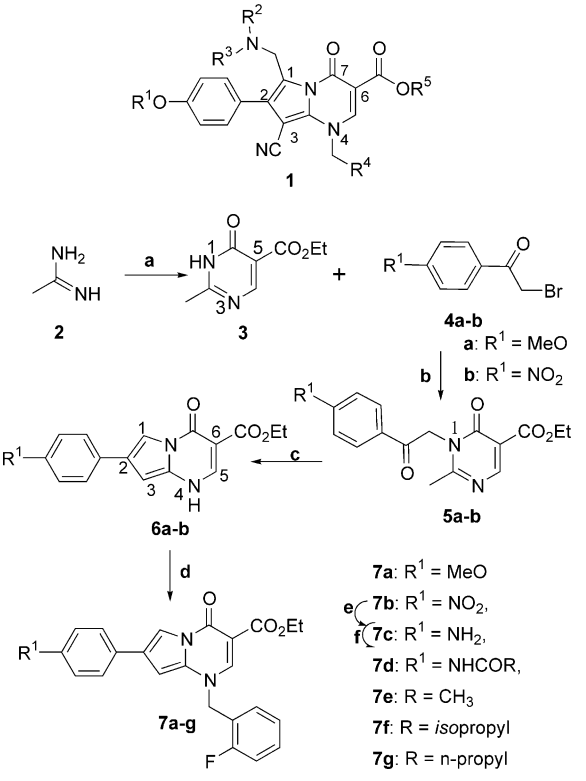
Abstract—In the process of developing GnRH receptor antagonists, a novel base-catalyzed cyclization of compounds **5a**–**b** was discovered, which led to the formation of the 2-aryl pyrrolo[1,2-*a*]pyrimid-7-one core stuctures **6a**–**b**. These intermediates were further modified at positions 1, 2, 4 and 6 to afford a series of potent GnRH antagonists with low nanomolar K_i values. © 2002 Elsevier Science Ltd. All rights reserved.

In the previous letter,[1] we discussed the initial SAR study of a novel series of 1-aminomethyl-2-aryl-3-cyano-pyrrolo[1,2-*a*]pyrimid-7-ones (**1**) as human gonadotropin-releasing hormone (hGnRH) receptor antagonists. Here, we report a novel synthesis of the bicyclic 2-arylpyrrolo[1,2-*a*]pyrmid-7-one core structure as well as further SAR studies of its derivatives as potent hGnRH receptor antagonists.

The novel synthesis of 2-arylpyrrolo[1,2-*a*]pyrimid-7-ones, represented by **6a**–**b**, is outlined in Scheme 1. Amidine **2** was refluxed with diethyl ethoxymethylene malonate in the presence of EtONa in EtOH[2] to afford the corresponding pyrimidone **3**. Compound **3** was then treated separately with α-bromoacetophones **4a** and **4b** in the presence of tetrabutylammonium fluoride (TBAF) in THF to give pyrimidones **5a** and **5b**, respectively. The regioselective N^1-alkylation was confirmed by NOE experiments. Catalyzed by NaH in THF, pyrimidones **5a**–**b** underwent intramolecular cyclization to give the bicyclic core structures **6a**–**b** in good yields. Intermediates **6a**–**b** were then exposed to 2-fluorobenzyl bromide and 1 M TBAF in THF to form compounds **7a**–**b**. Compound **7b** was further reduced by hydrogenation over Pd/C to yield the corresponding aniline **7c**. Subsequent acylation with carboxylic anhydrides gave the amides **7d** (**7e**–**g**).



Scheme 1. Reagents and conditions: (a) diethyl ethoxymethylene malonate, EtONa/EtOH reflux; (b) TBAF, THF; (c) NaH, THF; (d) TBAF/THF, 2-fluorobenzyl bromide; (e) H_2, Pd/C, HOAc, 30 psi; (f) $(RCO)_2O$, Et_3N, THF.

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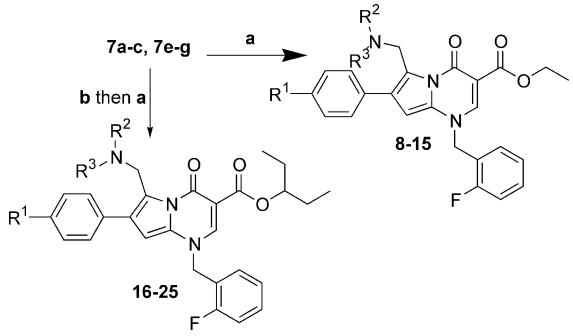
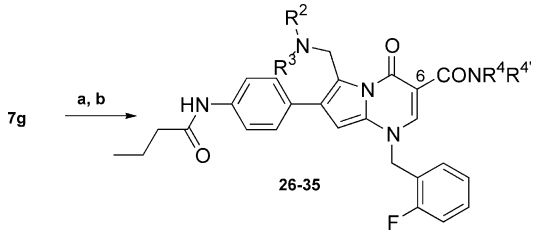
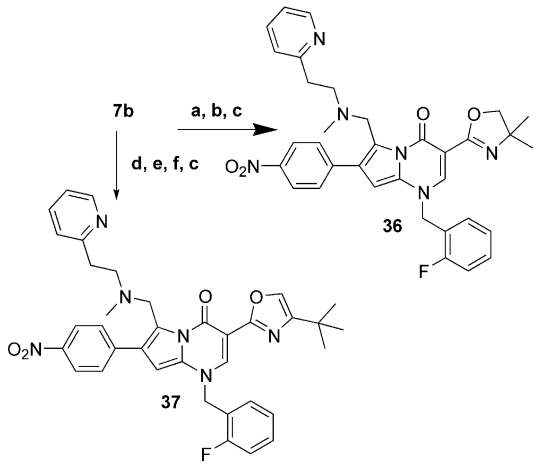


Scheme 2. Reagents and conditions: (a) R^2R^3NH, CH_2O in water, EtOH; (b) 3-pentanol, BuLi, THF.



Scheme 3. Reagents and conditions: (a) $R^4R^{4'}NH$, Et_3Al, DCE, reflux; (b) R^2R^3NH, CH_2O in water.

As illustrated in Scheme 2, advanced intermediates **7a**–**c** and **7e**–**g** were diversely modified at position 1 via a simple Mannich reaction[3] with various amines in the presence of formaldehyde. The structures of a selected series of final compounds **8**–**15** are presented in Table 1. In addition, modifications of the 6-ethoxy-carbonyl group on **7a**–**c** and **7e**–**g** were also explored (Scheme 2) and the ethyl esters were *trans*-esterified to the corresponding 3-pentyl esters by lithium 3-pentoxide, formed in situ from butyl lithium and 3-pentanol in THF, followed by Mannich reactions to afford the amines **16**–**25** as shown in Table 2. Furthermore, ethyl ester **7g** was converted to a variety of carboxamides by reaction with pre-formed triethyl aluminum and amine ($R^4R^{4'}NH$) complexes in 1,2-dichloroethane at 90 °C, followed by Mannich reactions to give products **26**–**35** as depicted in Scheme 3.



Scheme 4. Reagents and conditions: (a) NaH, 2-amino-2-methyl-propanol, THF; (b) $SOCl_2$, THF; (c) 2-(2-methylaminoethyl)pyridine, CH_2O in water; (d) LiOH, THF/MeOH/ H_2O; (e) K_2CO_3, 1-bromopinacolone, DMF; (f) NH_4OAc/HOAc, 100 °C.

Scheme 4 shows the synthesis of two heterocyclic derivatives (**36** and **37**) of the 6-carboxylic ester. Reaction of **7b** with pre-mixed NaH and 2-amino-2-methyl-propanol solution in THF, followed by treatment with thionyl chloride formed an oxazoline,[4] which was then subjected to Mannich conditions to yield **36**. Compound **7b** was hydrolyzed by LiOH in a mixture of THF, MeOH and water and the resulting acid was treated with 1-bromopinacolone in the presence of K_2CO_3, followed by reflux in HOAc with NH_4OAc to produce an oxazole.[5] Mannich reaction yielded the desired compound **37**.

Table 1. Binding affinities of compounds **8**–**15** on the hGnRH receptor[7]

Compd	R^1	R^2R^3NH	K_i (nM) human
8	MeO	BnNHMe	400
9	MeO	(2-pyr)-$(CH_2)_2$NHMe	55
10	NO_2	BnNHMe	450
11	NH_2	BnNHMe	190
12	MeCONH	BnNHMe	42
13	*i*-PrCONH	BnNHMe	44
14	*i*-PrCONH	(2-pyr)-$(CH_2)_2$NHMe	14
15	*n*-PrCONH	(2-pyr)-$(CH_2)_2$NHMe	1.2

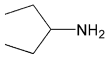
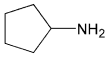
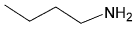
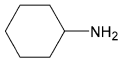
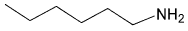
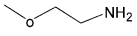
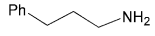
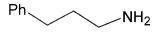
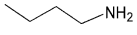
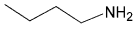
Table 2. Binding affinities of compounds **16**–**25** on the hGnRH receptor[7]

Compd	R^1	R^2R^3NH	K_i (nM) human
16	MeO	BnNHMe	62
17	MeO	(2-pyr)-$(CH_2)_2$NHMe	14
18	NH_2	BnNHMe	100
19	MeCONH	BnNHMe	4.0
20	MeCONH	(2-pyr)-$(CH_2)_2$NHMe	2.8
21	*n*-PrCONH	BnNHMe	3.8
22	*n*-PrCONH	(2-pyr)-$(CH_2)_2$NHMe	2.7
23	*n*-PrCONH	Me_2NH	130
24	*n*-PrCONH	$Et_2N(CH_2)_2$NH-Me	5.8
25	*n*-PrCONH	$PhCH_2)_2$NHMe	2.1

All of the synthesized compounds were evaluated for their ability to compete for des-Gly^{10}[^{125}I-Tyr,^{5}DLeu,^{6}NMeLeu,^{7}Pro^{9}-NEt]GnRH radioligand binding to the cloned human receptor.[6] The binding assay results on varying the R^1 group with either *N*-methyl-benzylamine or *N*-methyl-*N*-(2-pyridyl)ethylamine as R^2R^3NH are presented in Table 1.[7] Compounds **8** and **9** were substantially more potent than their corresponding 3-cyano analogues.[1] These data suggest that position 3 of the bicyclic core prefers not to be substituted. Replacement of the electron donating methoxy group of R^1 with a strong electron withdrawing nitro group had no substantial impact on potency (**10**, 450 nM). However, reduction of the nitro compound to the corresponding amino analogue **11** yielded a 2-fold improvement in the binding affinity. A hydrogen bond acceptor together with a lipophilic group seems to be the preferred R^1 group as the acylated analogues **12** and **13** had 5-fold improvement in potency, in comparison with the anilino compound **11**. Furthermore, a 3-fold enhancement of potency was obtained by use of *N*-methyl-*N*-2-(2-pyridyl)ethylamine as R^2R^3NH (**14**, $K_i = 14$ nM). Surprisingly, a slight change from the branched *iso*-butyrylamino group (*i*-PrCONH) to a linear *n*-butyrylamino group (*n*-PrCONH) of R^1 provided a 10-fold increase in the binding affinity (**15** vs **14**).

Table 2 lists the binding affinity data of compounds **16**–**25**, in which all molecules contain the 3-pentyl carboxylate instead of the ethyl carboxylate at position 6. A direct comparison between compounds **16**, **17**, **18** and compounds **8**, **9**, **11** reveals that the 3-pentyl carboxylate significantly enhanced the binding affinity. Consequently, combination of 3-pentyl carboxylate and acetamido groups in compound **19** gave a K_i value of 4 nM, while its corresponding ethyl carboxylate **12** was 10-fold less potent. Interestingly, the binding enhancement by incorporation of the *N*-methyl-*N*-2-(2-pyridyl)ethylamine at the 1-methyl position in the ethyl carboxylates was no longer observed here, as compound **20** was only slightly more potent than compound **19**. This trend was also observed between compounds **21** and **22**. Further evaluation of different substituents on the basic amine revealed that the simple dimethylamino analogue **23** had respectable potency ($K_i = 130$ nM). A non-aromatic side chain containing a basic amine (**24**) was only 2-fold less potent than compound **22**. The binding affinity of **25** provided more evidence that the *N*-methyl-*N*-2-(2-pyridyl)ethylamine was no longer crucial for high potency, since phenethyl replacement of the 2-pyridylethyl group as the substituent on the basic amine side chain gave very similar potency. It was worth noting that such replacement led to complete loss of binding affinity in the previous SAR study on compound **1**.[1]

Table 3. Binding affinities of compounds **26–35** on the hGnRH receptor[7]

Compd	R_2R_3NH	$R_4R_4'NH$	K_i (nM) human
26	(2-pyr)-$(CH_2)_2$NHMe		21
27	(2-pyr)-$(CH_2)_2$NHMe		9.2
28	(2-pyr)-$(CH_2)_2$NHMe		10
29	(2-pyr)-$(CH_2)_2$NHMe		32
30	(2-pyr)-$(CH_2)_2$NHMe		3.3
31	(2-pyr)-$(CH_2)_2$NHMe		630
32	(2-pyr)-$(CH_2)_2$NHMe		1.1
33	BnNHMe		17
34	BnNHMe		154
35	$Et_2N(CH_2)_2NH$–Me		440

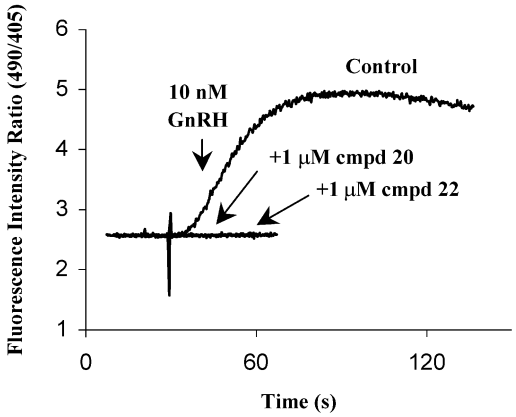


Figure 1. Inhibition of GnRH stimulated Ca^{++} flux by compounds **20** and **22**.

Table 4. Binding affinities of compounds **36** and **37** on the hGnRH receptor[7]

Compd	K_i (nM) human
36	270
37	64

With these results, subsequent SAR studies were focused on replacing the 6-carboxylates with carboxamides. As shown in Table 3, 3-pentyl carboxamide **26** was substantially less potent than its 3-pentyl ester analogue **22**. However, cyclopentyl analogue **27** was 2-fold more potent than **26**. While expanding the ring from cyclopentyl to cyclohexyl (**29**) caused a decrease in potency, a linear butyl carboxamide **28** was equally potent as the cyclopentyl analogue **27**. Further extension of the butyl to hexyl (**30**) provided a 3-fold increase in binding affinity. However, insertion of an oxygen atom in the butyl chain for reducing lipophilicity also reduced potency (**31**). The preferred side chain from this limited optimization study was a 3-phenylpropyl carboxamide group (**32**) which yielded another 3-fold increase in potency in comparison with its hexyl analogue **30**. Unlike the 3-pentyl ester, incorporation of carboxamide at position-6 required the presence of the *N*-methyl-*N*-[2-(2-pyridyl)]ethylamine on 1-methyl position for high potency again. For example, **33** decreased almost 17-fold in potency only due to switch of side chain of the basic amine from a 2-(2-pyridyl)ethyl group to a benzyl group. Similar results were observed in **34** and **35**. Compared to **28**, the potencies of these two compounds were dramatically reduced simply because benzyl and 2-diethylaminoethyl instead of 2-(2-pyridyl)ethyl were substituted on the basic amine.

Since position 6 was well tolerated for modification, a limited study was undertaken to explore the use of more stable heterocyclic groups to replace the esters and amides. The resulting compounds **36** and **37** (Table 4) had K_i values of 270 and 64 nM, respectively, which were comparable to the potency of the corresponding esters and amides. These results promoted us to perform more modifications at position 6 using aryl groups and the results will be presented elsewhere in the near future.

In order to demonstrate functional antagonism, selected compounds were evaluated for their ability to inhibit GnRH stimulated calcium flux.[8] As shown in Figure 1, compounds **20** and **22** at a concentration of 1 μM were able to completely block Ca^{++} flux stimulated by 10 nM GnRH. No indication of stimulatory activity for these or other compounds tested was observed.

In conclusion, we have developed a novel and efficient two-step synthesis for 2-arylpyrrolo[1,2-*a*]pyrimid-7-ones. Further modifications of these structures led to the discovery of a series of highly potent hGnRH receptor antagonists. SAR study of these antagonists indicated that hydrogen is more preferred substituent than a cyano group at position 3 of this bicyclic core structure. Position 6 was amenable to substitution by a variety of groups without compromising the binding affinity to the hGnRH receptor.

References and Notes

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6. Human GnRH receptor was stably expressed in HEK293 cells and a 96-well filtration assay was used.
7. 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. Most of compounds reported here were assayed 2–8 times.
8. HEK293 cells stably expressing the hGnRH receptor were loaded with the calcium sensitive dye Indo-1 then pre-incubated with compound **20**, **22** 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.