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Synthesis and Initial Structure–Activity Relationships of a Novel Series of Imidazolo[1,2-*a*]pyrimid-5-ones as Potent GnRH Receptor Antagonists

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Abstract—SAR studies of 2-arylimidazolo[1,2-*a*]pyrimid-5-ones **10a**–**m**, which were derived from initial lead **3a**, resulted in the discovery of a series of potent nonpeptide human GnRH receptor antagonists. Compounds with good potency (e.g., **10e**, $K_i = 7.5$ nM) were prepared by introduction of a 2-(2-pyridyl)ethyl at the basic nitrogen and a 3-pentyl ester at the 6-position of the bicyclic core.

Gonadotropin-releasing hormone (GnRH), or luteinizing hormone-releasing hormone, is a linear decapeptide amide, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, originally isolated and characterized from porcine[1] and ovine[2] hypothalami. Its action at the level of the pituitary is to stimulate the secretion of the gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) via interaction with its cell surface receptor, which belongs to the G-protein couple receptor superfamily.[3] These gonadotropins, in turn, act on the reproductive organs where they participate in the regulation of steroid production, gametogenesis and ovulation.[4] GnRH has been the subject of wide interest in the search for nonsteroidal contraceptive agents because of its important role in the regulation of both male and female reproduction.[5] Several disease conditions such as endometriosis and prostate cancer can be treated by suppression of the pituitary–gonadal axis, and gonadotropin-releasing hormone superagonists represented by leuprorelin,[6] which down regulates the receptor, are currently used in the treatment of these conditions.[7]

Clinical evidence shows that peptidic GnRH antagonists directly lower gonadal sex hormone levels alleviating disease symptoms without the concomitant flare effect, which can be caused by super agonists. Several small molecule GnRH receptor antagonists have appeared in the literature. T-98475 and its analogues are the first small molecules reported to have high affinity for the human GnRH receptor ($IC_{50} = 0.2$ nM) but are less potent at the rat receptor ($IC_{50} = 60$ nM).[8] An erythromycin A derivative, A-198401, was reported as a highly potent GnRH antagonist ($pK_i = 9.2$ and 8.7 on rat and human receptors, respectively), and having 15.2% oral bioavailability in rats (Fig. 1).[9] Compound **1** has very potent binding affinity to the human ($IC_{50} = 0.44$ nM) as well as rat GnRH receptor ($IC_{50} = 4$ nM),[10] and very recently, indole derivatives such as **2** were reported to be potent and orally bioavailable GnRH antagonists.[11]

We have recently reported the SAR of the pyrrolopyrimidone core as potent GnRH antagonists (i.e., **3b**) from modification of an initial lead compound **3a**.[12] For example, compound **3b** had a K_i of 2.7 nM against the human GnRH receptor and selected compounds from this series inhibited GnRH stimulated Ca^{++} flux.[12b] In this paper, we describe the design and synthesis of imidazolopyrimidones (**10a**–**m**) as potent GnRH antagonists. The main focus of this study is an investigation of the SAR for the replacement of the pyrrolo ring with an imizadolo moiety in the belief that such a change would increase the stability of the targeted compounds while maintaining their GnRH antagonist acivities.[13]

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A general synthesis of 7-alkylimidazolo[1,2-*a*]pyrimid-5-ones **6** based on a cyclization of a 2-aminopyrimidine with α-bromoketone is outlined in Scheme 1. Thus, ethyl 2-amino-4-hydroxypyrimidne-5-carboxylate **4** was treated with α-bromoacetophenones in the presence of sodium hydride in tetrahydrofuran at room temperature, followed by ammonium hydroxide. After a simple aqueous workup, the desired 8*H*-imidazolo[1,2-*a*]pyrimid-5-ones **5a** and **5b** were crystallized from methanol. *N*-Alkylation of the imidazolo[1,2-*a*]pyrimid-5-ones **5a** or **5b** with 2-fluorobenzyl bromide in the presence of tetrabutylammonium fluoride in DME at room temperature gave the desired 2-aryl-7-(2-fluorobenzyl)-imidazolo[1,2-α]pyrimd-5-ones **6** and **7**, which were purified by chromatography on silica gel (ethyl acetate/hexanes). The structure of compounds **6** and **7** were confirmed by NOE experiments in which the NOE between the benzylic proton and the proton at the 6-position of the bicyclic system was observed. Transesterification of the ethyl ester **7** in the presence of 3-hydroxypentane and sodium hydride in THF at room temperature gave the corresponding 3-pentyl ester **8**.

The nitro group of **7** or **8** was easily converted to the corresponding amide **9a** or **9b**, respectively, by hydrogenation using Raney-Ni catalyst under hydrogen, followed by acylation of the crude anilino compounds with various acid anhydrides or acid chlorides (Scheme 2). The imidazolo[1,2-*a*]pyrimid-5-ones were then subjected to a Mannich reaction to afford the desired products **10a**–**m** which were purified by preparative TLC chromatography on silica gel (Scheme 3).[14]

The synthesized compounds were evaluated for their ability to inhibit [^{125}I-Tyr^5,DLeu^6,NMeLeu^7,Pro^9-NEt]GnRH agonist binding to the cloned human GnRH receptor, and rat GnRH receptor as described previously.[15] All compounds with inhibition better than 50% at 100 μM were titrated on a six-point curve in duplicate, and their data are reported as K_i values using the Cheng–Prusoff equation.[16] The initial radioligand binding data for **9a** and **9b** suggested these two compounds were only weakly active (K_i 30 and 28 μM, respectively); the human GnRH receptor binding data for the analogues **10** are reported in Table 1.

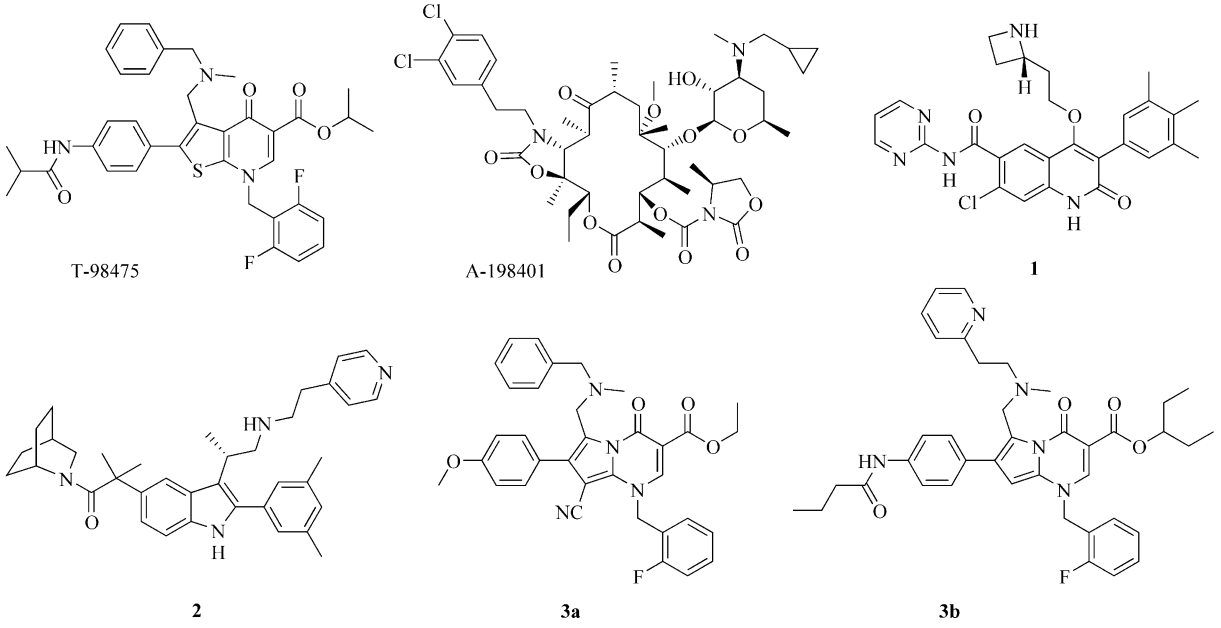
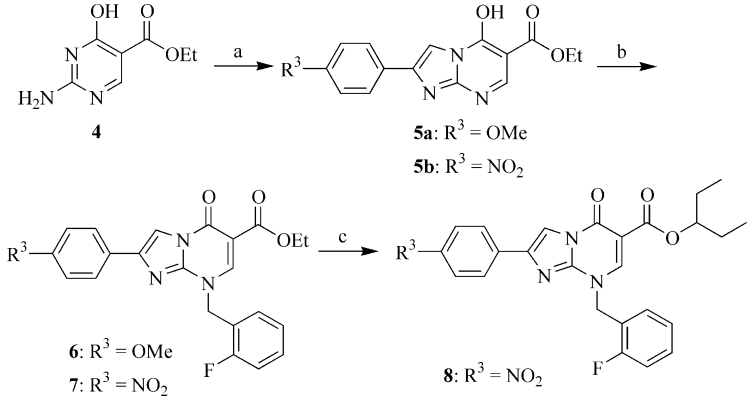
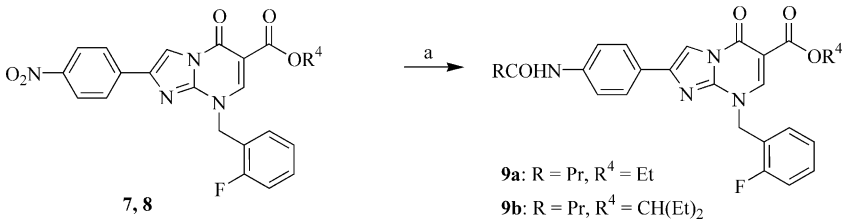


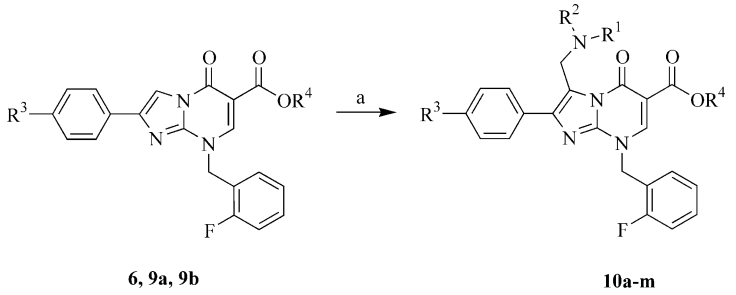
Figure 1. Small molecule GnRH antagonists.



Scheme 1. Reagents and conditions: (a) α-bromo-4′-methoxyacetophenone, or α-bromo-4′-nitroacetophenone, NaH, DME, rt; then NH_4OH, rt; (b) TBAF/THF, then 2-fluorobenzyl bromide, rt; (c) NaH/3-hydroxypentane, THF, 0 °C to rt.



Scheme 2. Reagents and conditions: (a) Raney Ni, H_2, MeOH; then RCOCl, Et_3N, CH_2Cl_2, rt.

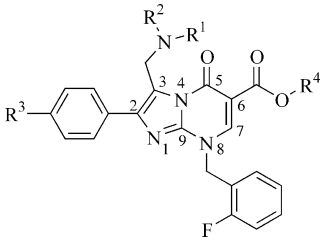


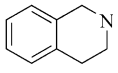
Scheme 3. Reagents and conditions: (a) R^1R^2NH, CH_2O, AcOH, rt to reflux.

The prototypical compound in this series was 2-(4-butyroylamidophenyl)-3-(*N*-benzyl-*N*-methylaminomethyl)-6-ethoxycarbonyl-8-(2-fluorobenzyl)imidazolo[1,2-*a*]pyrimid-5-one **10c**, which was found to have good affinity for GnRH receptor ($K_i = 80$ nM). This result indicated the importance of the basic nitrogen atom and the benzyl group attached (**9a** had a K_i of 30 μM). Replacement of the butyroylamido on the 4-position of the phenyl group (R^3) with a methoxy group resulted in approximately 12-fold loss of activity (**10a**, $K_i = 950$ nM). In addition to a possible hydrogen-bonding site from the oxygen or nitrogen atom, a small lipophilic group in this area seems to be very important for high binding affinity. Replacement of the benzyl group on the 3-aminomethyl functionality of the core structure with a 2-(2-pyridyl)ethyl group gave an analogue with over 4-fold increase in activity (**10b**, $K_i = 230$ nM). A similar result was obtained when the benzyl group of **10c** was replaced by the 2-(2-pyridyl)ethyl group (**10d**, $K_i = 12$ nM). The ethyl ester in **10d** could be replaced by the more bulky 3-pentyl ester with a suggestive, albeit modest, increase in activity (**10e**, $K_i = 7.5$ nM), leaving open the opportunity for further exploration at this site. Replacement of the 2-pyridyl group of compound **10e** with a nonsubstituted phenyl group only slightly decreased the binding activity (**10f**, $K_i = 14$ nM), but replacement of the 2-(2-pyridyl)ethyl with 3-pyridylmethyl group caused a 7-fold loss of activity (**10g**, $K_i = 52$ nM). Similar results were obtained when furanmethyl or 2-methoxyethyl group was used to replace the 2-(2-pyridyl)ethyl group (**10h** and **10i**, $K_i = 29$ and 36, respectively). However, introduction of cyanomethyl group caused over 40-fold decrease in binding affinity for the human GnRH receptor (**10j**, $K_i = 300$ nM), possibly because of the reduction in pKa of the amine by electron withdrawing cyano group.

On the other hand, introduction of a cyclic tetrahydroisoquinoline group decreased activity almost 40-fold (**10k**, $K_i = 290$ nM), while the non-aromatic pyrrolidine analogue showed much less activity (**10l**, $K_i = 2.2$ μM). Finally, the 4-isobutyroylamidophenyl analogue gave similar activity (**10m**, $K_i = 41$ nM), compared with the corresponding *n*-butyroylamidophenyl analogue such as **10g** and **10h**. From these studies, a number of the

Table 1. Structure–activity relationships for the 2-arylimidazolo[1,2-*a*]pyrimid-5-ones



Compd	R^1NR^2	R^3	R^4	h-GnRH K_i (nM)
9a	(−)	NHCOPr	Et	30,000
9b	(−)	NHCOPr	$CH(Et)_2$	28,000
10a	$PhCH_2NMe$	OMe	Et	950
10b	$2\text{-}PyCH_2CH_2NMe$	OMe	Et	230
10c	$PhCH_2NMe$	NHCOPr	Et	80
10d	$2\text{-}PyCH_2CH_2NMe$	NHCOPr	Et	12
10e	$2\text{-}PyCH_2CH_2NMe$	NHCOPr	$CH(Et)_2$	7.5
10f	$PhCH_2CH_2NMe$	NHCOPr	$CH(Et)_2$	14
10g	$3\text{-}PyCH_2NMe$	NHCOPr	$CH(Et)_2$	52
10h	$2\text{-}FuranCH_2NMe$	NHCOPr	$CH(Et)_2$	29
10i	$MeOCH_2CH_2NMe$	NHCOPr	$CH(Et)_2$	36
10j	$NCCH_2NMe$	NHCOPr	$CH(Et)_2$	300
10k		NHCOPr	$CH(Et)_2$	290
10l		NHCOPr	$CH(Et)_2$	2200
10m	$PhCH_2NMe$	NHCOPr-i	$CH(Et)_2$	41

imidazolo[1,2-α]pyrimid-5-ones having high binding affinity for the human GnRH receptor were identified.

As we expected, this series of compounds are much more stable than their counterpart from the pyrrolopyrimidone series **3b**. For example, When **10f** was incubated in 0.2 N HCl solution at 37 °C, there was no significant degradation (< 5% of parent).

All the compounds have very low binding affinity towards the rat GnRH receptor. For example, **10g** had a K_i of 3.5 μM. This is consistent with the similar observation by Cho and co-workers for the thienopyridinone analogue T98475.[8]

The binding activity of the initial compounds (**9a** and **9b**) was greatly improved by incorporation of a basic tertiary amine at the 3-position of the bicyclic system. This modification, we suspect, may initiate a crucial interaction between the ligand and its receptor, involving an acidic residue within the putatively helical domains. Based on a predictive model of the membrane spanning regions within the protein, the candidate for this site is aspartic acid 302 on helix 7.[3] Interestingly, the corresponding residue in the rat GnRH receptor is glutamic acid and this may contribute to the species selectivity between the human and rat GnRH receptors we observed for **10a**–**m**. The results of the SAR study suggest that a variety of tertiary amines are tolerated while the 2-(2-pyridyl)ethyl group on the 3-aminomethyl functionality of the imidazolo[1,2-*a*]pyrimid-4-one core structure gives the best result.

In conclusion, a series of 2-arylimidazolo[1,2-*a*]pyrimid-5-ones exemplified by **10a**–**m** was discovered, with some having good binding affinity for the human GnRH receptor. A hydrophobic ester at the 6-position is helpful for high binding affinity, and a small lipophilic group with possible hydrogen bonding site at the 4-position of the phenyl group is required for optimum human GnRH receptor binding affinity. Further structure activity relationships of this series of compounds will be reported in the following paper.[17]

Acknowledgements

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13. Unfortunately, it was subsequently shown that this compound (**3b**) and close related analogues suffered from the instability caused by the amino group attached to the benzylic-like position of pyrrole ring. For example, compound **3b**, after removing the electron-withdrawing cyano group at the 3-position of **3a,** only had a $t_{1/2}$ of 22 min in rat plasma at 37 °C.
14. All final compounds were characterized by HPLC-MS with two-wavelength (220 and 256) detection. All the compounds reported here passed purity of > 85% of both detection. The following is a typical procedure for synthesis of **10m**:

6-Ethoxycarbonyl-8-(2-fluorobenzyl)-2-(4-nitrophenyl)imidazolo[1,2-α]pyrimid-5-one: To a suspended solution of 2-amino-5-ethoxycarbonylpyrimid-4-one (10 g, 55 mmol) in DMF (100 mL) was added NaH (60% dispersion in mineral oil, 2.6 g, 65 mmol). The reaction was allowed to stir under nitrogen for 45 min and α-bromo-4-nitroacetophenone (14.09 g, 58 mmol) in DMF (20 mL) was added dropwise. This resulted in a purple then dark solution which was allowed to stir for 45 min at rt. To this solution was then added 50 mL of 30% aqueous ammonium hydroxide and the mixture was stirred at room temperature for 2 h. The resulting dark solution was concentrated in vacuo, and the residue was slowly poured into 300 mL of 2 M HCl solution, stirred for 0.5 h and the resulting orange solid was collected by vacuum filtration to yield the crude 6-ethoxycarbonyl-2-(4-nitrophenyl)-8*H*-imidazolo[1,2-*a*]pyrimid-5-one, which was used for next step, MS *m*/*e* 329 (MH^+).

6-Ethoxycarbonyl-2-(4-nitrophenyl)imidazolo[1,2-*a*]pyrimid-5-one (10 g, 30 mmol) was suspended in DME (100 mL) and treated with TBAF (1 M in THF, 1.3 equiv) dropwise at rt. This mixture was allowed to stir for 0.5 h before 2-fluorobenzyl bromide (1.1 mmol in 5 mL of DME) was introduced. The reaction was stirred at room temperature overnight, concentrated and extracted with chloroform (2×200 mL). The organic layers were combined, washed with brine, filtered,

dried over sodium sulfate, and concentrated in vacuo to yield the title compound as a solid. Analytic sample was obtained by recrystallization of the product from ethyl acetate. ^{1}H NMR $CDCl_3$ δ 1.40 (t, 3H), 4.42 (q, 2H), 5.54 (s, 2H), 7.6–7.15 (m, 4H), 8.06 (d, 2H), 8.15 (s, 1H), 8.3 (d, 2H), 8.60 (s, 1H); MS *m*/*e* 437 (MH^+). Anal. ($C_{22}H_{17}FN_4O_5$) C, H, N.

3-(*N*-Benzyl-*N*-methyl)aminomethyl-6-(3-pentyloxycarbonyl)-8-(2-fluorobenzyl)-2-(4-isobutyroylaminophenyl)imidazolo[1,2-*a*]pyrimid-5-one: To a solution of 3-pentanol (500 mg, 5 mmol) in THF (10 mL) was added *n*-BuLi (2.5M, in hexanes, 2 mL) while stirring at 0 °C. This solution was stirred for 0.5 h and then warmed to rt. A suspension of ethoxycarbonyl-8-(2-fluorobenzyl)-2-(4-nitrophenyl)imidazolo[1,2-*a*]pyrimid-5-one (1 g, 2.3 mmol) in THF (10 mL) was added to the stirred solution. The reaction was stirred at rt for 3 h, quenched with ammonium chloride solution, extracted with chloroform and washed with brine. The extract was dried and concentrated in vacuo to yield 6-(3-pentoxycarbonyl)-8-(2-fluorobenzyl)-2-(4-nitrophenyl)imidazolo[1,2-*a*]pyrimid-5-one as a solid.

The crude solid obtained from above was hydrogenated at 50 psi in the presence of Raney nickel catalyst in ethanol at room temperature overnight. The reaction mixture was filtered and concentrated in vacuo to yield a crude semisolid which was dissolved in dichloromethane and stirred overnight in the presence of excess isobutyric anhydride. The reaction mixture was concentrated, partitioned between ethyl acetate and water, washed with brine, dried over sodium sulfate and concentrated. The crude amide was purified by column chromatography to yield 6-(3-pentoxycarbonyl)-8-(2-fluorobenzyl)-2-(4-isobutyroylaminophenyl)-imidazolo[1,2-*a*]pyrimid-5-one as a yellow oil.

6-(3-Pentoxycarbonyl)-8-(2-fluorobenzyl)-2-(4-isobutyoylaminophenyl) imidazolo[1,2-α]pyrimid-5-one (1.0 mmol) was added to a stirred solution of formaldehye (37% in water, 1.5 equiv), benzylmethylamine (1.5 mmol) and acetic acid (2 mL). The reaction mixture was heated to 50 °C for 2 h, cooled to rt and concentrated in vacuo. This crude oil was suspended between ethyl acetate and saturated sodium bicarbonate solution. The organic layer was separated and washed with brine, dried over sodium sulfate and concentrated in vacuo. The title compound was purified by chromatography on silica gel (95% ethyl acetate, 5% MeOH). ^{1}H NMR $CDCl_3$ δ 1.2 (m, 9H), 1.69 (m, 4H), 1.72 (m, 2H), 2.21 (s, 3H), 2.45 (m, 2H), 3.72 (s, 2H), 4.37 (s, 2H), 4.88 (m, 1H), 5.54 (s, 2H), 7.2–7.5 (m, 11H), 7.89 (m, 2H), 8.54 (s, 1H); MS *m*/*e* 652 (MH^+).

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