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

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Abstract—SAR studies of lead GnRH receptor antagonists **2a** and **2b** reported earlier resulted in the discovery of compound **10b** which showed much higher potency ($K_i = 4.6$ nM, compared with **2b**, $K_i = 230$ nM) in which the 7-position of the imidazolo[1,2-*a*]pyrimidone core was substituted with a methyl group, and the ester at the 6-position was replaced by the 3-methoxyphenyl group.

We have previously described the identification of pyrrolopyrimidones **1**[1] and 2-arylimidazolo[1,2-*a*]pyrimidone series **2**[2] as potent human GnRH receptor antagonists. Initial structure–activity relationship studies of 2-arylimidazolo[1,2-*a*]pyrimidones showed that the bulky ester substituent at the 6-position and a *para*-isobutyroylamidophenyl group at the 2-position of the bicyclic core are important for high binding affinity, and resulted in the discovery of a potent analogue (**2a**) with a K_i of 7.5 nM. Compounds from this series are much more stable than pyrrolopyrimidones **1** under acidic conditions, however, the ester functional group is hydrolyzed in vivo. For example, **1** had a $t_{1/2}$ of only 28 min in rat plasma when incubated at 37 °C, and the major metabolite was the corresponding acid. In order to replace the labile ester group at the 6-position, some heterocycles such as oxazole and oxazoline were successfully incorporated at this position of the pyrrolo[1,2-*a*]pyrimidones.[1] In this paper, we describe the design and synthesis of the 6-arylimidazolo[1,2-*a*]pyrimidones as potent antagonists of the human GnRH receptor.

8-Alkylated imidazolo[1,2-*a*]pyrimid-5-ones **8** were synthesized based on a cyclization reaction[3] as outlined in Scheme 1. Commercial 2-amino-5-bromo-6-methylpyrimid-4-one **4** was treated with α-bromo-4′-methoxyacetophenone in the presence of sodium hydride in anhydrous DMF at room temperature, followed by ammonium hydroxide to give the desired 8*H*-imidazolo[1,2-*a*]pyrimid-5-one **5**, which was purified by crystallization. Compound **5** was alkylated with 2-fluorobenzyl bromide in the presence of tetrabutylammonium fluoride in DME at room temperature. The desired compound **7** was separated from the minor *O*-alkylated by-product using chromatography on silica gel with ethyl acetate/hexanes. Palladium catalyzed cross-coupling reaction of the bromide **7** with a variety of arylboronic acids under Suzuki conditions gave the corresponding 6-arylimidazolo[1,2-*a*]pyrimidones **8** after a chromatographic purification. Alternatively, the bromo compound **5** could be first subjected to the Suzuki reaction with arylboronic acids to give **6**,[4] which were then alkylated with alkyl halides to give compounds **8**. The structures **7** and **8** were confirmed by NOE experiments in which the NOE between the benzylic proton and the methyl group at the 7-position of the bicyclic system was clearly observed. The structure of **7** was further confirmed by single crystal X-ray structure. Compounds **7** and **8** were then subjected to a Mannich reaction with a variety of secondary amines and formaldehyde in acetic acid to afford the desired products **9** and **10**.[5]

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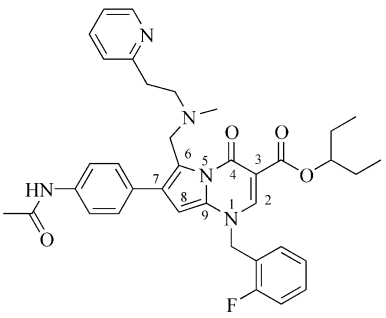

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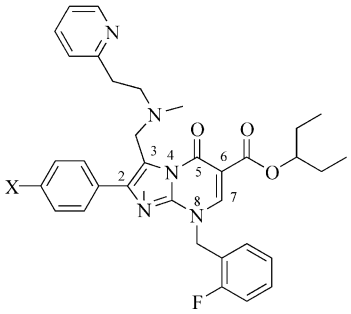
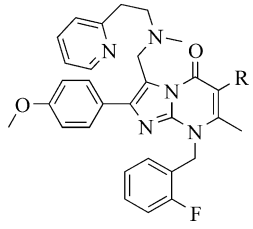


2a: X = PrCONH, K_i = 7.5 nM (hGnRH)
2b: X = MeO, K_i = 230 nM (hGnRH)

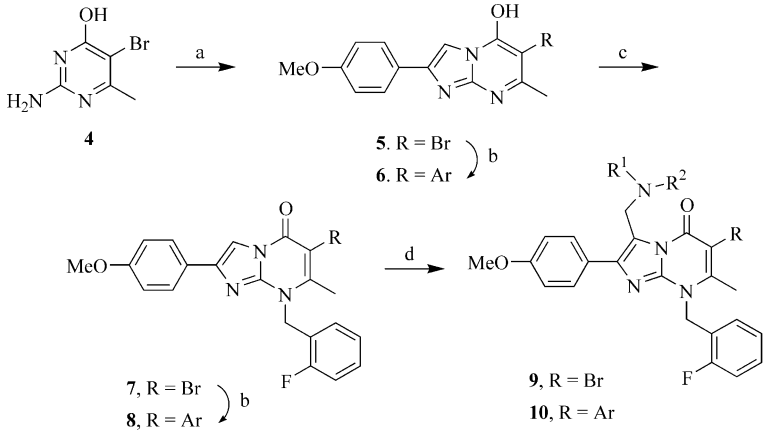
The synthesized compounds were evaluated in the cloned human and rat GnRH receptor assays for their ability to displace the binding of [^{125}I-Tyr^5,DLeu^6, NMeLeu^7,Pro^9-NEt]GnRH agonist.[6] All compounds with inhibition better than 50% at 10 μM were titrated on a 6-point curve in a duplicate, and the results are reported as K_i values.[7] The human GnRH receptor binding data for the analogues **10a**–**t** are reported in Tables 1 and 2.

Although **2a** has good potency, the 3-pentyl ester is potentially too labile to be used in a drug candidate. Since this ester may be functioning both as a lipophilic group and a hydrogen bonding acceptor, we designed the phenyl group bearing a hydrogen bonding acceptor group to replace it. The prototypical compound in this series was the 2-(4-methoxyphenyl)-3-{*N*-[2-(2-pyridyl) ethyl]-*N*-methylamino}methyl-6-(3-methoxyphenyl)-7-methyl-8-(2-fluorobenzyl)imidazolo[1,2-*a*]pyrimid-5-one **10b**, which was found to have high affinity for the human GnRH receptor (K_i = 4.6 nM). This compound has a 50-fold increase in binding affinity over the homologous **2b** (K_i = 230 nM). Changing the 3-methoxy group to the 2- or 4-position of the phenyl group resulted in less active compounds (**10a** and **10c**, K_i = 65 and 18 nM, respectively). Incorporation of an additional methoxy at the 4- or both 4- and 5-positions of the phenyl ring decreased activity almost 25- or 80-fold (**10e** and **10f**, K_i = 110 and 370 nM, respectively). Replacement of the bromo group of compound **9**, which had a K_i of 2.5 μM, with a methylenedioxyphenyl group resulted in over 200-fold increase of activity (**10d**, K_i = 11 nM). All other phenyl substitutents such as 3-fluoro-,

Table 1. Structure–activity relationships of 5-aryl substitution

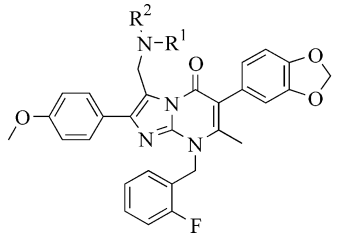


Compd	R	h-GnRH K_i (nM)
9	Bromo	2500
10a	2-Methoxyphenyl	65
10b	3-Methoxyphenyl	4.6
10c	4-Methoxyphenyl	18
10d	3,4-Methylenedioxyphenyl	11
10e	3,4-Dimethoxyphenyl	110
10f	3,4,5-Trimethoxyphenyl	370
10g	3-Fluorophenyl	79
10h	3-Trifluoromethylphenyl	140
10i	3,5-Dichlorophenyl	100
10j	3-Thiophenyl	56



Scheme 1. Reagents and conditions: (a) a-bromo-4′-methoxyacetophenone, NaH, DMF, rt; then NH_4OH, rt; (b) $ArB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, benzene–water–ethanol; (c) TBAF/THF-DME, then 2-fluorobenzyl bromide, rt; (d) R^1R^2NH, CH_2O, AcOH, rt to reflux.

Table 2. Effects of 3-alkylamino substituents



Compd	R^1NR^2	h-GnRH K_i (nM)
10d	*N*-Methyl-*N*-[(2-pyridyl)ethyl]amino	11
10k	*N*-Butyl-*N*-methylamino	220
10l	*N*-(Methoxyethyl)-*N*-methylamino	150
10m	*N*-(Diethylaminoethyl)-*N*-methylamino	130
10n	*N*-Benzyl-*N*-methylamino	47
10o	*N*-Phenethyl-*N*-methylamino	110
10p	*N*-(2-fluorophenethyl)-*N*-methylamino	67
10q	*N*-Methyl-*N*-[(4-pyridyl)ethyl]amino	1400
10r	Morpholin-1-yl	28,000
10s	4-Methylhomopiperazin-1-yl	9500
10t	*N*-(1-Methylpiperidin-4-yl)-*N*-methylamino	Inactive

3-trifluoromethyl- or 3,5-dichlorophenyl resulted in some loss of activity compared with the methoxy group substitution (**10g**, **10h** and **10i**, $K_i = 79$, 140 and 100 nM, respectively). The activity of the heteroaromatic 3-thiophenyl substituted analogous was comparable to the 2-methoxyphenyl compound. (**10j**, $K_i = 56$ nM). These results indicate the aromatic ring bearing a hydrogen-bonding acceptor substituent, such as 3-methoxyphenyl, at the 6-position of the imidazolo[1,2-*a*]pyrimid-5-one core, with the possible combination of the 7-methyl group, is important for high GnRH receptor activity.

The effect of variation in the basic side chain at the 3-position in **10**, using the methylenedioxyphenyl series as the template, was examined (Table 2). The analogue, having an *N*-butyl-*N*-methylaminomethyl group **10k**, was found to have moderate affinity for the human GnRH receptor ($K_i = 220$ nM). Introduction of a polar methoxy or diethylamino substituent at the β-position of basic amine center had similar binding affinity (**10l** and **10m**, $K_i = 150$ and 130 nM, respectively). However, replacement of the butyl group of **10k** with a benzyl group caused almost 5-fold increase of activity (**10n**, $K_i = 47$ nM), which was reduced in the phenethyl analogue (**10o**, $K_i = 110$ nM). Interestingly, activity was restored by introduction of a fluorine at the 2′-position of the phenethyl group (**10p**, $K_i = 67$ nM), when the phenyl group of **10o** was replaced by the 2-pyridyl, the compound gave much better binding affinity (**10d**, $K_i = 11$ nM), whereas the 4-pyridyl substitution showed a dramatic decrease in activity (**10q**, $K_i = 1.4$ μM). Finally, cyclic amines, such as morpholin, 1-methylpiperidine or 4-methylhomopiperizine showed either low activity or no activity at all (**10r**, **10s**, and **10t**, $K_i = 28$ μM, 9.5 μM, and inactive, respectively).

All compounds showed very poor binding affinity against the rat GnRH receptor. For example, **10d** had a K_i of 11 nM on the human GnRH receptor, but showed only a K_i of 12 μM on the rat GnRH receptor.

In a conclusion, a series of 2-(4-methoxyphenyl)-7-methyl-8-(2-fluorobenzyl)imidazolo[1,2-*a*]pyrimid-5-ones exemplified by **10a**–**10t** was discovered as having good binding affinity for the human GnRH receptor. The results of the SAR study suggest that the 2-(2-pyridyl)ethyl group on the 3-aminomethyl functionality of the imidazolo[1,2-*a*]pyrimidone core structure is required for optimum human GnRH receptor binding affinity. To replace the potentially labile ester group of **2**, we designed an alkoxy substituted phenyl group which, possibly in combination with the methyl at the 6-position, also increased binding affinity 50-fold. From these studies a number of 2-(4-methoxyphenyl)-3-dialkylaminomethyl-6-aryl-7-methyl-8-(2-fluorobenzyl) imidazolo[1,2-*a*]pyrimid-5-ones having low nanomolar binding affinity for the human GnRH receptor has been identified. More importantly, 6-aryl substituted imidazolo[1,2-*a*]pyrimidones were very stable when they were incubated with either 0.2 N HCl or rat plasma at 37 °C for 2 h. Further studies detailing the consequences of their antagonistic effects towards the GnRH receptor[8] in vitro and in vivo will be reported elsewhere.

Acknowledgements

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References and Notes

1. (a) Zhu, Y.-F.; Struthers, R. S.; Connors, P. J., Jr.; Gao, Y.; Gross, T. D.; Saunders, J.; Wilcoxen, K.; Reinhart, G. J.; Ling, N.; Chen, C. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 399. (b) Zhu, Y.-F.; Wilcoxen, K.; Saunders, J.; Guo, Z.; Gao, Y.; Connors, P. J., Jr.; Gross, T. D.; Tucci, F. C.; Struthers, R. S.; Reinhart, G. J.; Chen, C. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 403.

2. Wilcoxen, K.; Zhu, Y.-F.; Connors, P. J. Jr.; Saunders, J.; Gross, T. D.; Gao, Y.; Reinhart, G. J.; Struthers, R. S.; Chen C. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 2179.

3. An example of this cyclization was published recently, see: Laneri, S.; Sacchi, A.; Abignente, E. *J. Heterocycl. Chem*. **2000**, *37*, 1265.

4. This coupling gave low yield of the desired product **6**. Debromonation of **5** was the major by-product isolated.

5. All final compounds were characterized proton NMR and LC-MS and passed the purity of >85%.

6. Perrin, M. H.; Haas, Y.; Rivier, J. E.; Vale, W. W. *Mol. Pharmacol*. **1983**, *23*, 44.

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.

8. For antagonist activity compounds were tested for their ability to inhibit GnRH stimulated calcium flux in HEK 293 cells stably expressing the human GnRH receptor. Representative compound from this series was able to fully block GnRH (10 nM) stimulated Ca^{++} flux at a concentration of 1 uM antagonist, and did not show any evidence of agonistic activity.