Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 399–402

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Initial Structure–Activity Relationship Studies of a Novel Series of Pyrrolo[1,2-*a*]pyrimid-7-ones as GnRH Receptor Antagonists

Yun-Fei Zhu,* R. Scott Struthers, Patrick J. Connors, Jr., Yinghong Gao, Timothy D. Gross, John Saunders, Keith Wilcoxen, Greg J. Reinhart, Nicholas Ling and Chen Chen*

Neurocrine Biosciences, Inc., 10555 Science Center Drive, San Diego, CA 92121, USA

Received 4 October 2001; accepted 12 November 2001

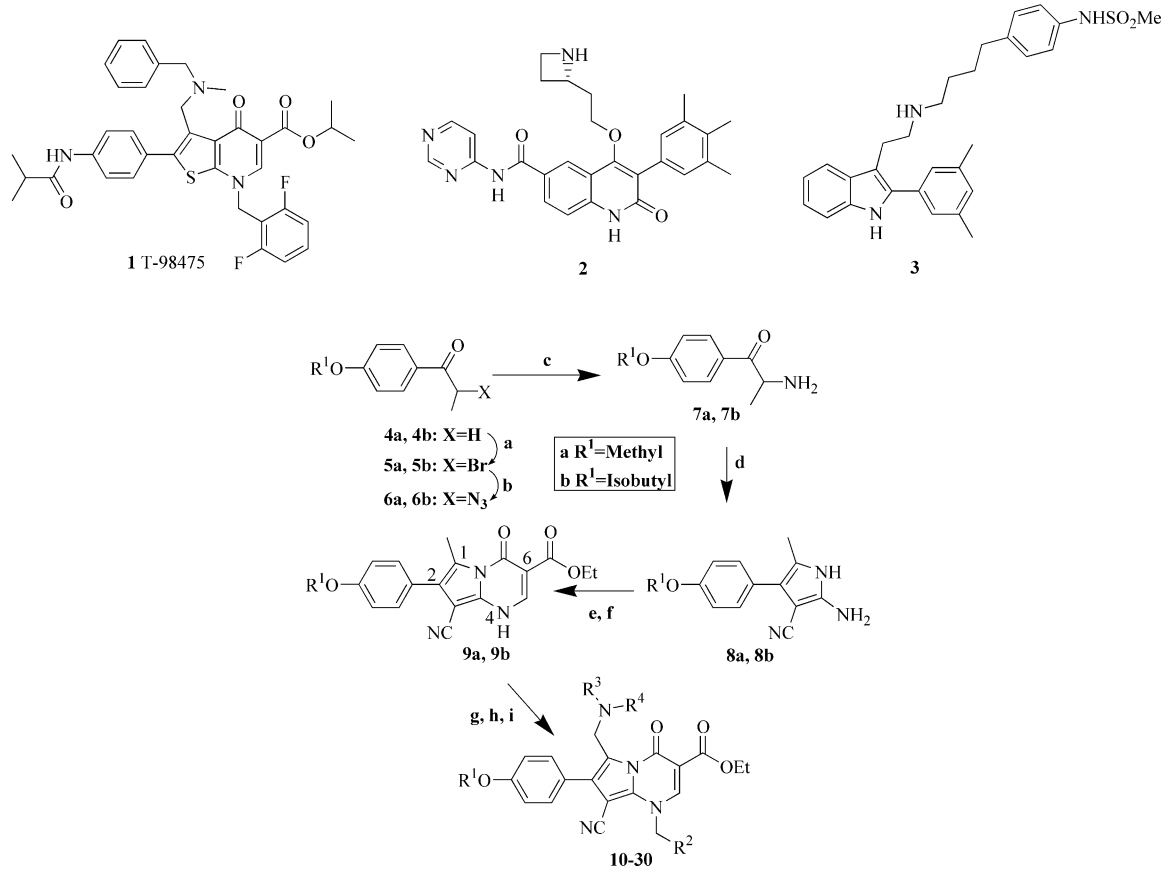
Abstract—Initial SAR studies on 1-aminomethyl-2-aryl-3-cyano-pyrrolo[1,2-*a*]pyrimid-7-one-6-carboxylates as human GnRH receptor antagonists were discussed. 2-(2-Methylaminoethyl)pyridine was discovered to be a key feature for generating active compounds. The best compound from the series had 25 nM (K_i) binding affinity to human GnRH receptor.

Gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone-releasing hormone (LHRH), is a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2), which was originally isolated and characterized from porcine[1] and ovine[2] hypothalami. It acts on the pituitary gland to stimulate the secretion of both luteinizing hormone and follicle-stimulating hormone. These gonadotropines in turn act on the reproductive organs, where they participate in the regulation of gonadal steroid production, spermatogenesis in male and follicular development in female. Several reproductive disease conditions such as endometriosis, uterine fibroids and prostate cancer can be treated by suppression of the pituitary-gonad hormonal axis. As matter of fact, depot form of peptidic GnRH agonists represented by leuprorelin© is currently used to treat such conditions through a receptor down-regulation mechanism to suppress gonadal steroid production.[3] However, recent clinical evidence has showed that peptidic GnRH antagonists can act immediately at the receptor to lower steroid level and therefore alleviate disease symptoms without the concomitant 'flare effect', which is exhibited by the peptide agonists due to their initial over-stimulation of the receptor. Nevertheless peptide antagonists still have their limitations such as poor oral bioavailability. Recently, small molecule GnRH antagonists have been described in the literature. Compound **1** (T-98475) and its analogues were reported[4] to have high potency on the human GnRH receptor ($IC_{50} = 0.2$ nM) albeit with the reduced binding affinity for the rat receptor ($IC_{50} = 60$ nM). In addition a series of papers on quinolones and tryptamines as potent GnRH antagonists were recently published.[5] For example, quinolone **2** possesses high affinity ($IC_{50} = 0.4$ nM) for the human GnRH receptor, but also shows reduced affinity ($IC_{50} = 4$ nM) for the rat receptor. While the tryptamine analogue **3** is a high-affinity inhibitor ($IC_{50} = 7$ nM) of the human GnRH receptor, but again is less potent for the rat receptor ($IC_{50} = 170$ nM). In this letter we report our initial efforts towards the design of orally active small molecule GnRH antagonists around a novel series of pyrrolo[1,2-*a*]pyrimidones.

2-Aryl-3-cyano-6-ethoxycarbonylpyrrolo[1,2-*a*]pyrimid-7-one[6] (**9a**, Scheme 1) was the core structure employed for our initial SAR study. To synthesize the core, the propanone **4a** was α-brominated by $CuBr_2$ in refluxing ethyl acetate/chloroform (1:1) and the resulting bromide **5a** was crystallized in ether at 0 °C. This was reacted with sodium azide to form azide **6a** in THF/H_2O at room temperature. The crude organic layer was transferred into a Parr bottle diluted with 1% HCl in ethanol and then hydrogenated over palladium (10% on carbon) at 36 psi to give the amino ketone **7a**. Subsequently, **7a** was refluxed with malonitrile under aqueous basic conditions to give the desired amino pyrrole

*Corresponding author. Tel.: +1-858-658-7745; fax: +1-858-658-7601; e-mail: fzhu@neurocrine.com

0960-894X/02/$ - see front matter
PII: S0960-894X(01)00779-X



Scheme 1. Reagents and conditions: (a) $CuBr_2$, EtOAc/$CHCl_3$, reflux; (b) NaN_3, THF/H_2O; (c) H_2, Pd/C, EtOH/HCl; (d) malonitrile, NaOH/H_2O–EtOH, reflux; (e) diethyl ethoxylethylene malonate, EtOH, reflux; (f) Dowtherm, 230 °C; (g) TBAF/THF, R^2CH_2Br, 60 °C; (h) NBS/CCl_4; (i) R^3R^4NH, CH_3CN, rt.

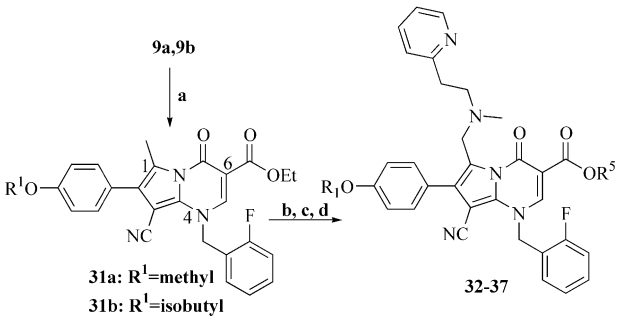
product **8a.** Heating **8a** first in ethanol with diethyl ethoxylethylene malonate and then in Dowtherm at 230 °C yielded the desired pyrrolo[1,2-*a*]pyrimidone **9a**.

Having synthesized **9a**, parallel synthesis was performed to explore two points of diversity at postions 1 and 4. In the presence of tetrabutylammonium fluoride (TBAF) in THF, **9a** underwent *N*-alkylation with a series of alkyl bromides (R^2CH_2Br), followed by radical bromination on the 1-methyl group (NBS, CCl_4, benzoyl peroxide). The bromide intermediates were treated with a variety of amines (R^3R^4NH) in CCl_4/CH_3CN (1:1), leading to the final products **10**–**22**. After obtaining the SAR information for the 1-aminomethyl and 4-alkyl groups, the investigation was expanded by optimizing the 2-(4-alkoxyphenyl) group. The approach for syntheses of compounds **23**–**30** was identical as that shown in Scheme 1, where 4-*iso*-butoxypheny replaced 4-methoxypheny at position 2. Compounds **10**–**30** were selected from a library of 400 compounds to facilitate the SAR discussion here.

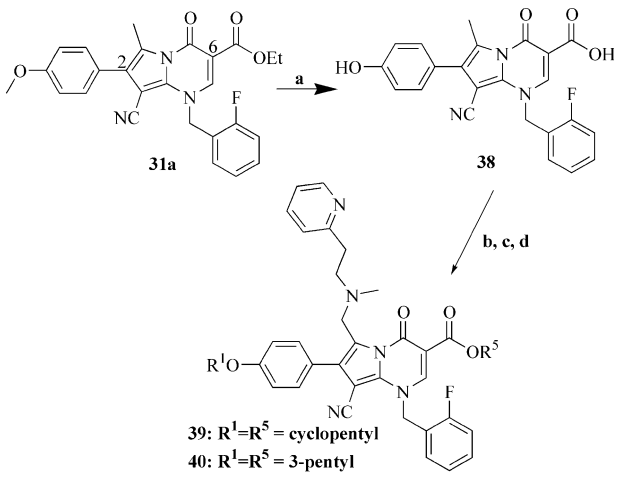
The ethyl esters **31a** and **31b**, obtained from **9a** and **9b**, respectively, by 4-benzylation with 2-fluorobenzyl bromide in the presence of TBAF in THF, were *trans*-esterified by different alcohols and *n*-butyl lithium under anhydrous conditions. The same methods outlined in Scheme 1 were then applied to introduce the 2-(2-methylaminoethyl)pyridyl group to give the desired products **32**–**37** as shown in Scheme 2. A simple approach to simultaneously introduce lipophilic groups at positions 2 and 6 was developed (Scheme 3). Compound **31a** was treated with boron tribromide at −78 °C to give **38**, which was di-alkylated with alkyl bromides (R^5Br) in dry DMF at 90 °C in the presence of K_2CO_3. Bromination with NBS and subsequently replacement by 2-(2-methylaminoethyl)pyridine yielded the desired products **39** and**40**.

All of the synthesized compounds were evaluated for their ability to compete for des-Gly^{10}[^{125}I-Tyr^5,D-Leu,^{6}NMeLeu,^{7}Pro^9-NEt]GnRH radioligand binding to the cloned human receptor stably expressed in HEK293 cells using a 96-well filtration assay format and several compounds were also tested using a cloned rat GnRH receptor binding assay.[7] The binding assay results for compounds **10**–**30** are listed in Table 1.[8] Notably, all non-basic compounds were not active (data not shown). Compound **10** with a simple benzyl substitution at position 4 and a dimethyl amine group at the 1-methyl position was not active. Weak activity (K_i = 42 μM) was observed when one of the methyl groups on **10** was replaced by a benzyl group as indicated in **11**. Binding activity was significantly enhanced, however, when a 2-(2-pyridyl)ethyl group was introduced to replace the benzyl group (**12**, K_i = 2.6 μM). Presumably an extra hydrogen bonding site and the hydrophobic aromatic ring are preferred in this area.

Simultaneous exploration of the benzyl group at position 4 led to the 2-fluorobenzyl substitutent as the best among these analogues with **16** showing 0.5 μM binding affinity. When a second fluoro group was introduced on the benzyl ring for all possible positions (**17**–**20**), no improvement on binding affinity was observed. The importance of benzyl group at position 4 was further indicated by **21**, a cyclopropylmethyl analogue, which totally lost the activity. Consequently the less active compound **22** ($K_i = 1.8$ μM) provided further evidence that the 2-fluorobenzyl group at position 4 and *N*-[2-(2-pyridyl)-ethyl]-*N*-methylaminomethyl group at the position 1 were the best substituents in this two points variation parallel synthesis study. Next, optimization of 2-aromatic ring showed that replacing the methoxyl with a more lipophilic group such as an isobutoxy generally increased the potency. For example, **23** was 3-fold more potent than its methoxyl analogue **15**. This same trend was observed for compounds **24**–**27**. Shifting the nitrogen of the pyridyl from the 2- to 3- or 4-position (**27** vs **28** or **29**) resulted in a considerable loss of binding affinity. The same was true for **30** where a phenyl was used to replace the 2-pyridyl group. These data suggested the 2-pyridyl nitrogen might act in concert with the basic amine to interact with the receptor. Apparently, none of the other pyridyl analogues can geometrically 'coordinate' such action.



Scheme 2. Reagents and conditions: (a) TBAF/THF, 2-fluorobenzyl bromide, 60 °C; (b) R^5OH, *n*-BuLi, THF; (c) NBS/CCl_4; (d) 2-(2-methylaminoethyl)pyridine, CH_3CN, rt.



Scheme 3. Reagents and conditions: (a) BBr_3, DCM; (b) R^5Br, K_2CO_3, MeOH, 50 °C; (c) NBS, CCl_4, reflux; (d) 2-(2-methyl-aminoethyl)pyridine, CH_3CN, rt.

Table 2 presents the data for a variety of esters. Changing the ethyl ester (**16**, 500 nM) to the isopropyl ester (**32**, 400 nM) did not alter the potency. However the cyclopentyl ester (**33**, 260 nM) and the 3-pentyl ester (**36**, 180 nM), yielded a 2-fold increase in potency. Both bulky dicyclopropymethyl ester **34** and less lipophoblic 3-tetrahydrofuryl ester **35** were less potent molecules. These results indicated that a medium sized lipophilic group was preferred at this position. Not surprisingly, the molecule with the combination of all four best substitutents (**37**) had very good binding affinity ($K_i = 25$ nM). Potent compounds were also obtained when the cyclopentyl or 3-pentyl moieties were introduced at both 2-(4-hydroxyphenyl) and 6-carboxyl groups (**39** and **40**). Similar to the literature data,[4,5] all the compounds exhibited much less potency on the rat GnRH receptor than the human receptor as exemplified by compounds **36**–**40**.

Table 1. Binding affinities of pyrrolo[1, 2-*a*]pyrimidones (**10**–**30**) on the human GnRH receptor[8]

Compd	R^1	R^2	R^3R^4N	K_i (μM) (human)
10	CH_3–	Ph–	$(CH_3)_2N$	> 50
11	CH_3–	Ph–	Bn–N–CH_3	42
12	CH_3–	Ph–	(2-Pyr)-CH_2CH_2–N–CH_3	2.6
13	CH_3–	2-CN–Ph–	(2-Pyr)-CH_2CH_2–N–CH_3	4.9
14	CH_3–	2-Cl–Ph–	(2-Pyr)-CH_2CH_2–N–CH_3	8.5
15	CH_3–	2-MeO–Ph–	(2-Pyr)-CH_2CH_2–N–CH_3	6.6
16	CH_3–	2-F–Ph–	(2-Pyr)-CH_2CH_2–N–CH_3	0.5
17	CH_3–	2.3-di-F–Ph–	(2-Pyr)-CH_2CH_2–N–CH_3	2.4
18	CH_3–	2.4-di-F–Ph–	(2-Pyr)-CH_2CH_2–N–CH_3	3.7
19	CH_3–	2.5-di-F–Ph–	(2-Pyr)-CH_2CH_2–N–CH_3	6.0
20	CH_3–	2.6-di-F–Ph–	(2-Pyr)-CH_2CH_2–N–CH_3	2.0
21	CH_3–	Cyclopropyl–	(2-Pyr)-CH_2CH_2–N–CH_3	> 50
22	CH_3–	2-F–Ph–	Bn–N–CH_3	1.8
23	Isobutyl	2-MeO–Ph–	(2-Pyr)-CH_2CH_2–N–CH_3	2.2
24	Isobutyl	2-F–Ph–	(2-Pyr)-CH_2CH_2–N–CH_3	0.1
25	Isobutyl	2.5-di-F–Ph–	(2-Pyr)-CH_2CH_2–N–CH_3	1.2
26	Isobutyl	2.6-di-F–Ph–	(2-Pyr)-CH_2CH_2–N–CH_3	0.8
27	Isobutyl	2.4-di-F–Ph–	(2-Pyr)-CH_2CH_2–N–CH_3	0.4
28	Isobutyl	2.4-di-F–Ph–	(3-Pyr)-CH_2CH_2–N–CH_3	28
29	Isobutyl	2.4-di-F–Ph–	(4-Pyr)-CH_2CH_2–N–CH_3	> 50
30	Isobutyl	2.4-di-F–Ph–	Ph–CH_2CH_2–N–CH_3	> 50

Table 2. Binding affinities of 1-*N*-[2-(2-pyridyl)ethyl-*N*-methyl]-aminomethyl-pyrrolo[1,2-*a*]pyrimidones (**32–40**) on the human and rat GnRH receptors[8]

Compd	R^1	R^5	K_i (nM) human	K_i (nM) rat
32	CH_3–		400	
33	CH_3–		260	
34	CH_3–		2500	
35	CH_3–		2100	
36	CH_3–		180	7700
37	$Me_2CH_2CH_2$–		25	7300
39			33	8900
40			31	7600

In conclusion, we have discovered a novel series of 1-aminomethyl-2-aryl-3-cyano-4-benzyl-pyrrolo[1,2-*a*]-pyrimid-7-ones as potent human GnRH receptor antagonists. The continued SAR studies led us to develop several more potent and novel small molecules, which will be presented in the following paper.

References and Notes

1. Matsuo, H.; Baba, Y.; Nair, R. M. G.; Arimura, A.; Schally, A. V. *Biochem. Biophys. Res. Commun.* **1971**, *43*, 1334.
2. Burgus, R.; Butcher, M.; Amoss, M.; Ling, N.; Monahan, M.; Rivier, J.; Fellows, R.; Blackwell, R.; Vale, W.; Guillemin, R. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 278.
3. Fujino, M.; Fukuda, T.; Shinagawa, S.; Kobayashi, S.; Yamazaki, I.; Nakayama, R.; Seely, J. H.; White, W. F.; Rippel, R. H. *Biochem. Biophys. Res. Commun.* **1974**, *60*, 406.
4. Cho, N.; Harada, M.; Toshihiro, I.; Imada, T.; Matsumoto, H.; Hayase, Y.; Sasaki, S.; Furuya, S.; Suzuki, N.; Okubo, S.; Ogi, K.; Endo, S.; Onda, H.; Fujino, M. *J. Med. Chem.* **1998**, *41*, 4190.
5. (a) DeVita, R. J.; Holling, D. D.; Goulet, M. T.; Wyvratt, M. J.; Fisher, M. H.; Lo, J.-L.; Yang, Y. T.; Cheng, K.; Smith, R. G. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2615. (b) DeVita, R. J.; Goulet, M. T.; Wyvratt, M. J.; Fisher, M. H.; Lo, J.-L.; Yang, Y. T.; Cheng, K.; Smith, R. G. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2621. (c) Walsh, T. F.; Toupence, R. B.; Young, J.; Huang, S. X.; Ujjainwalla, F.; DeVita, R. J.; Goulet, M. T.; Wyvratt, M. J.; Fisher, M. H.; Lo, J.-L.; Ren, N.; Yudkovitz, J. B.; Yang, Y. T.; Cheng, K.; Smith, R. G. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 443. (d) Lin, C.; Hutchins, J. E.; Weber, A. E.; Lo, J.-L.; Yang, Y. T.; Cheng, K.; Smith, R. G.; Fisher, M. H.; Wyvratt, M. J.; Goulet, M. T. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 509. (e) Lin, C.; Lo, J.-L.; Yang, Y. T.; Cheng, K.; Smith, R. G.; Fisher, M. H.; Wyvratt, M. J.; Goulet, M. T. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 515. (f) Young, J. R.; Huang, S. X.; Chen, I.; Walsh, T. F.; DeVita, R. J.; Wyvratt, M. J.; Goulet, M. T.; Ren, N.; Lo, J.-L.; Yang, Y. T.; Yudkovitz, J. B.; Cheng, K.; Smith, R. G. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1723. (g) Lin, P.; Marino, D.; Lo, J.-L.; Yang, Y. T.; Cheng, K.; Smith, R. G.; Fisher, M. H.; Wyvratt, M. J.; Goulet, M. T. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1073. (h) Lin, P.; Parikh, M.; Lo, J.-L.; Yang, Y. T.; Cheng, K.; Smith, R. G.; Fisher, M. H.; Wyvratt, M. J.; Goulet, M. T. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1077. (i) DeVita, R. J.; Walsh, T. F.; Young, J. R.; Jiang, J. L.; Ujjainwalla, F.; Toupence, R. B.; Parkh, M.; Huang, S. X.; Fair, J. A.; Goulet, M. T.; Wyvratt, M. J.; Lo, J.-L.; Ren, N.; Yudkovitz, J. B.; Yang, Y. T.; Cheng, K.; Cui, J.; Mount, G.; Rohrer, S.; Schaeffer, J. M.; Rhodes, L.; Drisko, J. E.; McGowan, E.; MacIntyre, D. E.; Vincent, S.; Carlin, J. R.; Cameron, J.; Smith, R. G. *J. Med. Chem.* **2001**, *44*, 917.
6. Abdalla, G. M.; Sowell, J. W. *J. Heterocyclic. Chem.* **1987**, *24*, 297.
7. Perrin, M. H.; Haas, Y.; Rivier, J. E.; Vale, W. W. *Mol. Pharmacol.* **1983**, *23*, 44.
8. 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.