Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 509–513

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Initial Structure–Activity Relationship of a Novel Class of Nonpeptidyl GnRH Receptor Antagonists: 2-Arylindoles

Lin Chu,[a,*] Jennifer E. Hutchins,[a] Ann E. Weber,[a] Jane-Ling Lo,[b] Yi-Tien Yang,[b] Kang Cheng,[b] Roy G. Smith,[b] Michael H. Fisher,[a] Matthew J. Wyvratt[a] and Mark T. Goulet[a]

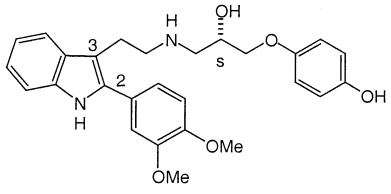
[a]*Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065-0900, USA*
[b]*Department of Biochemistry and Physiology, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065-0900, USA*

Received 24 October 2000; accepted 6 December 2000

Abstract—A nonpeptidyl GnRH receptor antagonist (**1**), with a unique 2-arylindole core, was identified through the Merck in-house screening for binding affinity on the rat GnRH receptor. SAR studies directed toward the alkoxy-ethanolamine and 2-aryl groups resulted in a simpler lead structure with improved activity. This compound **50** exhibits a 60-fold improvement in binding activity over our initial lead **1**.

Gonadotropin releasing hormone (GnRH, also known as luteinizing hormone releasing hormone, LHRH), identified by Schally in 1971,[1] is a decapeptide released by the hypothalamus in a pulsatile fashion. It acts on the pituitary gland to stimulate the biosynthesis and secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which are responsible for regulations of gonadal steroid production and reproductive development. Several endocrine-based diseases, such as endometriosis, prostate cancer, and uterine fibroids, can be treated with GnRH agonists,[2] which achieve the necessary suppression of the gonadal steroid production through a receptor down-regulation mechanism. One of the major drawbacks associated with receptor down-regulation is that the initial over-stimulation, the 'flare effect', tends to exacerbate the symptoms of the disorders. There have been several recent reports[3,4] showing that peptidyl GnRH antagonists directly lowered the production of sex steroids without the detrimental flare effect. These GnRH antagonists, being peptidyl in nature, have not been reported to exhibit useful oral activities, which could be more easily obtained with nonpeptidyl, small molecules. Recently, two different classes of nonpeptidyl GnRH receptor antagonists have been reported.[5–9] In this letter, we disclose a novel class of GnRH antagonists along with initial structure–activity studies toward improving the activity of this design.

In-house screening measuring binding affinity on the rat GnRH receptor[10] identified the 2-(3,4-dimethoxy-phenyl)indole compound **1** ($IC_{50}=3$ μM) as a GnRH receptor antagonist. A medicinal chemistry effort was initiated to determine the viability of this lead.

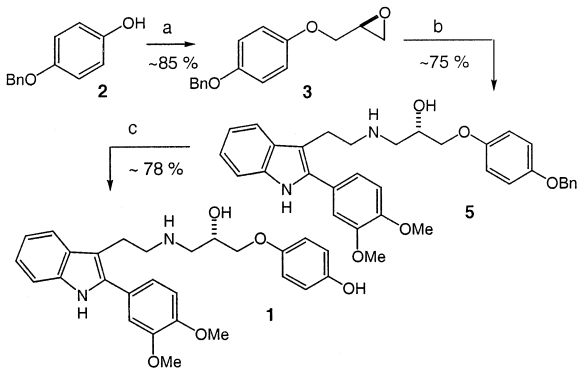


1, rGnRH binding IC_{50} = 3 μM

The lead compound **1** was prepared following the synthetic sequence shown in Scheme 1.[11] Commercially available 4-benzyloxy-phenol (**2**) was alkylated with (2*S*)-(+)-glycidyl 3-nitrobenzene-sulfonate to afford the epoxide **3**, which was reacted with 3-(2-aminoethyl)-2-(3,4-dimethoxyphenyl)indole (**4**) in refluxing methanol to form the epoxide-opened product **5** in good yields. The final deprotection was accomplished via palladium hydroxide catalyzed hydrogenolysis. The corresponding *R* enantiomer (**6**) was synthesized by substituting the 2*S*-glycidyl 3-nosylate with its *R* enantiomer.

*Corresponding author. Tel.: +1-732-594-1125; fax: +1-732-594-5966; e-mail: lin_chu@merck.com

0960-894X/01/$ - see front matter
PII: S0960-894X(00)00707-1

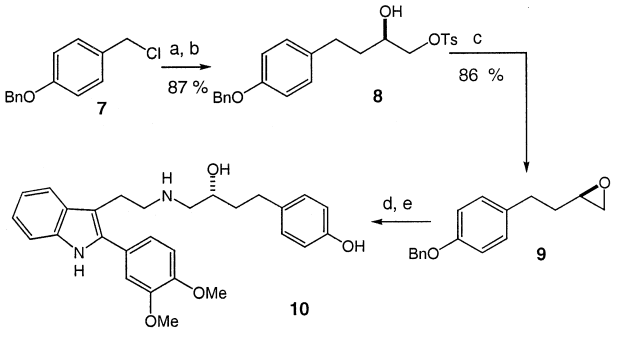


Scheme 1. Reagents and conditions: (a) NaH, (2*S*)-(+)-glycidyl 3-nitrobenzenesulfonate, DMF; (b) 3-(2-aminoethyl)-2-(3,4-dimethoxyphenyl)indole (**4**), MeOH, reflux; (c) $Pd(OH)_2$, H_2, MeOH.

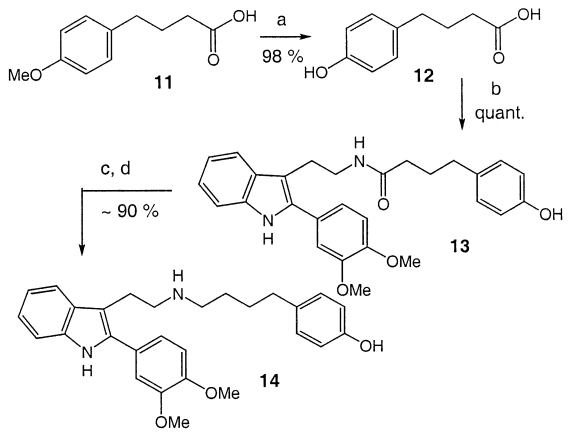
The synthesis of an analogue in which the oxygen of the ether linkage in the lead was substituted with a methylene unit (**10**) is outlined in Scheme 2. 4-Benzyloxybenzyl chloride (**7**) was converted to the corresponding Grignard reagent, which was then reacted with (*R*)-glycidyl tosylate to afford compound **8**. Upon treatment with potassium carbonate, compound **8** cyclized to the epoxide **9**, which was reacted with excess tryptamine **4** in refluxing methanol and deprotected with palladium hydroxide catalyzed hydrogenolysis to reveal the target compound **10**.

To obtain a fully deoxygenated tether, 4-(4-methoxyphenyl)butyric acid (**11**, Scheme 3) was demethylated with boron tribromide to give 4-(4-hydroxyphenyl)butyric acid (**12**), which was subsequently coupled with tryptamine **4** to afford amide **13**. Amide **13** was reduced to the dialkylamine with borane·THF. It is worth noting that the initial product from this borane reduction was a very stable boron–amine complex, which could be isolated by standard silica gel chromatography. Due to stability and solubility issues, *N*,*N*-dimethylethanolamine was used to break down the boron–amine complex, and the amine product **14** could be isolated in excellent overall yield.

The synthetic sequence outlined in Scheme 3 was also applied toward the preparation of analogues of **14**, starting with 4-hydroxybenzoic acid (**15**), 2-(4-hydroxyphenyl)acetic acid (**16**), 3-(4-hydroxyphenyl)propionic



Scheme 2. Reagents and conditions: (a) Mg, Et_2O; (b) (*R*)-glycidyl tosylate, LiCl, $CuCl_2$, THF; (c) K_2CO_3, MeOH, CH_2Cl_2; (d) **4**, MeOH, reflux; (e) $Pd(OH)_2$, H_2.

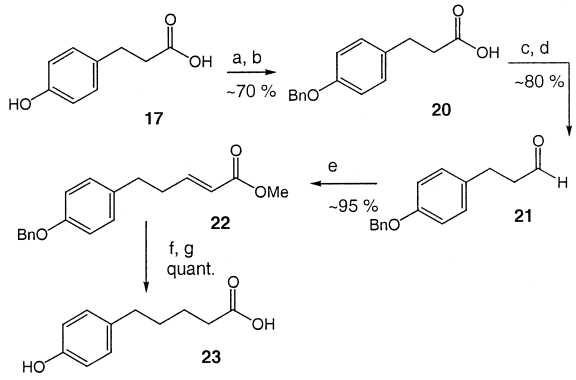


Scheme 3. Reagents and conditions: (a) BBr_3, CH_2Cl_2; (b) HOBT, EDAC, **4**, DMF/CH_2Cl_2; (c) BH_3·THF, THF, reflux, 2 h; (d) excess *N*,*N*-dimethylethanolamine, MeOH/THF, reflux, 3–5 h.

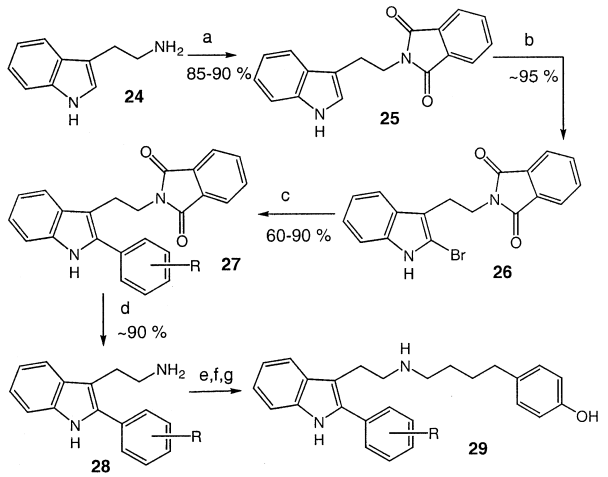
acid (**17**), 5-(4-hydroxyphenyl)pentanoic acid (**18**), and 6-(4-hydroxyphenyl)hexanoic acid (**19**). The latter two were readily obtainable from 2-carbon homologations of acids **17** and **12**, respectively, as shown in Scheme 4.

To introduce a wide range of aryl substituents to the 2-position of the indole, a versatile and high-yielding protocol employing a modified Suzuki coupling reaction as the key step was developed.[12] Protection of the amino group of tryptamine (**24**) with *N*-carbethoxyphthalimide (Scheme 5) in refluxing THF for 1–2 days afforded pure phthalimide **25** in high yield after trituration with hexane/CH_2Cl_2 (3:1). Selective bromination of phthalimide **25** on the 2-position of the indole was accomplished with pyridine hydrobromide perbromide, and the product bromide, compound **26**, was subjected to modified Suzuki coupling conditions to afford the coupled compound **27** in good yields. Deprotection of **27** with aqueous hydrazine yielded tryptamine **28**, which was subsequently carried to the targeted analogue **29** through transformations described in Scheme 3.

All analogues prepared were tested in the rat GnRH receptor binding assay for the ability to displace [^{125}I]-radio-labeled buserelin.[11] Those with binding activities



Scheme 4. Reagents and conditions: (a) NaH, BnBr, DMF; (b) NaOH, MeOH/H_2O; (c) HOBT, EDAC, *N*,*O*-dimethylhydroxylamine hydrochloride, Et_3N, DMF/CH_2Cl_2; (d) DIBAL, THF, −78 °C; (e) methyl (triphenylphosphoranylidene) acetate, CH_2Cl_2; (f) $Pd(OH)_2$, H_2, MeOH/EtOAc; (g) NaOH, MeOH/H_2O.



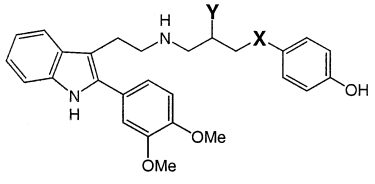
Scheme 5. Reagents and conditions: (a) *N*-carbethoxyphthalimide, THF, reflux; (b) pyridine hydrobromide perbromide, THF/$CHCl_3$, 0 °C; (c) boronic acid, Na_2CO_3, LiCl, $Pd(PPh_3)_4$, toluene/EtOH (1:1), reflux, 3–5 h; (d) hydrazine in H_2O, THF/EtOH, overnight; (e) **12**, HOBT, EDAC, DMF/CH_2Cl_2; (f) BH_3•THF, THF, reflux; (g) *N*,*N*-dimethylethanolamine, MeOH/THF, reflux, 3 h.

better than 10 μM were titrated on a four-point curve, and their IC_{50} values were calculated.

To examine the importance of stereochemistry in the lead compound **1**, the corresponding *R* enantiomer **6** was synthesized and tested. Interestingly, the *R* isomer **6** was equipotent to the lead **1** (Table 1). We next substituted the oxygen of the ether linkage with a methylene unit (**10**) to evaluate the role of the oxygen on receptor binding. Again, compound **10** exhibited similar activity as the lead **1**. Since neither the absolute stereochemistry of the molecule nor the ether linkage appeared significant for GnRH receptor binding, simplified analogue **14** was prepared. We were pleased to see that even with this simplified tether connecting the phenyl and the central amine, no loss of GnRH binding activity was observed.

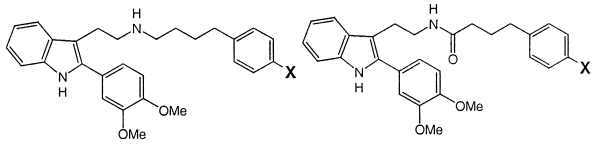
Several analogues containing phenol replacements were also prepared using the synthetic sequence outlined in Scheme 3, and selected ones are listed in Table 2, together with their amide counterparts. It is apparent that the basic amine was superior to an amide linker for maintaining affinity for the GnRH receptor.

Table 1.



Analogue	–X–	–Y–	IC_{50} (μM)
1	–O–	····OH , S	3
6	–O–	—OH , R	3
10	$–CH_2–$	····OH , S	2
14	$–CH_2–$	—H	2

Table 2.



X	Analogue	IC_{50} (μM)	X	Analogue	IC_{50} (μM)
–OH	**14**	2	–OH	**13**	>30
–OBn	**30**	30	–OBn	**31**	—[a]
$–NO_2$	**32**	5	$–NO_2$	**33**	>30
$–NH_2$	**34**	10	$–NH_2$	**35**	30

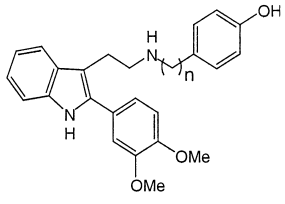
[a]0% Inhibition at 10 μM.

A series of analogues with various tether lengths was synthesized in order to determine the optimal spacing between the phenol and the central amine. As shown in Table 3, analogues containing tether lengths of two to five carbon atoms (**14** and **37**–**39**) had essentially equal affinity for the GnRH receptor.

The investigation of the SARs in the indole region was most rewarding. Table 4a shows some of the compounds that we prepared with zero or one substituent on the aryl group at the 2-position of the indole. *para*-Substitutions were detrimental to binding activity; even a small fluoro group decreased the binding affinity substantially. From the study of disubstituted aryls (Table 4b), we discovered that the 3,5-dimethylphenyl group provided a dramatic increase in binding activity (**50**). Further investigation on bicyclic aryls (Table 4c) also showed that several of these analogues bound better than the 2-phenyl indole **41**, but none was as active as the 3,5-dimethylphenyl containing analogue **50**.

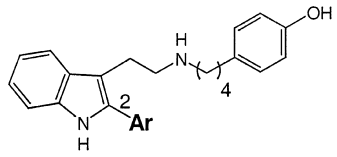
With the potency-enhancing, 3,5-dimethylphenyl group on the 2-position of the indole, another tether length study (Table 5) was conducted following the aforementioned synthetic sequence. The result of this study indicated that, in this context, the four-carbon tether length is at least 4-fold more potent than all others tested.

Table 3.



n	Analogue	IC_{50} (μM)
1	**36**	20
2	**37**	4
3	**38**	6
4	**14**	2
5	**39**	2
6	**40**	10

Table 4a.



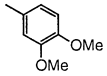
Analogue	Ar	IC_{50} (μM)	Analogue	Ar	IC_{50} (μM)
14		2.0	**44**		0.5
41		1.3	**45**		0.2
42		11.0	**46**		0.9
43		9.0	**47**		0.6

Table 4b.

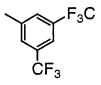
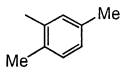
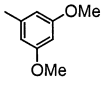
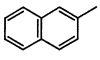
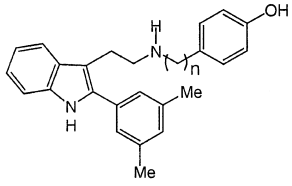
Analogue	Ar	IC_{50} (μM)	Analogue	Ar	IC_{50} (μM)
48		1.5	**52**		1.7
49		0.17	**53**		0.4
50		0.05	**54**		0.9
51		0.7			

Table 4c.

Analogue	Ar	IC_{50} (μM)	Analogue	Ar	IC_{50} (μM)
55		0.13	**58**		0.30
56		2.5	**59**		2.5
57		1.9	**60**		2.8

The initial SAR study on the novel 2-arylindole GnRH antagonist lead **1**, simplified the ether-ethanolamine linker to a four-carbon tether without any loss in binding affinity. It was also demonstrated that the basic amine was important for maintaining affinity for the GnRH receptor. However, a variable distance between this amine and the phenol pharmacophore was tolerated. Furthermore, a wide range of aryl groups at the 2-position of the indole were explored, which led to the discovery of the 3,5-dimethylphenyl group being the most potency-enhancing group. A subsequent tether length study indicated that a four-carbon spacer between the amine and phenol was optimal. A 60-fold improvement in binding affinity was accomplished through our initial SAR studies. In conclusion, we have demonstrated the viability of this novel class of 2-arylindoles as promising nonpeptidyl GnRH receptor antagonists. Further progress in this structural class is described in the accompanying report.

Table 5.



n	Analogue	IC_{50} (μM)
1	**61**	9.0
2	**62**	0.20
3	**63**	0.25
4	**50**	0.05
5	**64**	0.20
6	**65**	0.60

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. Kardamakis, E.; Tzingounis, V. In *Biomed. Health Res.*; IOS Press, Vol. 22 (*Bioactive Peptides in Drug Discovery and Design: Medical Aspects*), 1999; pp 275–285, and references cited therein.
3. Kutscher, B.; Bernd, M.; Beckers, T.; Polymeropoulos, E. E.; Engel, J. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2149 and references cited therein.
4. Behre, H. M.; Nordhoff, V.; Nieschlag, E. Ovul. Induct. Update '98, Proc. World Conf., 2nd, 1997. Filicori, M., Flamigni, C., Eds.; Parthenon Publishing: Carnforth, UK (English) 1998; pp 107–113.
5. Cho, N.; Harada, M.; Imaeda, T.; 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.
6. DeVita, R. J.; Hollings, 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.
7. DeVita, R. J.; Goulet, M.; Wyvratt, M. J.; Fisher, M. H.; Lo, J.-L.; Yang, Y.-T.; Cheng, K.; Smith, R. G. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2621.
8. Walsh, T. F.; Toupence, R. B.; Young, J. R.; 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.
9. 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.
10. Crude membranes prepared from rat pituitary glands were the GnRH receptor source and [^{125}I]buserelin (a peptidyl

GnRH analogue) was used as the radio-labeled ligand. The competitive binding was conducted in a Tris–HCl based buffer at 4 °C for 90 min. The activities are presented as the IC_{50} for the inhibition of [^{125}I]buserelin binding to the receptors. A similar assay is described in ref 5 using a different radioligand.

11. Weber, A. E.; Mathvink, R. J.; Perkins, L.; Hutchins, J. E.; Candelore, M. R.; Tota, L.; Strader, C. D.; Wyvratt, M. J.; Fisher, M. H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1101.
12. Chu, L.; Fisher, M. H.; Goulet, M. T.; Wyvratt, M. J. *Tetrahedron Lett.* **1997**, *38*, 3871 and references cited therein.