Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3467–3470

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

The Discovery of Novel Small Molecule Non-peptide Gonadotropin Releasing Hormone (GnRH) Receptor Antagonists

David R. Luthin,* Yufeng Hong, Ved P. Pathak, Genevieve Paderes, Karen D. Nared-Hood, Mary A. Castro, Haresh Vazir, Haitao Li, Eileen Tompkins, Lance Christie, John M. May and Mark B. Anderson

Pfizer Global Research and Development-La Jolla/Agouron Pharmaceuticals, Inc., 10724 Science Center Drive, San Diego, CA 92121, USA

Received 5 June 2002; accepted 12 August 2002

Abstract—A novel series of non-peptide derivatives **1**, **14**, and **15** that bind with high affinity to the human GnRH receptors is discussed. The discovery was made from screening our in-house libraries that contained the active structure **2** along with a trace amount of a second active structure **1** that was derived from an acid-induced rearrangement. From this structure type **1**, a series of guanidine and non-guanidine containing analogues were prepared and tested as GnRH receptor antagonists. Compounds derived from this series bind to both human and rat GnRH receptors and antagonize GnRH-mediated increases in inositol phosphate production in cells containing recombinant human receptors. These compounds or their analogues may be useful as therapeutic agents for the treatment of hormone-dependent pathologies including prostate, breast and ovarian cancers.

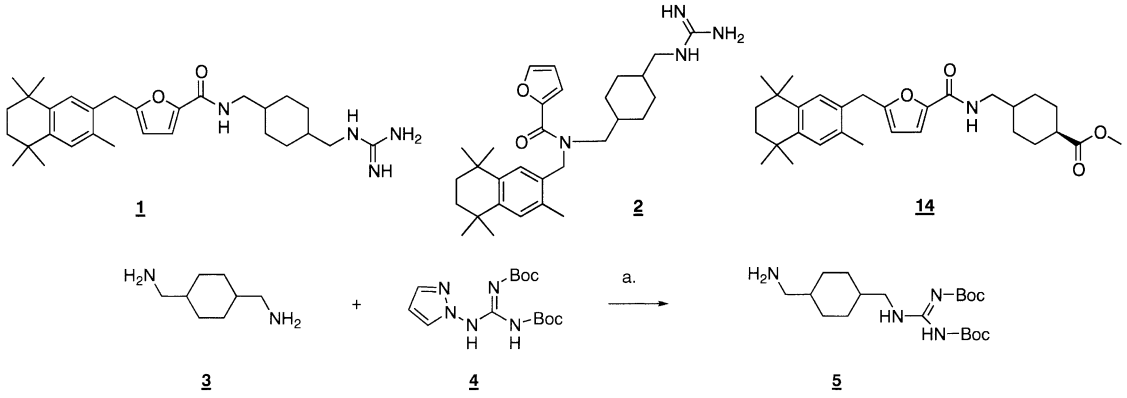
GnRH[1] is a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) that is synthesized in the hypothalamus and acts upon receptors in the anterior pituitary where it triggers[2] the synthesis and release of LH and follicle-stimulating hormone (FSH). LH and FSH enter the systemic circulation and bind to receptors in the gonads where they stimulate steroidogenesis and gametogenesis in testes and ovaries.[3] Peptide GnRH receptor antagonists have been studied by various groups,[4] due to their potential therapeutic benefit[5–8] in treating hormone-dependent diseases such as prostate, breast and ovarian cancers with additional possibilities in fertility disorders, endometriosis, uterine fibroids, and precocious puberty. Several companies have recently reported the discovery of nonpeptide GnRH antagonists.[9–14] We report here the identification of a novel series of non-peptide GnRH receptor antagonists that might have utility as therapeutic agents for treating hormone-dependent disease.

The synthetic pathway for the preparation of the guanidine containing core structure is shown in Scheme 1. The synthesis involves the slow addition of the solid bis-BOC protected *N*-(1*H*-pyrazol-1-yl)guanidine **4** to a dilute THF solution of the diamine **3** in order to ensure monofunctionalization to a bis-BOC protected guanidine **5** in an overall good yield. Once **5** was prepared, 2-furoyl chloride **10** was used to acylate the free amine followed by deprotection to give *N*-{[4-({[amino(imino) methyl]amino}methyl)cyclohexyl]methyl} - 5 - [(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)methyl]-2-furamide **1** (Scheme 3) using TFA in dichloromethane at room temperature. Deprotected **1** was then purified by HPLC. The preparation of **9** involved a Friedel–Crafts alkylation of toluene using 2,5-dichloro-2,5-dimethylhexane **6**[15] which gave 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene **7**. This alkylation was followed by a second Friedel–Crafts alkylation using methyl 5-(chloromethyl)-2-furoate **8**. The resulting methyl 2-furoate **9** was saponified to its corresponding carboxylic acid and then transformed to 2-furoyl chloride **10** using thionyl chloride (Scheme 2). Additional analogues like methyl 4-[({5-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)methyl]-2-furoyl}amino) methyl]cyclo hexanecarboxylate **14** (Scheme 4) were prepared in a similar fashion and are shown in Table 1.[16]

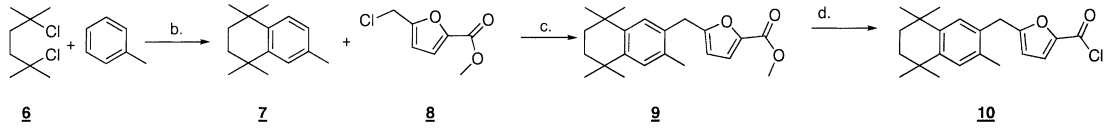
*Corresponding author. Tel.: +1-858-526-4968; fax: +1-858-526-4121; e-mail: david.luthin@pfizer.com

0960-894X/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)00755-2

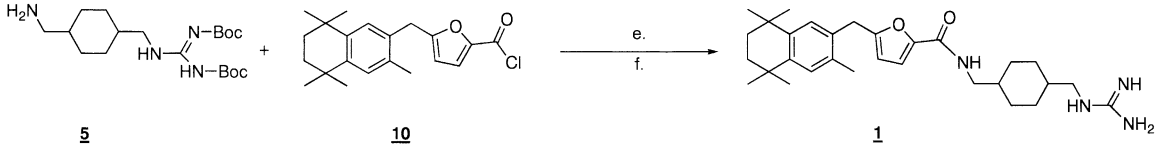
Although the guanidine-containing analogues were potent GnRH receptor antagonists (Fig. 1), it was perceived that guanidines, due to potential absorption issues, may be a liability in developing them as potential therapeutics. Therefore, we wanted to determine if we could remove and/or replace the guanidine moiety with a small variety of other functional groups and still retain activity against the rat and human GnRH receptors. Two clear examples of potent guanidine-containing compounds stand out from our efforts. The initial **1** was a mixture of *cis* and *trans* isomers and bound to human and rat receptors with K_i's of 40 and 520 nM, respectively. However, separation of the *cis* **11** and *trans* **12** isomers did not result in appreciable potency changes for either pure isomer, suggesting that the *cis* or *trans* configuration did not play a significant role in binding affinities in these specific examples. However, removal of the guanidine functionality completely as in **13**, reduced affinity at both human and rat receptors with K_i's of 180 and 1550 nM, respectively. Adding a simple methyl ester **14** afforded comparable affinity at human but slightly reduced affinity at rat receptor with K_i's of 24 versus 830 nM, respectively, whereas amide **15** afforded similar activity to the guanidine moiety with human and rat K_i's of 13 and 270 nM, respectively. In general, most of these compounds showed a marked 5–60× preference for the human GnRH receptor with the exceptions of **19**, **20**, and **21** that showed similar affinities between species. Some of the higher-affinity compounds at the human receptor (**1**, **11**–**13**, and **15**–**18**) were tested for their ability to antagonize GnRH-stimulated increases in total inositol phosphate production and were determined to be antagonists with comparable affinities to their binding K_i's (see Fig. 1; **1**: $K_B = 30$ nM, $K_i = 40$ nM). Although these are potent against the human GnRH receptor, most of these compounds did not have sufficient binding affinity to the rat GnRH receptor to warrant extensive animal studies. Further exploration of



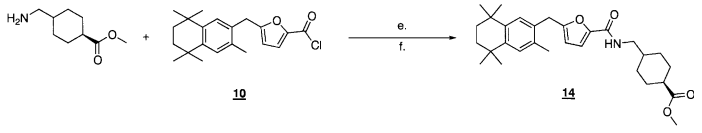
Scheme 1. Reagents and conditions: (a) 0.01 M solution in THF (ratio A/B 1/1.1 equiv), 2 h, rt.



Scheme 2. Reagents and conditions: (b) 0.5 equiv $AlCl_3$, CH_2Cl_2, reflux, 97% yield; (c) 1.0 equiv $AlCl_3$,CH_2Cl_2, reflux, 2 h 68%; (d) 5 equiv LiOH, 50:25:25 THF/MeOH/H_2O, 4 h, 2. 20 equiv SO_2Cl_2 CH_2Cl_2 reflux, 5 h overall 71%.



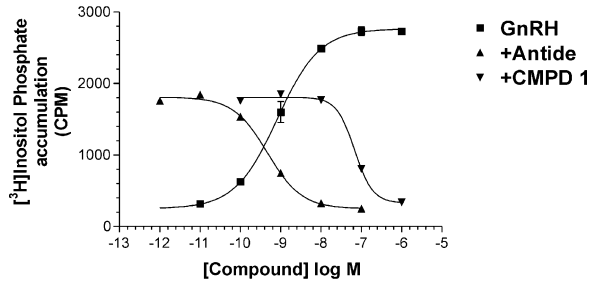
Scheme 3. Reagents and conditions: (e) ethyl acetate, triethyl amine, 1–12 h, rt; (f) 50% TFA, 30 min, rt.



Scheme 4. Reagents and conditions: (e) ethyl acetate, triethyl amine, 1–12 h, rt.

these series is under investigation to improve multi-species metabolic stability and potency. Substitution around the cyclohexyl ring system exhibited species differences as well as clear potency differences. The SAR suggests that the guanidine portion was not critical to the overall binding affinity of the structure. These compounds represent the seminal set of chemical leads from which to develop potential GnRH receptor antagonists.

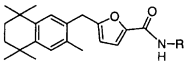
In this study, 5-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)methyl]-2-furoyl chloride **10** was a key structural building block. The combination of this building block with the amines shown in Table 1 was responsible for the general activity in the GnRH receptors. Compounds were potent antagonists of human GnRH receptors in vitro. These agents or their

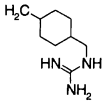
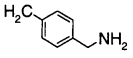
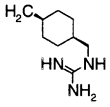
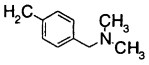
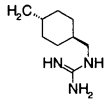
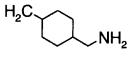
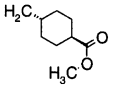
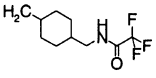
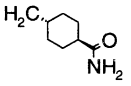
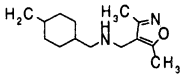
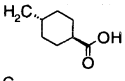
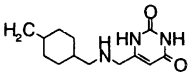
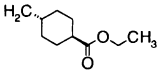
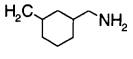
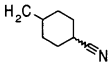
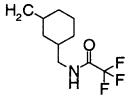
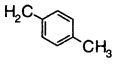
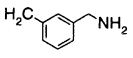
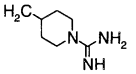
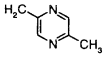
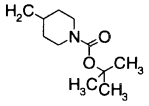


Compound	EC_{50}(nM)	K_B(nM)
GnRH	0.83	
Antide		0.2
CMPD1		30

Figure 1. Inhibition of GnRH-stimulated total inositol phosphate accumulation by 1 or the peptide antagonist, antide.[16]

Table 1. Binding affinity constants of various compounds binding to human and rat GnRH receptors



Analogue	R	Human K_i (nM)	Rat K_i (nM)	Analogue	R	Human K_i (nM)	Rat K_i (nM)
1		40±5	520±70	**22**		140±11	280±50
11		40±6	730±44	**23**		79±20	140±35
12		16±3	385±12	**24**		102±14	240±40
13		180±16	1550±340	**25**		42±7	730±95
14		24±3	830±120	**26**		66±5	2720±210
15		13±4	270±23	**27**		180±30	1200±220
16		250±26	3000±630	**28**		24±2	865±230
17		51±7	2730±380	**29**		174±13	890±120
18		17±3	560±46	**30**		20±2	250±12
19		83±4	60±13	**31**		700±130	5210±710
20		91±17	94±30	**32**		220±21	2040±300
21		56±16	55±8	**33**		54±6	3260±410

analogues may serve as useful therapeutic agents for treating hormone-dependent pathologies including hormone-dependent prostate and breast cancer. Further investigations of analogues in this area are being pursued.

Acknowledgements

We thank Sandra Barnum for her excellent cell culture work and Stuart Sealfon (Mt. Sinai School of Medicine) for providing the cell line expressing recombinant human GnRH receptors.

References and Notes

1. Stanislaus, D.; Pinter, J. H.; Janovick, J.; Conn, P. M. *Mol. Cell. Endocrinol.* **1998**, *144*, 1.
2. Millar, R.; Assefa, D.; Ott, T.; Pawson, A.; Troskie, B.; Wakefield, I.; Katz, A. *Horm. Front. Gynecol.* **1998**, *5*, 385.
3. Kaiser, U. B.; Conn, P. M.; Chin, W. W. *Endocrine Rev.* **1997**, *18*, 46.
4. Bouchard, P. In *GnRH Analogues: The State of the Art 1996*; Lunenfeld, B.; Insler, V., Eds.; Parthenon: New York, 1996; p 21.
5. Huirne, J. A. F.; Lambalk, C. B. *Lancet* **2000**, *358*, 1793.
6. Harris, K. A.; Small, E. J. *Exp. Opin. Invest. Drugs* **2001**, *10*, 493.
7. Cook, T.; Sheridan, W. P. *Oncologist* **2000**, *5*, 162.
8. Leibowitz, R. L.; Tucker, S. J. *Oncologist* **2001**, *6*, 177.
9. 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.
10. (a) 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. (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) Devita, R. J.; Walsh, T. F.; Young, J. R.; Jiang, J.; Ujjainwalla, F.; Toupence, R. B.; Parikh, 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. P.; 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.
11. 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.
12. (a) Ashton, W. T.; Sisco, R. M.; Yang, Y. T.; Lo, J.-L.; Yudkovitz, J. B.; Cheng, K. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1723. (b) Ashton, W. T.; Sisco, R. M.; Yang, Y. T.; Lo, J.-L.; Yudkovitz, J. B.; Gibbons, P. H.; Mount, T. R.; Ren, R. N.; Butler, G. S.; Cheng, K.; Goulet, M. T. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1727. (c) Ashton, W. T.; Sisco, R. M.; Kieczykowski, G. R.; Ynag, Y. T.; Yudkovitz, J. B.; Cui, J.; Mount, G. R.; Ren, R. N.; Wu, T.-J.; Shen, X.; Lyons, K. A.; Mao, A.-H.; Carlin, J. R.; Karanam, B. V.; Vincent, S. H.; Cheng, K.; Goulet, M. T. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2597.
13. (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.; Gross, T. D.; Tucci, F. C.; Struthers, R. S.; Reinhart, G. J.; Xie, Q.; Chen, C. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 403.
14. Besecke, L. M.; Diaz, G. J.; Segreti, J. A.; Mohning, K. M.; Cybulski, V. A.; Rao, M.; Bush, E. N.; Randolph, J. T.; Waid, P. I.; Haviv, F.; Wegner, C. D.; Greer, J. *Drug Dev. Res.* **2001**, *52*, 485.
15. Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Himi, T.; Shudo, K. *J. Med. Chem.* **1988**, *31*, 2182.
16. Detailed experimentals and analytical data can be found in: Anderson, M. B.; Vazir, H. N.; Luthin, D. R. Paderes, G. D.; Pathak, V. P.; Christie, L. C.; Hong, Y.; Tompkins, E. V.; Li, H.; Faust, J. WO 00/20358, 2000; *Chem Abstr.* **2000**, *132*, 279106.