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Characterization of Mono- and Diaminopyrimidine Derivatives as Novel, Nonpeptide Gonadotropin Releasing Hormone (GnRH) Receptor Antagonists

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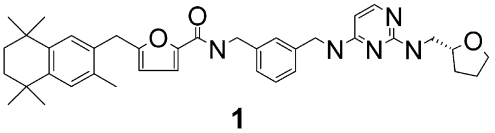
Abstract—A novel series of derivatives of mono- and diaminopyrimidines **1** potently displaced binding of a radiolabeled GnRH analogue to human and rat GnRH receptors. Analogues from these series competitively antagonized GnRH-stimulated increases in extracellular acidification in vitro and suppressed GnRH-mediated increases in circulating luteinizing hormone (LH) in castrated rats and testosterone in intact rats. These compounds or their analogues may be useful in treating sex hormone-dependent disease.

GnRH plays a central role in the biology of reproduction. The GnRH decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) is synthesized in the hypothalamus and acts upon receptors in the anterior pituitary where it triggers 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.[1] Peptide GnRH receptor antagonists have been studied by various groups[2] due to their potential therapeutic benefit in treating hormone-dependent diseases including fertility disorders, endometriosis, uterine fibroids, precocious puberty, and various hormone-dependent cancers such as prostate, ovarian, and breast cancer. Several companies have recently reported the discovery of nonpeptide GnRH antagonists.[3–6] We report here the identification of a novel series of nonpeptide GnRH antagonists that might have utility as therapeutic agents for treating hormone-dependent disease.

The synthetic pathway for the preparation of compounds **1** and **13–15** is illustrated in Schemes 1 and 2. Amination of 2,4-dichloropyrimidine with tetrahydrofurylamine afforded compounds **2** and **3** in a ratio of 3:1. Mono-protection of *m*-xylene diamine was carried out in THF in the presence of trifluoroacetate. The reaction gave **4** in 53% yield and the bis-protected amine side product was also observed. The remaining amino group was protected as ethylcarbamate **5** and the trifluoroacetylamide was cleaved to give mono amino carbamate **6**.[7] Amination of compound **3** followed by cleavage of the carbamate afforded the bis-substituted pyrimidine **8**.[8] The synthesis of **1** was accomplished by coupling **8** with **9.** Compounds **16–38** (Table 1) were prepared following the general chemistry procedure illustrated in Scheme 3. The acylation and deprotection steps gave almost quantitative yields. The yields of the reaction from **11** to **12** were 30–70%, depending upon the substitution on the pyrimidine.[9]



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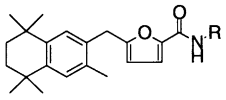
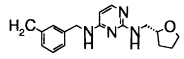
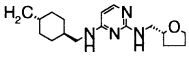
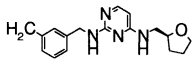
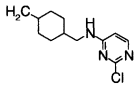
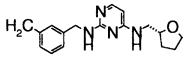
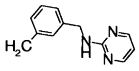
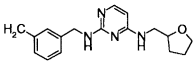
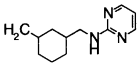
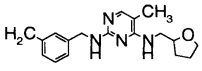
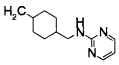
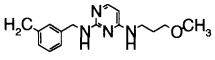
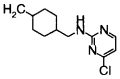
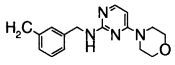
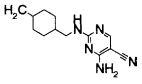
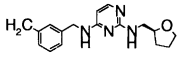
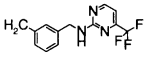
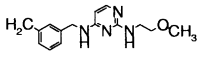
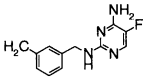
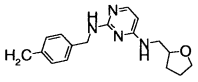
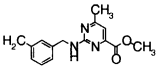
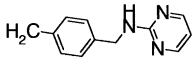
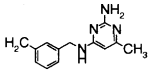
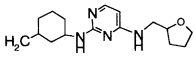
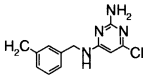
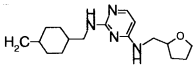
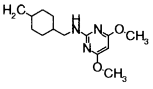
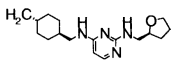
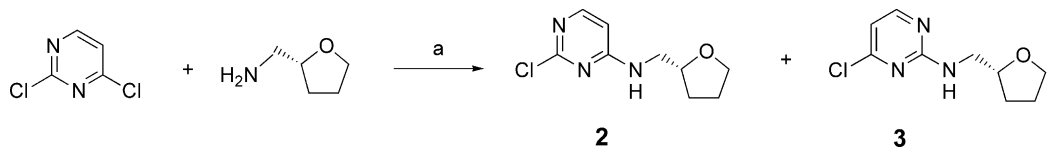
In this report, we have optimized the guanidine GnRH receptor antagonist analogues presented in the accompanying report for potency at both human and rat GnRH receptors. Modifications on the left-hand portion of the amide bond will be presented in future reports. Although the mono- and diaminopyrimidines appear in essence as 'caged' guanidines, they confer unique characteristics to the structure–activity relationships of the series. The mono-aminopyrimidine compounds (**27**–**31**) had similar affinity and preference (~5–40×) for the human versus the rat receptor as the parent guanidines reported in the adjoining paper. Others[3,6] have reported species differences with very different chemical series, which can be problematic when utilizing animal models with surrogate marker endpoints (e.g., testosterone suppression). The di-aminopyrimidines were the most potent compounds generated within the series and in contrast to the mono-aminopyrimidines (**27**–**31**), several exhibited either similar (**16**, **17**, **20**, and **21**) binding between rat and human receptors or a notable preference (5–10×; **13**–**15**, **18**, **19**, and **23**) for the rat receptor versus the human counterpart. 4-amino substitutions

Table 1. Binding affinity constants of various mono- and diaminopyrimidine analogues to human and rat GnRH receptors[10]

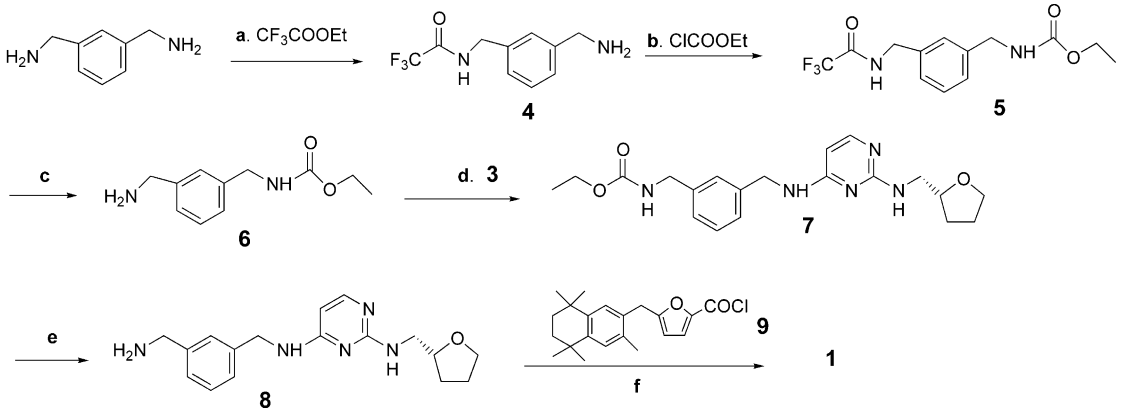


Compd	R	Human K_i (nM)[a]	Rat K_i (nM)	Compd	R	Human K_i (nM)	Rat K_i (nM)
1		9.3±0.9	4.7±0.4	**26**		175±45	235±49
13		8±0.8	1.6±0.7	**27**		103±18	2140±500
14		56±8	3±0.6	**28**		130±10	510±30
15		33±9	6±0.7	**29**		67±7	1540±360
16		14±1.5	7±1.5	**30**		150±40	1760±60
17		11±1.7	11±0.7	**31**		170±16	5210±1250
18		80±7	7.5±1.5	**32**		63±15	1490±340
19		10±3	2.5±1	**33**		780±33	ND
20		44±7	22±2	**34**		220±23	ND
21		40±7	33±10	**35**		510±55	ND
22		110±10	400±40	**36**		29±5	55±7
23		57±4	9±0.3	**37**		35±10	380±40
24		74±19	26±5	**38**		300±80	ND
25		28±8	370±90				

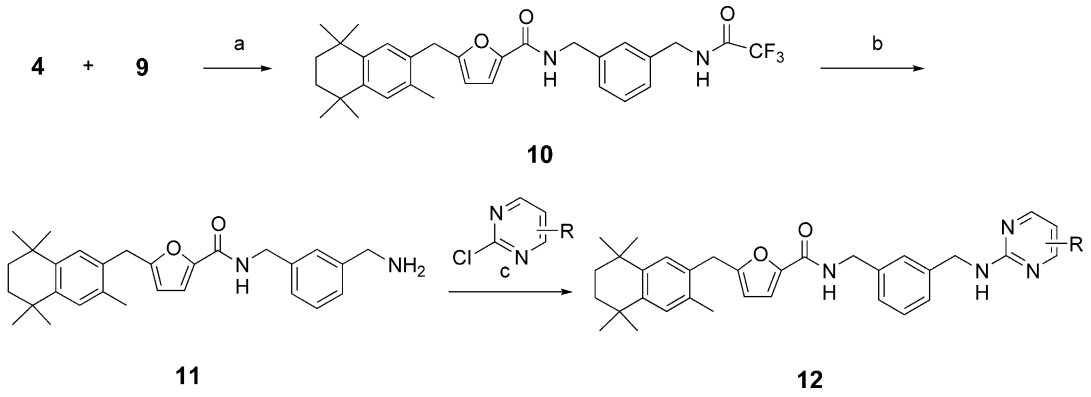
[a]Values are means of at least three experiments±SE; K_i values were determined from IC_{50} values;[10] ND = not determined.



Scheme 1. Reagents and conditions: (a) Et_3N, THF, rt, 2 h, 85%.



Scheme 2. Reagents and conditions: (a) THF, rt, overnight, 53% yield; (b) Et_3N, CH_2Cl_2, rt, 20 min, 95%; (c) 20% K_2CO_3, MeOH, rt, 3 h, 90%; (d) Et_3N, chlorobenzene, reflux, overnight 60%; (e) 20% KOH, ethyleneglycol, 130 °C, 70%; (f) Et_3N, CH_2Cl_2, rt, 90%.



Scheme 3. Reagents and conditions: (a) Et_2N, CH_2Cl_2, rt, 95%; (b) 20% K_2CO_3, MeOH, 85%; (c) Et_2N, chlorobenzene, reflux, overnight, 30–70%.

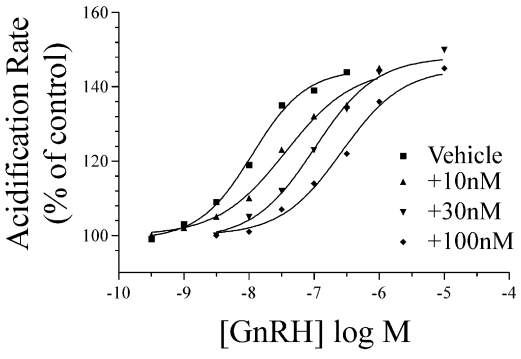


Figure 1. Effect of **1** (10, 30 or 100 nM) on GnRH-stimulated increases

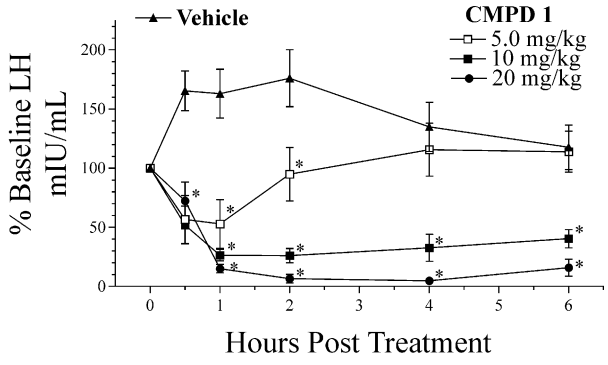


Figure 2. Effect of **1** (5, 10, 20 mg/kg iv) on plasma LH in castrated

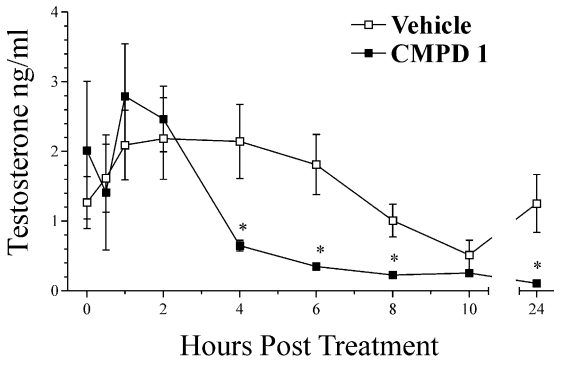


Figure 3. Suppression of testosterone in gonad-intact male rats by **1** (20 mg/kg i.m.). *$p \leq 0.05$ Compound **1** versus vehicle.

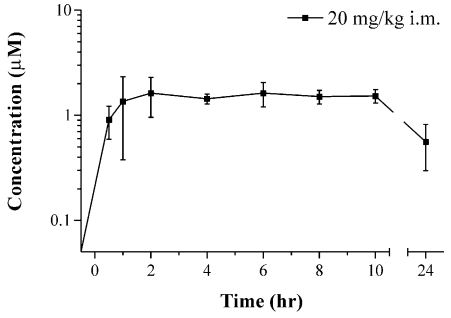


Figure 4. Pharmacokinetic profile of **1** (20 mg/kg i.m.).

(**13**–**18**, **21**, **23**, and **24**) or 2-amino substitutions (**1**, **19**, **20**, **25**, and **26**) in 2, 4-diaminopyrimidines with ether-containing groups afforded the most potent compounds, particularly at rat receptors. These group extensions, such as morpholino (**18**), methoxyalky (**17** and **20**), and tetrahydrofurfuryl (**1**, **13**–**16**, **19**, **21**, and **23**–**26**) presumably provide additional hydrophobic interactions with both the human and rat receptors. The benzyl linker (**1**, and **13**–**22**) from the pyrimidine group to the amide bond afforded more potency to human and rat receptors than did a cyclohexyl linker (**23**–**27**, **30**–**32**, and **38**). In cells expressing recombinant rat GnRH receptors, **1** was a competitive inhibitor of GnRH-stimulated extracellular acidification with a pA_2 value of 8.25 ± 0.11 ($N = 3$) similar to its pK_i (8.33) at rat pituitary receptor (Fig. 1). In male rats, surgical removal of the gonads eliminates the negative feedback of testosterone on the hypothalamus, producing a rat model with GnRH-mediated elevations of circulating LH.[11] In castrated male rats, **1** was well tolerated and dose-dependently suppressed circulating LH levels[9] (Fig. 2). When administered to gonad-intact male rats, **1** significantly lowered circulating testosterone[9] levels for up to 24 h after a single dose (Fig. 3). This effect was reversible after 24 h (data not shown).

In a similar experiment as in Figure 3, plasma levels of **1** were sustained at $>1\,\mu M$ for over 10 h after a single dose (Fig. 4), suggesting that this compound is fairly stable in rats. In selectivity assays, **1** was profiled in >40 receptor, enzyme and channel assays (Novascreen Peripheral SEP™, Hanover, MD). With the exceptions of D_2 dopamine[12] receptors (160 nM) and sodium channels (site 2; 200 nM), **1** was 50 to $>100\times$ selective for human GnRH receptors in all other assays performed.

In this report, we have identified and characterized two novel series of nonpeptide, mono- and diamino-pyrimidine derivatives that potently bind to human and rat GnRH receptors. Compound **1** was a selective, competitive antagonist of GnRH receptors in vitro and lowered testosterone levels in male rats in vivo. These agents or their analogues may serve as useful therapeutic agents for treating hormone-dependent pathologies including hormone-dependent prostate and breast cancer.

Acknowledgements

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References and Notes

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