Discovery of a Novel, Orally Active, Small Molecule Gonadotropin-Releasing Hormone (GnRH) Receptor Antagonist

Haitao Li,* Kenna L. Anderes, Eugenia A. Kraynov, David R. Luthin, Quyen-Quyen Do, Yufeng Hong, Eileen Tompkins, Eric T. Sun, Ranjan Rajapakse, Ved P. Pathak, Lance C. Christie, Haresh Vazir, Rosemary Castillo, Margaret L. Gregory, Mary Castro, Karen Nared-Hood, Genevieve Paderes, and Mark B. Anderson

Department of Medicinal Chemistry, Research Pharmacology, and Pharmacokenetics, Dynamics & Metabolism, Pfizer Global Research & Development, 10770 Science Center Drive, San Diego, California 92121

Received January 5, 2006

Gonadotropin releasing hormone (GnRH) plays an important role in the biology of reproduction. The use of GnRH receptor antagonists has been reported in the literature for the treatment of breast, ovarian, and prostate cancers. In this article, we report the synthesis, in vitro characterization, pharmacokinetics, and pharmacodynamics of an orally bioavailable, potent, small molecule GnRH receptor antagonist *N*-{4,6-dimethoxy-2-[(3-morpholin-4-ylpropyl)amino]pyrimidin-5-yl}-5-[3,3,6-trimthyl-2,3-dihydro-*1H*-inden-5-yl)oxy]-2-furamide (compound **1**).

Introduction

Gonadotropin-releasing hormone (GnRH) plays an important role in the biology of reproduction.[1] The GnRH decapeptide (pyro-Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) is produced in the hypothalamus. The decapeptide is released into the pituitary, where GnRH interacts with high-affinity receptors (7-transmembrane G-Protein coupled receptors). In the pituitary, GnRH triggers the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH).[2] GnRH, as the primary regulator of LH, is consequently the primary regulator of the sex hormones testosterone and estrogen.[3] GnRH and its analogues have stimulated much interest because of their potential therapeutic benefit in treating sex-hormone-dependent diseases, such as prostate, ovarian, and breast cancer as well as endometriosis, uterine fibroids, benign prostate hyperplasia, fertility disorders, and precocious puberty.[4−7]

GnRH receptor agonists have been clinically used in the treatment of sex-steroid-dependent diseases. The agonists mimic GnRH to stimulate receptors on the pituitary gland, mediating the release of LH and FSH. After a transient rise in hormone production or the flare effect, the administration of GnRH receptor agonists results in a down-regulation of GnRH receptors. GnRH receptor down-regulation leads to a decrease in circulating levels of LH, testosterone, and estrogen.

In recent years, peptide GnRH receptor antagonists have been available for clinical evaluation. They directly lower the hormones levels without the observed flare effect associated with the agonists.[8] Several laboratories have reported active nonpeptide GnRH receptor antagonists;[9−17] few have shown oral activity in animal models.[18−21] In this article, we report the synthesis, in vitro characterization, pharmacokinetics, and pharmacodynamics of an orally bioavailable, potent, small molecule GnRH receptor antagonist (compound **1**). Our effort toward the discovery of small molecule GnRH receptor antagonists started with the screening of our exploratory library. Compound **2** was obtained as an initial lead.[22,23] Following this discovery, an efficient combination of targeted library syntheses and medicinal chemistry approaches resulted in compound **1** as a potent, orally active GnRH receptor antagonist.

* To whom correspondence should be addressed. Tel: 858-526-4659. Fax: 858-526-4508. E-mail: haitao.li@pfizer.com.

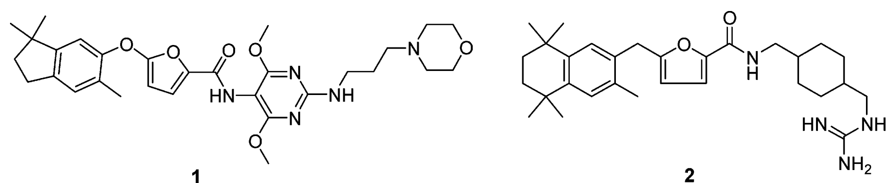
Results and Discussion

Chemistry. The synthesis of compound **1** is outlined in Scheme 1. Friedel−Crafts alkylation of *o*-cresol **3** with 3,3-dimethylacrylic acid provided indanone **4**, which was reduced by hydrogenation to yield indanol **5**. Alkylation of phenol **5** with methyl 5-bromo-2-furoate afforded **6**. Saponification of **6** provided furoic acid **7**, which was acylated with 4,6-dimethoxy-*N*-(3-morpholin-4-yl-propyl)-pyrimidine-2,5-diamine (**11**) to furnish compound **1**. The synthesis of diamine **11** is shown in Scheme 2. Nitration of 2-chloro-4,6-dimethoxy-pyrimidine (**8**) provided nitro-pyrimidine **9**. Nucleophilic aromatic substitution of **9** with 3-morpholin-4-yl-propylamine afforded compound **10**. The nitro group was then reduced by hydrogenation to provide compound **11**.

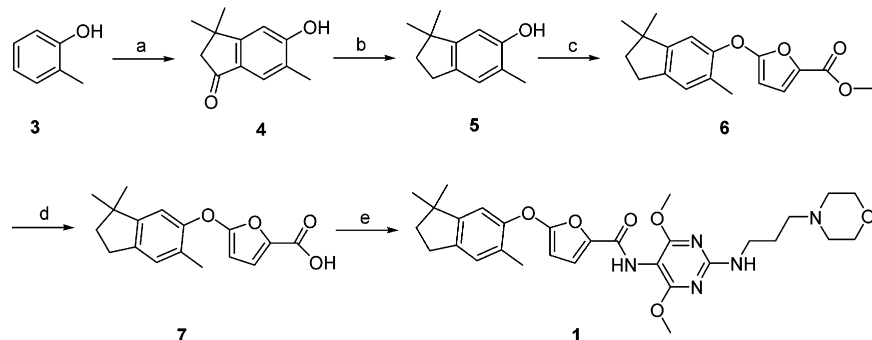
In Vitro Characterization. Compound **1** was characterized in competition radioligand-binding assays utilizing ^{125}I-GnRH-A as previously described.[24] Compound **1** bound to rat pituitary and recombinant human GnRH receptors with identical binding K_i values of 0.4 ± 0.1 nM. In cell-based functional assays, compound **1** was an antagonist of GnRH-stimulated total inositol phosphate accumulation with IC_{50} values of 3.3 ± 0.9 nM (Hill coefficient of 0.96 ± 0.08; 1 nM GnRH in assay) and 8.8 ± 1.5 nM (Hill coefficient of 0.83 ± 0.15; 100 nM GnRH in assay) for recombinant human and rat receptors, respectively. GnRH stimulated increases in total inositol phosphates with EC_{50} values of 53 ± 16 nM ($N = 4$; Hill coefficient of 1.2 ± 0.2) and 1.0 ± 0.2 ($N = 4$; Hill coefficient of 0.72 ± 0.2) for recombinant rat and human receptors, respectively. In selectivity assays, compound **1** was examined in 54 different assays including ion channels, GPCRs, and monoamine transporters. The K_i values were determined for hits at 10 μM. These are depicted in Table 1. With the exceptions of the Ca^{2+} channel assays, GnRH selectivity of compound **1** was more than 1000-fold for all 54 assays examined. Compound **1** was subsequently profiled for effects on basal (agonist) and KCl-stimulated Ca^{2+} channel activity. Compound **1** produced no effect on basal Ca^{2+} channel activity and inhibited KCl-stimulated Ca^{2+} flux with an IC_{50} value of 6.3 μM (n_H 1.6), suggesting that although compound **1** readily binds to these channels, it has only modest effects as a Ca^{2+} channel antagonist. Compound **1** has low potential for drug−drug interactions mediated by CYP 1A2, 2C19, 2D6, and

10.1021/jm060012g CCC: $33.50 © 2006 American Chemical Society
Published on Web 04/26/2006

Chart 1

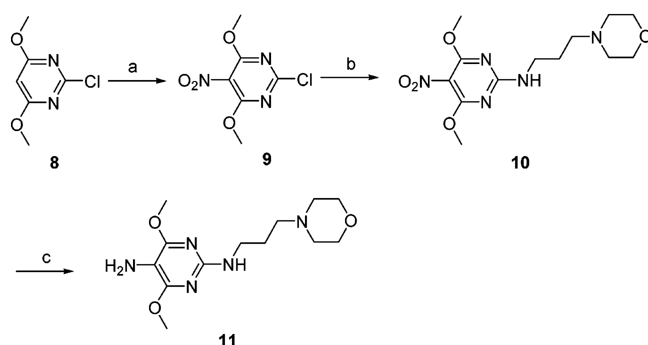


Scheme 1[a]



[a] Reagents and conditions: (a) 3,3-dimethylacrylic acid, polyphosphoric acid, 105 °C; (b) H_2, Pd/C, H_2SO_4, MeOH; (c) methyl 5-bromo-2-furoate, Cs_2CO_3, DMF; (d) NaOH, MeOH; (e) compound **11**, HATU, DIEA, DMF.

Scheme 2[a]



[a] Reagents and conditions: (a) tetramethylammonium nitrate, triflic anhydride, DCM; (b) 3-morpholin-4-yl-propylamine, DMF; (c) H_2, Pd/C, MeOH

3A4 (IC_{50} > 30 μM). The IC_{50} value for CYP2C9 inhibition was 3 μM (in-house data).

Pharmacokinetics. When compound **1** was given to intact male rats, it showed long $T_{1/2}$ (5.1 h), low CL[a] (0.33 L/hr/kg), and medium V_{ss} (1.1 L/kg). The compound had an oral bioavailability of 26% (Figure 1).

In Vivo Efficacy. In gonad intact rats, compound **1** dose-dependently suppressed testosterone to below castrate levels for 12 h following a single oral dose (Figure 2). In gonad intact rats, ≥2 μM plasma concentration of compound **1** was required to sustain the suppression of testosterone at or below castrate levels. In castrated rats, compound **1** dose-dependently suppressed luteinizing hormone levels to below the detection limit (0.12 mIU/mL) (Figure 3). Complete suppression of LH was obtained in castrated rats at ≥1.2 μM concentration. The plasma concentration was different between intact and castrated rats because of the hormone-dependent metabolism of compound **1** as described in Iatsimirskaia et al.[25] Following seven days of oral treatment with compound **1** in intact rats, testosterone suppression was sustained at or below castrate levels, and the plasma concentration was maintained at 2 μM. Testosterone levels returned to normal 24 h after the final dose (Figure 4).

[a] Abbreviations: bid, twice a day dosing; p.o., oral dosing; i.v., intravenous dosing; CL, clearance; V_{ss}, volume of distribution.

Conclusion

We have reported the discovery of a novel, small molecule GnRH receptor antagonist, *N*-{4,6-dimethoxy-2-[(3-morpholin-4-ylpropyl)amino]pyrimidin-5-yl}-5-[3,3,6-trimthyl-2,3-dihydro-*1H*-inden-5-yl)oxy]-2-furamide (compound **1**). Compound **1** exhibited picomolar affinities to human and rat GnRH receptors and was more than 1000-fold selective for GnRH receptors versus 54 G-protein-coupled receptors, other enzymes, and ion channels. In gonad intact male rats, compound **1** dose-dependently suppressed testosterone to below castrate levels for 12 h following a single oral dose. These data suggest that compound **1** is a potent and selective, orally active GnRH receptor antagonist that may have potential in treating sex-hormone dependent diseases, such as prostrate cancer and endometriosis.

Experimental Section

Chemistry. 5-Hydroxy-3,3,6-trimethyl-indan-1-one (4). To a three-necked round-bottom flask assembled with a condenser, thermometer, and mechanic stirrer under nitrogen, *o*-cresol **3** (1.197 mol, 124 mL) and 3,3-dimethylacrylic acid (1.52 mol, 154 g) were added. The mixture was gently stirred and heated at 40 °C while polyphosphoric acid (3.9 L) was added. After the addition of polyphosphoric acid was completed, the contents were rapidly heated to 105 °C and then promptly removed from the heating mantle. The reaction was quenched by pouring the hot mixture into a large bucket of ice water with constant stirring. The aqueous layer was extracted with ethyl acetate. The organic layer was dried over sodium sulfate and filtered, and the solvent was removed in vacuo. The crude product was crystallized with ethyl acetate to obtain 21.0 g of compound **4** (9%). ^{1}HNMR (300 MHz, $CDCl_3$) δ 1.36 (s, 6H), 2.27 (s, 3H), 2.55 (s, 2H), 6.06 (bs, 1H), 7.50 (s, 1H), 7.83 (s, 1H). APCI-MS: calcd for $C_{12}H_{14}O_2$ 190; found, 191, $[M + H]^+$.

3,3,6-Trimethyl-indan-5-ol (5). To a solution of 5-hydroxy-3,3,6-trimethyl-indan-1-one (21.5 mmol, 4.1 g) and sulfuric acid (290 μL) in methanol (150 mL) was added Pd/C (4.3 mmol, 0.63 g). The ketone was reduced overnight under 40 psi of H_2. The reaction mixture was filtered through Celite. The filtrate was taken to dryness to give a brown oil that was redissolved in ethyl acetate and washed with water and brine. The organic layer was dried over sodium sulfate and filtered, and the solvent was removed in vacuo to yield a light-yellow oil. The crude product was purified by flash column chromatography (ethyl acetate/hexane 1:3) to afford 3.6 g

Table 1. Selectivity of Compound **1** in Broad Ligand Profile[a]

assay	compd **1** K_i (nM)	compd **1** n_H	ref compd	ref comp K_i (nM)	ref compd n_H	GnRH fold selectivity
A3[b]	628	2.0	IB-MECA	0.8	0.9	1570
NE transporter[b]	5090	2.8	protriptyline	11	1.5	12725
AT1[b]	3710	1.2	saralasin	0.76	1.0	9275
D1[b]	1620	2.1	SCH 23390	0.53	1.0	4050
D3[b]	1180	1.8	(+)butaclamol	3.8	1.3	2950
D4.4[b]	2510	2.4	clozapine	35	1.1	6275
DA transporter[b]	1800	2.7	GBR12909	6.2	2.0	4500
H2	1220	1.0	cimetidine	877	0.8	3050
M1[b]	4120	2.0	pirenzepine	14	1.0	10300
M4[b]	3680	2.3	4-DAMP	1.2	1.4	9200
NK1[b]	1250	1.3	[Sar9,Met(O_2)11]-SP	1.1	0.9	3125
Kappa opiate	1960	1.7	U50488	0.28	1.3	4900
Mu opiate[b]	1400	1.4	DAMGO	0.42	1.0	3500
PAF	1060	1.2	WEB 2086	1.2	0.7	2650
5HT2A[b]	583	1.5	ketanserin	0.67	1.1	1458
5HT2C[b]	964	1.3	mesulergine	0.65	1.1	2410
5HT7[b]	1750	1.7	serotonin	0.43	1.1	4375
5HT transporter[b]	1430	1.9	imipramine	1.9	1.0	3575
L-type Ca2+ DHP site	145	1.2	nitrendipine	0.57	1.4	363
L-type Ca2+ diltiazem site	370	2.0	diltiazem	15	1.1	925
L-type Ca2+ verapamil site	166	1.0	D600	4.2	0.9	415
N-type Ca2+	5240	1.7	ω-conotoxin GVIA	0.003	1.3	13100

[a] Assays all performed at Cerep. [b] human isoform utilized for assay.

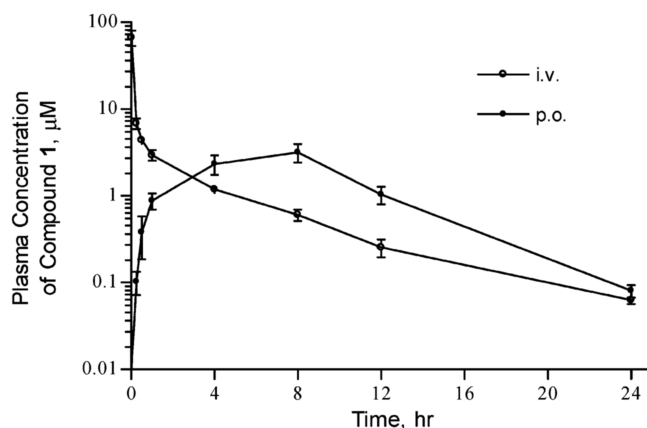


Figure 1. Plasma concentrations of compound **1** in intact rats after i.v. (5 mg/kg) and p.o. (20 mg/kg) administration. Data are means ± SD for $n = 3$.

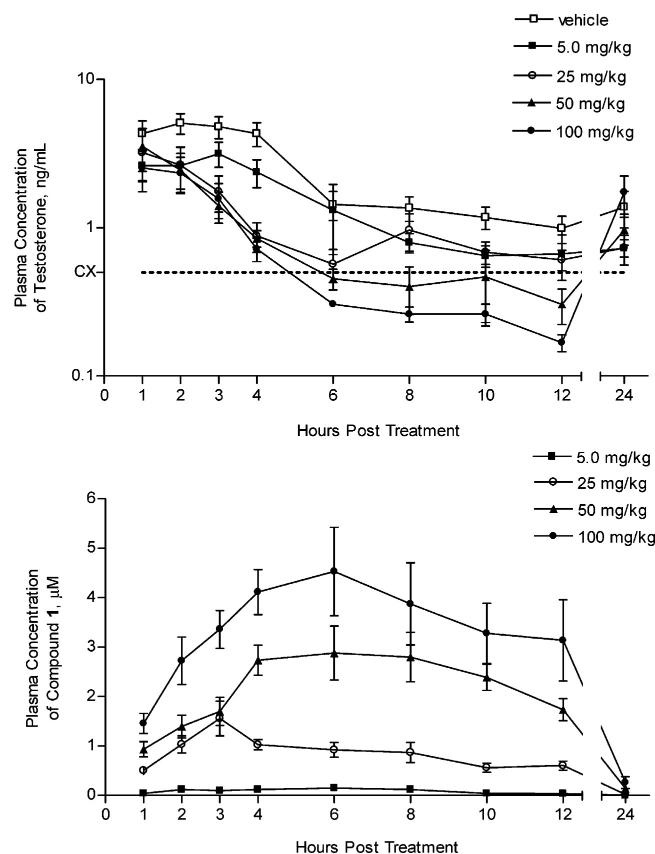


Figure 2. (a) Effect of a single oral dose of compound **1** (5.0, 25, 50, and 100 mg/kg) on testosterone time course in intact male rats. The testosterone level below 0.5 ng/mL is equivalent to the castrated levels denoted by CX. (b) Plasma concentration (μM) of compound **1** (5.0, 25, 50, and 100 mg/kg) following a single oral dose to intact male rats.

of compound **5** as a light-yellow solid (93%). ^{1}HNMR (300 MHz, $CDCl_3$) δ 1.21 (s, 6H), 1.89 (t, 2H), 2.21 (s, 3H), 2.78 (t, 2H), 4.58 (s, 1H), 6.57 (s, 1H), 6.94 (s, 1H). Anal. ($C_{12}H_{16}O$) C, H.

5-(3,3,6-Trimethyl-indan-5-yloxy]-furan-2-carboxylic Acid Methyl Ester (6). In a round-bottom flask assembled with a condenser and gas outlet, a solution of compound **5** (22.73 mmol, 4.0 g), methyl 5-bromo-2-furoate (17.6 mmol, 3.6 g) and cesium carbonate (22.8 mmol, 7.4 g) in DMF (45 mL) was heated to 100 °C for 7 h under a N_2 atomosphere. The mixture was cooled to room temperature and neutralized with 1 M HCl. The content was extracted with ethyl acetate. The organic layer was dried over sodium sulfate and filtered, and the solvent was removed in vacuo. The crude product was purified by column chromatography (ethyl acetate/hexane 1:5) to afford 5.8 g of compound **6** as a yellow oil (84%). Compound **6** was partially pure and used in the next reaction step without any further purification. ^{1}HNMR (300 MHz, $CDCl_3$) δ 1.21 (s, 6H), 1.89 (t, 2H), 2.20 (s, 3H), 2.77 (t, 2H), 3.86 (s, 3H), 5.22 (d, 2H), 6.56 (s, 1H), 6.93 (s, 1H), 7.12 (d, 1H). APCI-MS: calcd for $C_{18}H_{20}O_4$ 300; found 301 $[M + H]^+$.

5-(3,3,6-Trimethyl-indan-5-yloxy)-furan-2-carboxylic Acid (7). To a solution of compound **6** (19.3 mmol, 5.8 g) in methanol (10 mL) was added 4 M NaOH solution (20 mL). The reaction mixture was stirred at room-temperature overnight. The clear brown solution was acidified with 2 M HCl and extracted with ethyl acetate. The combined organic layer was washed with brine, dried over sodium

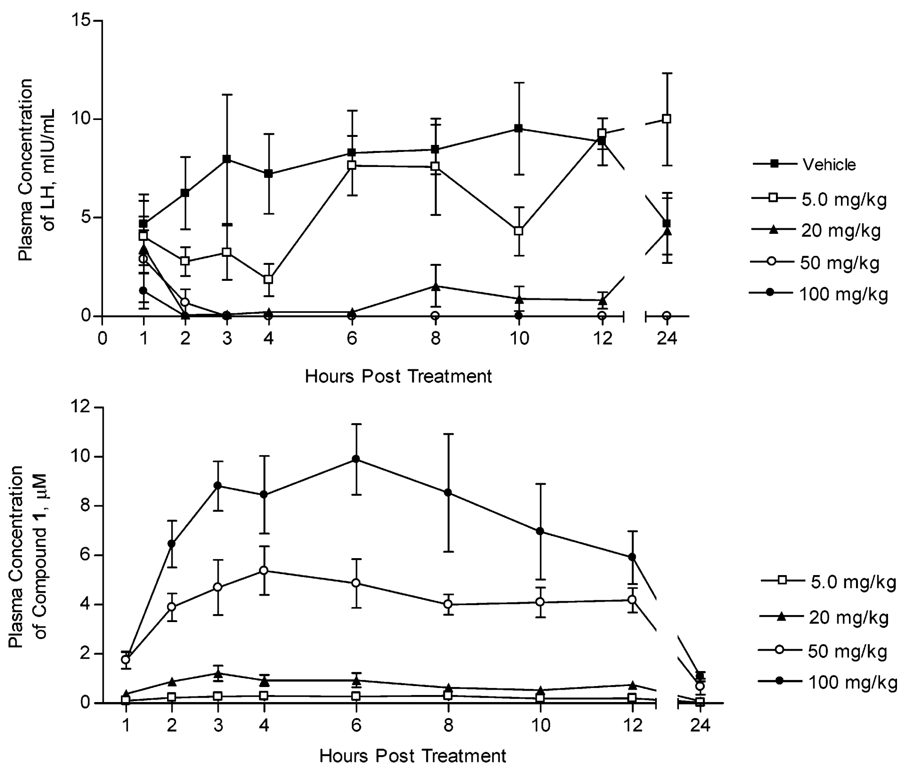


Figure 3. (a) Effect of a single oral dose of compound **1** (5.0, 20, 50, and 100 mg/kg) on luteinizing hormone time course in castrated male rats. (b) Plasma concentration of compound **1** (5.0, 20, 50, and 100 mg/kg) in castrated male rats following a single oral dose.

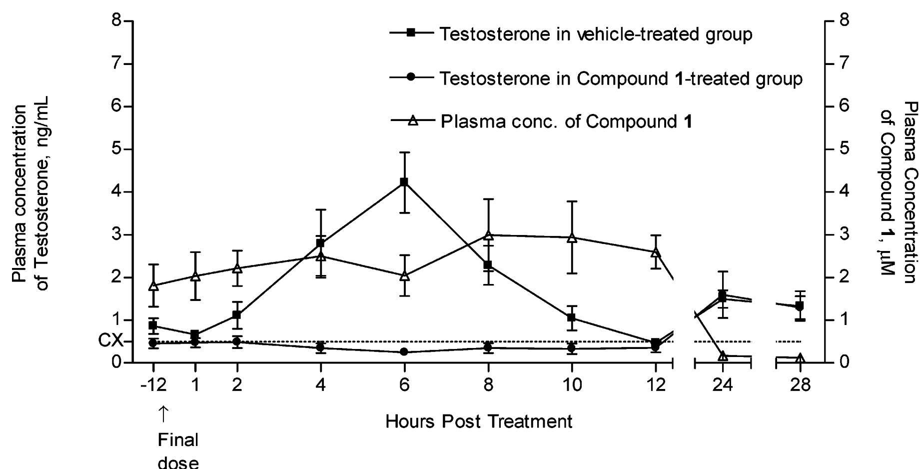


Figure 4. Effect of seven-day pretreatment with compound **1** (100 mg/kg, p.o., bid) on testosterone time course in intact rats. The plasma samples were collected following the last dose. The testosterone level below 0.5 ng/mL is equivalent to the castrated levels denoted by CX.

sulfate, filtered and the solvent was removed in vacuo. The crude product was crystallized with CH_3CN to afford 3.18 g compound **7** as a light yellow solid (57%). ^{1}HNMR (300 MHz, $CDCl_3$) δ 1.21 (s, 6H), 1.93 (t, 2H), 2.20 (s, 3H), 2.84 (t, 2H), 6.85 (s, 1H), 7.05 (s, 1H), 7.26 (d, 1H). APCI-MS: calcd for $C_{17}H_{18}O_4$ 286; found, 287 $[M + H]^+$.

5-(3,3,6-Trimethyl-indan-5-yloxy)-furan-2-carboxylic Acid [4,6-dimethoxy-2-(3-morpholin-4-yl-propylamino)-pyrimidine-5-yl]-amide (1). To a solution of compound **7** (9.74 mmol, 2.8 g), **11** (8.0 mmol, 2.4 g), and HATU (10.2 mmol, 3.4 g) in DMF (18 mL), diisopropylethylamine (33.5 mmol, 6 mL) was slowly added via a syringe under a N_2 atomosphere. The reaction mixture was stirred at room-temperature overnight. The solvent was removed in vacuo. The crude mixture was purified by HPLC (32−95% in 70 min, CH_3CN in water with 0.1M NH_4OAc) to give 2.84 g of compound **1** as a white solid (52%). It was dissolved in methylene chloride and the solvent was removed in vacuo to give an amorphous solid. ^{1}HNMR (300 MHz, $CDCl_3$) δ 1.21 (s, 6H), 1.81 (t, 4H), 1.93 (t, 2H), 2.23 (s, 3H), 2.49 (m, 6H), 2.84 (t, 2H), 3.48 (bt, 2H), 3.75 (t, 4H), 3.87 (s, 6H), 5.29 (d, 1H), 5.72 (bs, 1H), 6.82 (s, 1H), 6.95 (s, 1H), 7.05 (s, 1H), 7.10 (d, 1H). Anal. ($C_{30}H_{39}N_5O_6 \cdot 1.0H_2O$) C, H, N, O.

2-Chloro-4,6-dimethoxy-5-nitro-pyrimidine (9). In a 12 L flask assembled with an overhead stirrer, thermometer, N_2 inlet, and an additional funnel were added tetramethylammonium nitrate (587 g, 4.31 mol) and dichloromethane (4 L). The contents were stirred under a N_2 atomosphere for 1 h at room temperature (20 °C). Triflic anhydride (1216 g, 4.31 mol, 725 mL) was added dropwise in 45 min so that the temperature was not raised above 25 °C. The contents were stirred at room temperature for 2 h. The reaction mixture was then cooled to −78 °C in a dry ice/acetone bath. Compound **8** (500 g, 2.87 mol) was dissolved in minimal amount of DCM (3 L) and added dropwise over a 1.5 h time period. The flow rate was critical in controlling the temperature and preventing it from rising more than 5 °C. After the addition of compound **8**, the ice bath was removed, and the reaction was stirred over the weekend under a N_2 atomosphere. The reaction was quenched by pouring the reaction mixture into ~2 kg of ice. The contents were

neutralized with $NaHCO_3$ solution and extracted with DCM (2 × 1 L). The combined organic layer was washed with water (2 × 1 L), dried over $MgSO_4$, and filtered, and the solvent was removed in vacuo to afford 592.8 g of compound **9** as a white solid (94%). ^{1}HNMR (300 MHz, $CDCl_3$) δ 4.09 (s, 6H). APCI-MS: calcd for $C_6H_6ClN_3O_4$ 219; found, 220 $[M + H]^+$. Anal. ($C_6H_6ClN_3O_4$) C, H, N, Cl.

(4,6-Dimethoxy-5-nitro-pyrimidine-2-yl)-(3-morpholin-4-yl-propyl)-amine (10). To compound **9** (92.32 mmol, 20.2 g) in DMF (23 mL) was added dropwise 3-morpholin-4-yl-propylamine (92.4 mmol, 13.5 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred overnight under a N_2 atomosphere. The organic solvent was removed in vacuo, and the crude product was purified by column chromatography (methanol/ethyl acetate 2:5) to give 18 g of compound **10** as a bright-yellow solid (60%). ^{1}HNMR (300 MHz, CD_3OD) δ 2.00−2.04 (m, 2H), 3.04−3.14 (m, 4H), 3.25 (t, 2H), 3.48 (t, 2H), 3.68−3.72 (bm, 1H), 3.83 (bm, 4H), 3.89 (s, 3H), 3.97 (s, 3h). APCI-MS: calcd for $C_{13}H_{21}N_5O_5$ 327; found, 328 $[M + H]^+$.

4,6-Dimethoxy-*N*-(3-morpholin-4-yl-propyl)-pyrimidine-2,5-diamine (11). To a solution of compound **10** (55 mmol, 18 g) in methanol (500 mL) was added Pd/C (5.5 mmol, 0.8 g). The nitro group was reduced overnight under 30 psi of H_2. The reaction mixture was filtered through Celite, and the solvent was removed in vacuo. The product was dried under high vacuum to afford 15 g of compound **11** as a brown solid (90%). ^{1}HNMR (300 MHz, CD_3OD) δ 1.83−1.92 (q, 2H), 2.75−2.82 (m, 6H), 3.25−3.26 (m, 4H), 3.35 (t, 2H), 3.74 (t, 4H), 3.87 (s, 6H). APCI-MS: calcd for $C_{13}H_{23}N_5O_3$ 297; found, 298 $[M + H]^+$.

Animals. Adult male Sprague−Dawley (SD) rats were purchased from Harlan Sprague Dawley (San Diego, CA). All animals were maintained in a temperature-controlled room (22 ± 2 °C) with a photoperiod of 12 h light/12 h dark (lights on at 6:00 a.m.). Rat chow (Teklad rat diet) and tap water were provided ad libitum. Protocols followed those described in the *Principles of Laboratory Animal Care* (NIH publication #85-23, revised 1985).

Pharmacokinetic Studies. The rats were prepared with indwelling jugular vein catheters as described earlier and allowed to recover overnight with free access to standard rat chow and water.[25] Compound **1** was administered acutely at 5 mg/kg (i.v.) as 5 mg/mL solution in 70% PEG400/30% H_2O, or 20 mg/kg (p.o.) as 20 mg/mL suspension in 0.5% CMC. Blood samples were withdrawn at specific times, the plasma was immediately separated (100 μL), and the compound was extracted with ethyl acetate (1.4 mL) containing an internal standard. The supernatant was dried under a flow of nitrogen gas and the residue reconstituted in 80 μL of mobile phase, and 50 μL was analyzed by HPLC.

HPLC Analysis. The samples were analyzed on a Betabasic C_{18} column, 3 μm, 4.6 × 50 mm (Western Analytical Products, Inc., Murrieta, CA) using a Hewlett-Packard HPLC system (model 1100, Palo Alto, CA). A linear gradient of 50−70% ACN in 10 mM ammonium phosphate buffer at pH 7 over 10 min was used with UV detection at 260 nm. The flow rate was 1 mL/min.

Data Analysis. The pharmacokinetic parameters were calculated using WinNonlin software (Scientific Consulting, Inc.). The bioavailability was calculated as AUCp.o./AUCi.v., where AUCp.o. and AUCi.v. are areas under the plasma concentration-time curve after p.o. and i.v. administration, respectively.

Pharmacodynamics. Male SD (200−225 g) rats were castrated via the scrotal approach under halothane anesthesia. All castrated animals were housed two per cage and allowed 14 days of postoperative recovery prior to study. Intact male SD (300−325 g) animals for testosterone studies were single-housed to avoid the dominant male effect.[26] Three days prior to study, the animals were anesthetized with halothane and instrumented with indwelling jugular vein microrenathane cannula. Details of the cannulation procedure have been described previously.[27] On the day of the study, the animals were allowed to acclimate to the procedure room, while residing in their home cage. Basal blood samples were drawn from all animals between 7:00−7:30 am. Immediately following basal sampling, the vehicle or compound **1** was orally administered to gonad intact male rats (5.0, 25, 50, and 100 mg/kg) or castrated male rats (5.0, 20, 50, and 100 mg/kg) (n = 6/dose) to determine the efficacy of hormone suppression in vivo. The test compound was formulated as a suspension in 0.5% CMC in water.

Considering the circadian rhythm of testosterone,[28] multiple samples were collected during the course of the experiment. Blood samples (400 μL) were drawn into heparin containing tubes at predetermined post-treatment time points. The blood was centrifuged immediately, and the plasma was collected and stored in a −20 °C freezer until assayed. The samples collected for the plasma concentration determination of compound **1** were prepared for analysis as described above in Pharmacokinetics.

Hormone Measurements. LH and testosterone were measured using DSL-4600 ACTIVE LH coated-tubes or DSL-4000 ACTIVE testosterone coated-tube radioimmunoassay assay kits from Diagnostic Systems Laboratories, Inc., Webster, Texas.

Data Analysis. Luteinizing hormone and testosterone levels were expressed as means ± SEM. Significant differences between groups of animals were determined by two-way analysis of variance with post hoc Tukey multiple comparisons at each particular time point (SPSS software, Chicago, IL). Differences were considered significant if p <0.05.

References

(1) Stanislaus, D.; Pinter, J. H.; Janovick J. Conn, P. M. Mechanisms mediating multiple physiological responses to gonadotropin-releasing hormone. *Mol. Cell. Endocrinol.* **1998**, *144*, 1−10.

(2) Millar, R.; Assefa, D.; Ott, T.; Pawson, A.; Troskie, B.; Wakefield, I.; Katz, A. GnRH and GnRH analogues: structure, actions and clinical applications. *Horm. Front. Gynecol*. **1998**, *5*, 385−391.

(3) Kaiser, U. B.; Conn P. M.; Chin, W. W. Mechanisms mediating multiple physiological responses to gonadotropin-releasing hormone. *Endocr. Rev.* **1997**, *18*, 46−70.

(4) Huirne, J. A. F.; Lambalk, C. B. Gonadotropin-releasing-hormone-receptor antagonists. *Lancet* **2001**, *358*, 1793−803.

(5) Harris, K. A.; Small, E. J. Hormonal treatment for prostate cancer. *Expert Opin. Invest. Drugs* **2001**, *10*, 493−510.

(6) Cook, T.; Sheridan, W. P. Development of GnRH antagonists for prostate cancer: new approaches to treatment. *Oncologist* **2000**, *5*, 162−168.

(7) Leibowitz, R. L.; Tucker, S. J. Treatment of localized prostate cancer with intermittent triple androgen blockade: preliminary results in 110 consecutive patients. *Oncologist* **2001**, *6*, 177−182.

(8) Schultze-Mosgau, A.; Griesinger, G.; Altgassen, C.; von Otte, S.; Hornung, D.; Diedrich, K. New developments in the use of peptide gonadotropin-releasing hormone antagonists versus agonists. *Expert Opin. Invest. Drugs* **2005**, *14*, 1085−1097.

(9) 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. Potent antagonists of gonadotropin releasing hormone receptors derived from quinolone-6-carboxamides. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 443−447.

(10) (a). Ashton, W. T.; Sisco, R. M.; Yang, Y. T.; Lo, J.-L, Yudkovitz, J. B.; Cheng, K. Substituted Indole-5-carboxamides and -acetamides as Potent Nonpeptide GnRH Receptor Antagonists. *Bioorg. Med. Chem. Lett*. **2001**, 11, 1723−1726. (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.; and Goulet, M. T. Potent nonpeptide GnRH receptor antagonists derived from substituted indole-5-carboxamides and -acetamides bearing a pyridine side-chain terminus. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 1727−1731. (c). Ashton, W. T.; Sisco, R. M.; Kieczykowski, G. R.; Yang, 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. Orally bioavailable, indole-based nonpeptide GnRH receptor antagonists with high potency and functional activity. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 2597−2602.

(11) 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. H.; Carlin, J. R.; Cameron, J.; Smith, R. G. A potent, nonpeptidyl 1H-quinolone antagonist for the gonadotropin-releasing hormone receptor. *J. Med. Chem*. **2001**, *44*, 917−922.

(12) Tucci, F. C.; Zhu, Y.-F.; Guo, Z.; Gross, T. D.; Connors, P. J.; Struthers, R. S.; Reinhart, G. J.; Saunders: J.; Chen, C. Synthesis and structure−activity relationships of 1-arylmethyl-3-(1-methyl-2-amino)ethyl-5-aryl-6-methyluracils as antagonists of the human GnRH receptor. *Bioorg. Med. Chem. Lett*. **2003**, *13*, 3317−3322.

(13) Tucci, F. C.; Zhu, Y.-F.; Guo, Z.; Gross, T. D.; Connors, P. J.; Struthers, R. S.; Reinhart, G. J.; Wang, X.; Saunders: J.; Chen, C. A novel synthesis of 7-aryl-8-fluoro-pyrrolo[1,2-a]pyrimid-4-ones as potent, stable GnRH receptor antagonists. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 3491−3495.

(14) Wilcoxen, K. M.; Zhu, Y.-F; Connors, P. J.; Saunders, J.; Gross, T. D.; Gao, Y.; Reinhart, G. J.; Struthers, R. S.; Chen, C. Synthesis and initial structure−activity relationships of a novel series of imidazolo[1,2-a]pyrimid-5-ones as potent GnRH receptor antagonists. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 2179−2183.

(15) Zhu, Y.-F; Struthers, R. S.; Connors, P. J.; Gao, Y.; Gross, T. D.; Saunders, J.; Wilcoxen, K.; Reinhart, G. J.; Ling, N.; Chen, C. Initial structure−activity relationship studies of a novel series of pyrrolo-[1,2-a]pyrimid-7-ones as GnRH receptor antagonists. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 399−402.

(16) Zhu, Y.-F; Wilcoxen, K.; Saunders, J.; Guo, Z.; Gao, Y.; Connors, P. J.; Gross, T. D.; Tucci, F. C.; Struthers, R. S.; Reinhart, G. J.; Xie, Q.; Chen, C. A novel synthesis of 2-arylpyrrolo[1,2-a]pyrimidin-7-ones and their structure−activity relationships as potent GnRH receptor antagonists. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 403−406.

(17) Zhu, Y.-F.; Chen, C. Recent advances in small molecule gonadotrophin-releasing hormone receptor antagonists. *Expert Opin. Ther. Pat.* **2004**, *14*, 187−199.

(18) Anderes, K. L.; Luthin, D. R.; Castillo, R.; Kraynov, E. A.; Castro, M.; Nared-Hood, K.; Gregory, M. L.; Pathak, V. P.; Christie, L. C.; Paderes, G.; Vazir, H.; Ye, Q.; Anderson, M. B.; May, J. M. Biological characterization of a novel, orally active small molecule gonadotropin-releasing hormone (GnRH) antagonist using castrated and intact rats. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 688−695.

(19) 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. Discovery of a novel, potent, and orally active nonpeptide antagonist of the human luteinizing hormone-releasing hormone (LHRH) receptor. *J. Med. Chem*. **1998**, *41*, 4190−4195.

(20) 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. L.; Haviv, F.; Wegner, C. D.; Greer, J. Pharmacological and endocrine characterization of A-198401, an orally active GnRH antagonist, in intact and castrate male rat models. *Drug Dev. Res*. **2001**, *52*, 485−491.

(21) 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. Orally bioavailable, indole-based nonpeptide GnRH receptor antagonists with high potency and functional activity. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 2597−2602.

(22) Luthin, D. R.; Hong, Y.; Pathak, V. P.; Paderes, G.; Nared-Hood, K. D.; Castro, M. A.; Vazir, H.; Li, H.; Tompkins, E.; Christie, L.; May, J. M.; Anderson, M. B. The discovery of novel small molecule non-peptide gonadotropin releasing hormone (GnRH) receptor antagonists. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 3467−3470.

(23) Luthin, D. R.; Hong, Y.; Tompkins, E.; Anderes, K. L.; Paderes, G.; Kraynov, E.; Castro, M. A.; Nared-Hood, K. D.; Castillo, R.; Gregory, M.; Vazir, H.; May, J. M.; Anderson, M. B. Characterization of mono- and diaminopyrimidine derivatives as novel, nonpeptide gonadotropin releasing hormone (GnRH) receptor antagonists. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 3635−3639.

(24) 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. Non-peptide GnRH agents, methods and intermediates for their preparation. WO 00/20358, 2000, CA 132:279106.

(25) Iatsimirskaia, E. A.; Gregory, M. L.; Anderes, K. L.; Castillo, R.; Milgram, K. E.; Luthin, D. R.; Pathak, V. P.; Christie, L. C.; Vazir, H.; Anderson, M. B.; May, J. M. Effect of testosterone suppression on the pharmacokinetics of a potent GnRH receptor antagonist. *Pharm. Res*. **2002**, *19*, 202−208.

(26) Johnson, M.; Everitt, B. *Essential Reproduction,* 3rd ed.; Blackwell Scientific Publications: Cambridge, MA, 1988.

(27) Harms, P. G.; Ojeda, S. R. A rapid and simple procedure for chronic cannulation of the rat jugular vein. *J. Appl. Physiol*. **1974**, *36*, 391−398.

(28) Mock, E.; Norton, H.; Frankel, A. Daily rhythmicity of serum testosterone concentration in the male laboratory rat. *Endocrinol*ogy **1978**, *103*, 1111−1121.

JM060012G