Letters

3-(2-Aminoalkyl)-1-(2,6-difluorobenzyl)-5-(2-fluoro-3-methoxyphenyl)-6-methyluracils as Orally Bioavailable Antagonists of the Human Gonadotropin Releasing Hormone Receptor

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Received March 15, 2004

Abstract: Uracils possessing N-3 side chains derived from various amino alcohols were designed and synthesized as potent human gonadotropin releasing hormone receptor antagonists. The compounds herein presented displayed superior metabolic stability than their predecessor molecules. Selected compounds from this series featured good oral bioavailability in mice and cynomolgus monkeys.

Gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone-releasing hormone (LHRH), is a decapeptide that is synthesized in neurons of the hypothalamus from a 92 amino acid precursor molecule. GnRH is released to the local vasculature in a pulsatile manner and activates specific receptors in the anterior pituitary gland, thus leading to the release of gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). These hormones in turn are released into the general circulation and bind to receptors on specialized cells in the ovaries or testes to stimulate steroidogenesis. The gonads respond to this activation by synthesizing and secreting the sex steroid hormones (estrogen and progesterone in the female and testosterone in the male), which in turn exert their classical effects on secondary sexual characteristic development throughout the body. Elucidation of the hypothalamic-pituitary−gonadal axis physiology led to the notion that antagonism of the GnRH receptor in the pituitary gland is an effective way to treat diseases that are aggravated by the presence of sex steroid hormones, such as endometriosis, uterine fibroids, and prostate cancer.[1] Currently, these conditions are treated with long-acting GnRH peptide agonists.[2] These work by an initial stimulation, followed by down-regulation of the receptor.

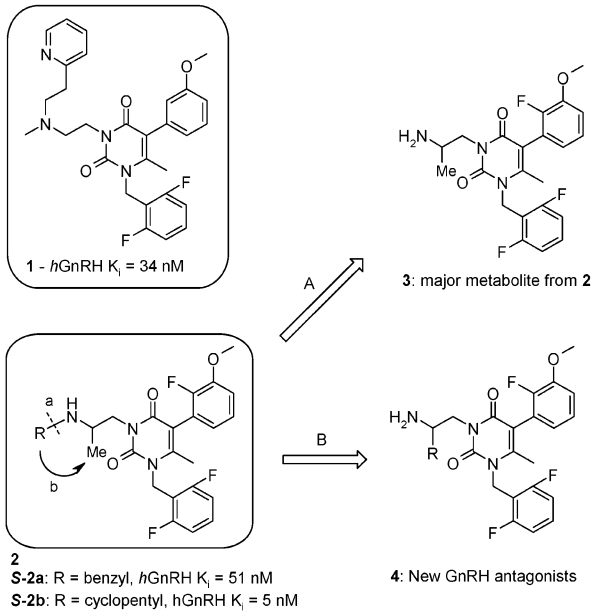

Figure 1. Design of new GnRH antagonists.

However, the lack of oral bioavailability and the unavoidable "flare effect" are the limitations of these peptides. In response to the need for convenient administration, we[3] and others[4] have initiated intensive efforts for the development of orally active, small-molecule GnRH antagonists.

Recently, we reported on the discovery of a new class of small-molecule GnRH antagonists, the 1-arylmethyl-3-(2-aminoethyl)-5-aryl-6-methyluracils, exemplified by **1**[3g] (Figure 1). This series of compounds was further optimized by introduction of a methyl group at the β-carbon of the N-3 side chain, e.g., **2**[3h,j] (Figure 1). Although potent functional GnRH antagonists such as (*S*)-**2a** and (*S*)-**2b** were identified from this series, we later found that these were relatively metabolically unstable. For instance, upon incubation with human liver microsomes (HLM), both (*S*)-**2a** and (*S*)-**2b** were rapidly consumed, leading mainly to the side chain dealkylation metabolite **3**. This compound in turn was much less active as a GnRH antagonist (path A, Figure 1). To obtain orally active, longer-acting small-molecule GnRH antagonists, we clearly needed to improve the metabolic stability profile of these uracil compounds. To accomplish this goal, we envisioned having the lipophilic side chain (e.g., the benzyl group in (*S*)-**2a** or the cyclopentyl in (*S*)-**2b**) originate from the 2-carbon on the N-3 side chain. This modification would result in branched primary amines where the side chain is connected by a carbon−carbon bond (path B, Figure 1), which we expect should be much more stable toward HLM metabolism. Here, we report on the synthesis, SAR studies, and preliminary pharmacokinetics of these novel GnRH antagonists.

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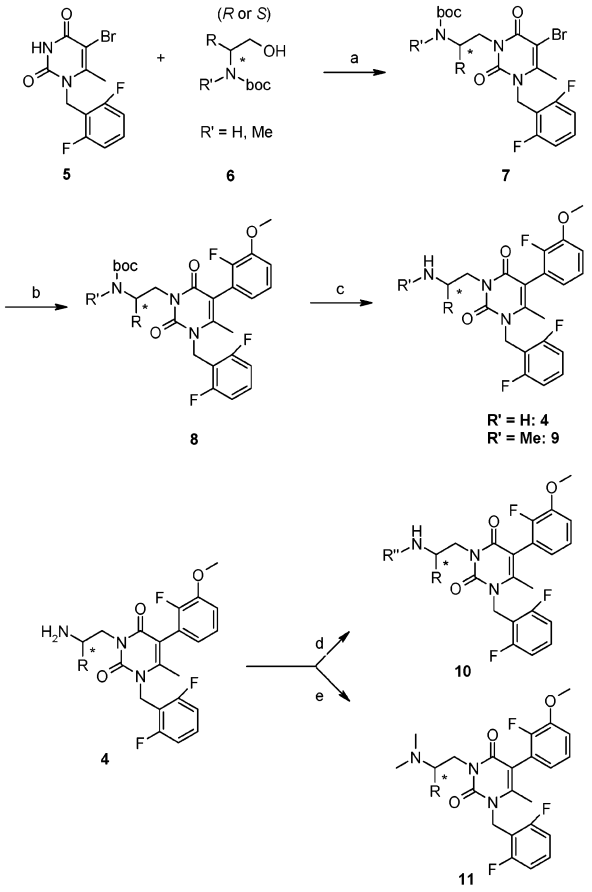
10.1021/jm049791w CCC: $27.50
Published on Web 05/28/2004

Scheme 1[a]



[a] Reagents: (a) diethyl azodicarboxylate, Ph_3P, THF, room temp, 5 h; (b) 2-fluoro-3-methoxyphenylboronic acid, $Pd[PPh_3]_4$, DME/PhH/EtOH, $Ba(OH)_2$ (aq), 80 °C, 16 h; (c) TFA, CH_2Cl_2, room temp, 1 h; (d) aldehyde or ketone, CH_2Cl_2, HOAc, $Na(OAc)_3BH$, room temp, 1 h; (e) CH_2O (aq), CH_2Cl_2, HOAc, $Na(OAc)_3BH$, room temp, 1 h.

4 and **9**−**11** were synthesized according to the procedure depicted in Scheme 1. Thus, the previously described 5-bromo-1-(2,6-difluorobenzyl)-6-methyluracil **5**[3h] was coupled, using Mitsunobu conditions, with the corresponding Boc-protected amino alcohols **6**, which are available either commercially or from the respective amino acids by lithium aluminum hydride reduction followed by Boc protection in an one-pot procedure. The products **7** were obtained in 80−90% isolated yields. Subsequent Suzuki couplings of **7** with 2-fluoro-3-methoxyphenylboronic acid gave **8** in 50−70% yields. These compounds were deprotected in the presence of trifluoroacetic acid in dichloromethane to furnish the primary amines **4** in quantitative yields. This procedure was also applied to *N*-methyl derivatives **9** because N-monomethylation of **4** was very difficult with conventional reductive alkylation methods. Selected compounds **4** were further alkylated under reductive conditions to give N-alkyl derivatives **10** or N,N-dimethylated **11** in very good yields.

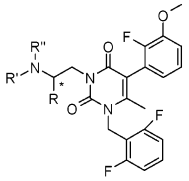
All compounds were then evaluated for their competitive binding to the cloned human GnRH (hGnRH) receptor expressed in HEK293 cells, using a 96-well filtration setup[5,6] and des-Gly[10][^{125}I-Tyr[5],D-Leu[6],NMeLeu[7],-Pro[9]-NEt]GnRH as the radiolabel. The results of the binding assay are presented in Tables 1 and 2.

In Table 1, we examined N-3 side chains containing a branched primary amine, e.g., **4**. Interestingly, the enantiomeric preference for the receptor varies according to the substitution pattern. For instance, the (*S*)-enantiomer of the phenylalanine analogue ((*S*)-**4f**) is about 4- fold more potent than its (*R*)-enantiomer counterpart ((*R*)-**4f**). Homologation of the (*S*)-isomer ((*S*)-**4g**) significantly reduced binding affinity. When alkyl-substituted side chains were employed, the opposite enantiomeric preference was observed. In all cases, the (*R*)-isomer was the preferred one. The affinity for the hGnRH receptor was proportional to the lipophilic content of the side chain, with cyclohexyl ((*R*)-**4c**) and isobutyl ((*R*)-**4d**) being the best. The lack (e.g., (*R*)-**3**) or excess (e.g., (*R*)-**4e**) of lipophilicity proved to be detrimental to binding.

Table 1. Binding Affinities of **3** and **4** toward the hGnRH Receptor

compd	R	stereochemistry	$K_i \pm$ SEM (nM)
(*R*)-**3**	Me	(*R*)	595 ± 87
(*S*)-**3**	Me	(*S*)	>10000
(*R*)-**4a**	*i*-Pr	(*R*)	51 ± 18
(*S*)-**4a**	*i*-Pr	(*S*)	973 ± 226
(*R*)-**4b**	*c*-Pent	(*R*)	10 ± 3.3
(*S*)-**4b**	*c*-Pent	(*S*)	94 ± 27
(*R*)-**4c**	*c*-Hex	(*R*)	6.5 ± 1.2
(*S*)-**4c**	*c*-Hex	(*S*)	46 ± 12
(*R*)-**4d**	*i*-Bu	(*R*)	6.4 ± 1.0
(*S*)-**4d**	*i*-Bu	(*S*)	39 ± 13
(*R*)-**4e**	*neo*-Pent	(*R*)	71 ± 15
(*R*)-**4f**	Bn	(*R*)	66 ± 11
(*S*)-**4f**	Bn	(*S*)	16 ± 1.1
(*S*)-**4g**	$PhCH_2CH_2$	(*S*)	965 ± 140

Table 2. Binding Affinities of **2** and **9**−**11** toward the hGnRH Receptor



2,9,10 and 11

compd	R	R′	R″	$K_i \pm$ SEM (nM)
(*S*)-**2a**	Me	Bn	H	51 ± 12
(*S*)-**2b**	Me	*c*-Pent	H	4.5 ± 0.6
(*R*)-**9d**	*i*-Bu	Me	H	1.2 ± 0.3
(*S*)-**9f**	Bn	Me	H	8.1 ± 0.5
(*R*)-**10b**	*c*-Pent	*i*-Pr	H	12 ± 0.2
(*R*)-**10c**	*c*-Hex	*i*-Pr	H	23 ± 3.0
(*R*)-**10d**	*i*-Bu	*i*-Pr	H	12 ± 2.7
(*R*)-**11b**	*c*-Pent	Me	Me	0.6 ± 0.1
(*S*)-**11b**	*c*-Pent	Me	Me	2.4 ± 0.7
(*R*)-**11c**	*c*-Hex	Me	Me	4.1 ± 1.0
(*S*)-**11f**	Bn	Me	Me	7.9 ± 1.5

Next, we investigated the effects of having small alkyl substituents on the basic nitrogen. The results are summarized in Table 2. Generally, these modifications led to enhancements in potency and in some cases to a reversal of enantiomeric preference for the receptor. Mono- and dimethylation of the phenylalanine derivative (*S*)-**4f** gave (*S*)-**9f** and (*S*)-**11f**, respectively, which showed modest improvements in binding affinity. While the derivative from (*R*)-leucinol ((*R*)-**4d**) was very potent (K_i = 6.4 ± 1.0 nM), monomethylation of its basic nitrogen resulted in a 5-fold increase in binding affinity ((*R*)-**9d**, K_i = 1.2 ± 0.3 nM). Interestingly, the highly potent dimethylated cyclopentyl derivatives (*R*)-**11b** and (*S*)-**11b**

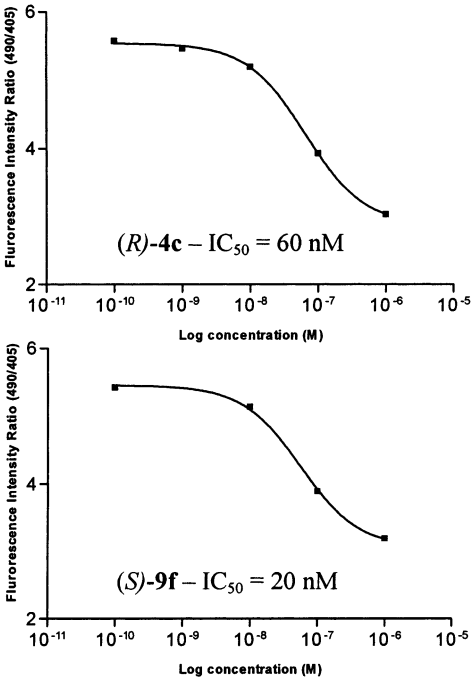


Figure 2. Dose−response curves of (*R*)-**4c** and (*S*)-**9f** inhibiting GnRH induced Ca^{2+} flux. The respective IC_{50} values are indicated for each case.

($K_i = 0.6 \pm 0.1$ and 2.4 ± 0.7 nM, respectively) are virtually equipotent toward the hGnRH receptor, contrasting with the marked preference for the (*R*)-isomer in the primary amine parent compounds (*R*)-**4b** and (*S*)-**4b**. The observation that N-alkylated or dialkylated derivatives of primary amines were equally or more potent than their parent compounds offers a good opportunity to adjust physicochemical properties of this class of compounds.

The functional antagonism of the compounds herein synthesized was confirmed by their ability to inhibit GnRH stimulated Ca^{2+} flux in transfected cells in a dose-dependent manner.[7] For instance, (*R*)-**4c** and (*S*)-**9f** were found to be functional antagonists with $IC_{50} =$ 60 and 20 nM, respectively (Figure 2).

These new compounds were metabolically more stable in the HLM assay. For example, upon incubation with HLM for 20 min, (*S*)-**4f** suffered a 4% loss whereas 33% of (*S*)-**2a** was degraded. This same trend was also observed with other compounds in this series, which shows that the strategy of connecting the lipophilic side chain via a carbon−carbon bond was warranted.

(*S*)-**2a**, (*R*)-**4c**, (*R*)-**4d**, (*S*)-**4f**, and (*S*)-**9f** were investigated for pharmacokinetic properties in mice. The compounds were given orally (po) and intravenously (iv) at a 10 mg/kg dose, and the data are shown in Table 3. All compounds showed low to good oral exposure in mice, despite their relatively high rate of clearance. (*S*)-**2a**, for instance, had the worst exposure among all of those tested, which can be partly attributed to its inherent metabolic instability. (*S*)-**9f** was far superior than the other compounds in terms of absolute oral bioavailability, even though it had the highest rate of clearance. On the basis of compound concentration in the hepatic portal vein, we estimated that 76% of (*S*)-**9f** was absorbed, translating in 55% of first-pass metabolism in the mice. Clearly, given the similar rates of clearance of all compounds, the main differences seen here most probably have to do with their ability to cross the gut wall. We can only speculate that (*S*)-**9f** may have an advantage in that respect because it only has one hydrogen bond donor, in contrast with its analogue (*S*)-**4f**, which has two.

Table 3. Mouse and Cynomolgus Monkey Pharmacokinetics Profiles of (*S*)-**2a**, (*R*)-**4c**, (*R*)-**4d**, (*S*)-**4f**, and (*S*)-**9f**

compd	oral bioavailability (*F*, %)	clearance (mL min^{-1} kg^{-1})	V_d (L kg^{-1})	$t_{1/2}$ (h)
		Mouse		
(*S*)-**2a**	3	81.3	7.9	1.1
(*R*)-**4c**	11	65.4	7.3	1.3
(*R*)-**4d**	6	65.7	3.9	0.7
(*S*)-**4f**	11	65.6	4.9	0.9
(*S*)-**9f**	42	97.2	6.9	0.8
		Monkey		
(*S*)-**9f**	22	13.5	3.6	3.1

To address the species differences with respect to clearance, we also performed a pharmacokinetic evaluation of (*S*)-**9f** in cynomolgus monkeys (Table 3). (*S*)-**9f** showed good oral bioavailability (22%), with a clearance of 13.5 mL min^{-1} kg^{-1}, much lower than hepatic blood flow rate.

We were unable to perform the in vivo evaluation of efficacy in mice, rats, and cynomolgus monkeys because of the lack of sufficient affinity of our compounds for their GnRH receptors. For example, (*S*)-**9f** had K_i values of 200 nM in the cynomolgus monkey receptor and 11 000 nM in the rat receptor. This type of species selectivity is consistent with our previous findings.[3h−j] Further optimization toward good potency on the monkey GnRH receptor and in vivo efficacy results of those compounds will be reported in due course.

In summary, we have designed and synthesized a novel series of uracils containing modified N-3 side chains, as potent hGnRH receptor antagonists. These are metabolically more stable, and selected compounds from this series displayed good oral bioavailability in mice and cynomolgus monkeys. Although the half-lives of these compounds are still short for desirable pharmacokinetic properties in these species, the in vitro metabolism data in HLM were encouraging, suggesting that these compounds should have longer half-lives in humans because of a lower rate of metabolism.

Acknowledgment. This work was partly supported by NIH Grants 1-R43-HD38625-01 and 2-R44-HD38625-02. We are grateful to Sue K. Sullivan and Lee Carter for pharmacological support.

Supporting Information Available: Experimental details of the pharmacokinetic experiments and experimental procedures for the synthesis and characterization of (*R*)-**4c**, (*R*)-**4d**, (*S*)-**4f**, and (*S*)-**9f**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) On each assay plate a standard antagonist of affinity comparable to those being tested was included as a control for plate-to-plate variability. All compounds were assayed in three to eight independent experiments.

(7) For experimental details of the hGnRH functional assay, please refer to the Supporting Information.

JM049791W