Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 3301–3305
Bioorganic & Medicinal Chemistry Letters

Potent and orally bioavailable zwitterion GnRH antagonists with low CYP3A4 inhibitory activity

Chen Chen,[a,*] Yongsheng Chen,[a] Joseph Pontillo,[a] Zhiqiang Guo,[a] Charles Q. Huang,[a] Dongpei Wu,[a] Ajay Madan,[c] Takung Chen,[c] Jenny Wen,[c] Qiu Xie,[b] Fabio C. Tucci,[a] Martin Rowbottom,[a] Yun-Fei Zhu,[a] Warren Wade,[a] John Saunders,[a] Haig Bozigian[c] and R. Scott Struthers[b]

[a]*Department of Medicinal Chemistry, Neurocrine Biosciences, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*
[b]*Department of Endocrinology, Neurocrine Biosciences, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*
[c]*Department of Preclinical Development, Neurocrine Biosciences, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*

Received 18 March 2008; revised 10 April 2008; accepted 11 April 2008
Available online 26 April 2008

Abstract—Incorporation of a carboxylic acid into a series of uracil derivatives as *h*GnRH-R antagonists resulted in a significant reduction of CYP3A4 inhibitory activity. Highly potent *h*GnRH antagonists with low CYP3A4 inhibitory liability, such as **8a** and **8d**, were identified. Thus, **8a** had a K_i of 2.2 nM at GnRH-R and an IC_{50} of 36 μM at CYP3A4.

The relationship between cytochrome P450 (CYP) inhibition and drug–drug interactions is well understood, therefore, modern drug discovery has incorporated CYP inhibition detection at an early stage.[1] One of the major liver enzymes responsible for the metabolism of many drugs is CYP3A4.[2] Previously, we identified a series of uracils as potent antagonists of the human gonadotropin-releasing hormone receptor (*h*GnRH-R), and found that a trifluoromethyl substitution of the 1-benzyl group of the uracil results in a substantial increase in potency.[3] Thus, compound **1a** (Fig. 1) possesses a K_i value of 0.64 nM, which is about ninefold better than its fluoro analog (**1b**, K_i = 5.3 nM).[3] However, **1a** potently inhibited the CYP3A4 enzyme with an IC_{50} of 0.1 μM, showing potential drug–drug interaction liability. We then embarked on a study to address this issue by incorporating a polar group into the molecule. We reasoned that acid groups in the ligand may be tolerated because of the presence of several basic residues (e.g., Lys-121, Arg-299, Lys-115) in the ligand binding pocket.[4] In this paper, we describe a series of butyrate derivatives of **1**, exemplified by **8a** and **8d**, which possesses high GnRH antagonist potency and good oral bioavailability, but low CYP3A4 inhibitory activity.

The synthesis of compounds **4**–**6** started from the 5-bromouracil **2**[3] (Scheme 1). Suzuki coupling reactions of **2** with 4-substituted phenylboronic acids and palladium (0) afforded the desired products **3a–c**. Coupling reactions of the benzyl alcohol **3a** with carboxylic acids or acid chlorides gave, after Boc-deprotection with trifluoroacetic acid, esters **4a–c** in good yields. Ether **6a** was prepared from **3a** by alkylation and Boc-removal, while thioether **6b** was made by a conversion of **3a** to the isopropylsulfonate, followed by displacement with mercaptoacetic acid, and Boc-deprotection. Alkylation and deprotection of benzylamine **3b** gave ester **5**, and the corresponding carboxylic acid **6c**. Finally, **6d** was formed by reductive amination and deprotection of the benzaldehyde **3c**.

Compounds **8** were synthesized according to Scheme 2. Alkylations of **1a**–**f**[3] with methyl 4-bromobutyrate provided the secondary amines **7**, which were then hydrolyzed under basic conditions to give the desired carboxylic acids **8a**–**f**.

The synthesized compounds were screened in a GnRH receptor binding assay as previously described.[5] These

Keywords: Zwitterion; Gonadotropin-releasing hormone; Receptor; Antagonist; CYP3A4; Inhibition; Structure–activity relationship.
* Corresponding author. Tel.: +1 858 617 7600; fax: +1 858 617 7967; e-mail: cchen@neurocrine.com

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.04.036

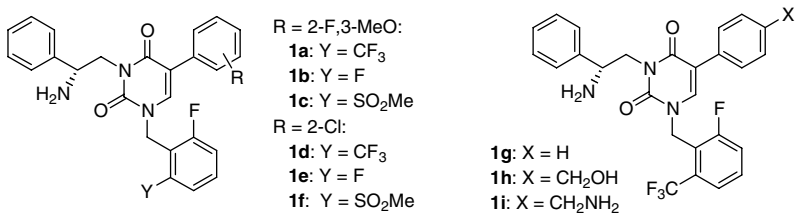
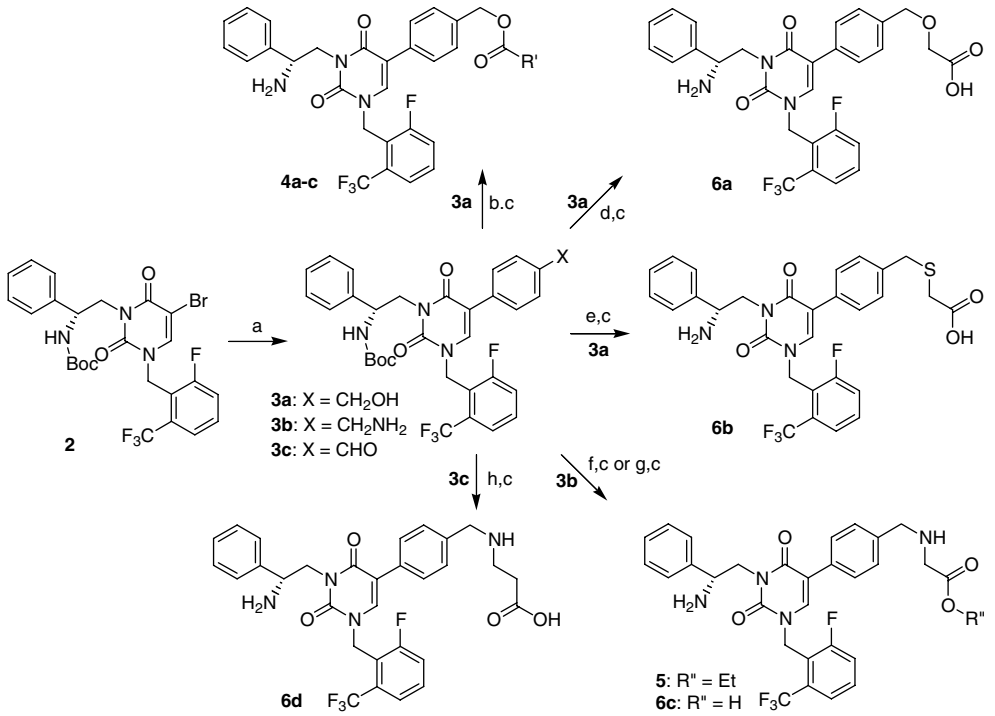
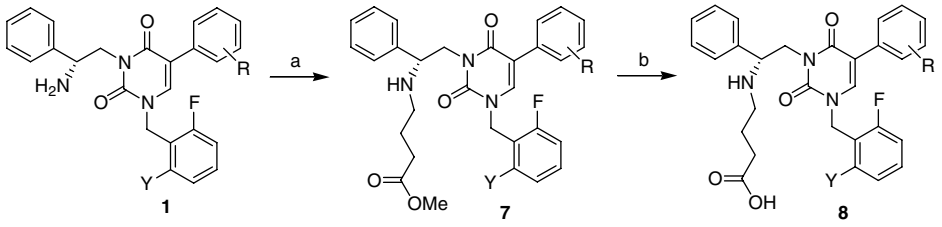


Figure 1. 2*R*-Aminophenethyl uracil GnRH antagonists.



Scheme 1. Reagents: (a) 4-$XC_6H_4B(OH)_2$/$Pd(PPh_3)_4$/aq Na_2CO_3; (b) R′COCl/Et_3N/DCM or R′COOH/CDI/DMF; (c) TFA/DCM; (d) KO*t*Bu/$BrCH_2COO^tBu$/THF; (e) i— *i*$PrSO_2Cl$/Et_3N/DCM; ii— $HSCH_2COOH$; (f) $BrCH_2COOEt$/ACN; (g) $BrCH_2COO^tBu$/ACN; (h) $NH_2(CH_2)_2COOtBu$/$NaBH_3CN$/DCM.

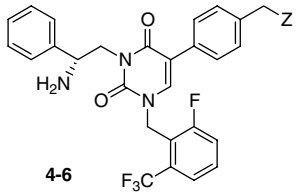


Scheme 2. Reagents: (a) $BrCH_2CH_2CH_2COOMe/Na_2CO_3$/THF–$H_2O$; (b) NaOH/EtOH–$H_2O$.

were also screened for their ability to inhibit ligand binding to the CYP3A4 enzyme in an in vitro assay.[6] These data are summarized in Tables 1 and 2. Mean, standard error, and number of replicates associated with these data for key compounds are given in the text.

The lipophilic cyclopropanecarboxylate **4a** (K_i = 3.3 nM) and hydroxyacetate **4b** (K_i = 10 nM) exhibited potencies similar to the unsubstituted 5-phenyluracil **1g** (K_i = 7.8 nM), but higher than the hydro-xymethyl analog **1h** (K_i = 69 nM) at *h*GnRH-R, The weakly basic aminoacetate **4c** (K_i = 77 nM) was substantially less potent compared to **1g**. These results suggest that this region can tolerate a large and non-polar group. Like **1g** (IC_{50} = 0.3 μM), these compounds showed high inhibitory activity toward the CYP3A4 enzyme (IC_{50} 0.048–0.80 μM). The highly hydrophilic benzylamine **1i** (K_i = 1100 nM) possessed poor binding affinity at *h*GnRH-R, but also low inhibition at CYP3A4 (IC_{50} = 9.1 μM). Compared to **1i**, the more lipophilic glycine ester **5** (K_i = 110 nM) had 10-fold improvement, while zwitterion **6c** (K_i = 3900 nM) was fourfold less potent at *h*GnRH-R

Table 1. SAR of the substituted 5-phenyl group of uracils **1** and **4–6** at *h*GnRH-R and CYP3A4



4-6

Compound	Z	GnRH K_i (nM)	CYP3A4 IC_{50} (μM)
1g		7.8	0.30
1h	OH	69	0.80
1i	NH_2	1100	9.1
4a	OCOPr-c	3.3	0.048
4b	$OCOCH_2OH$	10	0.12
4c	$OCOCH_2NH_2$	77	0.67
5	$NHCH_2COOEt$	110	0.35
6a	OCH_2COOH	>10,000	20
6b	SCH_2COOH	3700	16
6c	$NHCH_2COOH$	3900	36
6d	$NHCH_2CH_2COOH$	>10,000	31

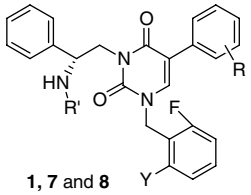
and the propionic acid **6d** showed no binding. However, zwitterions **6c** and **6d** had insignificant inhibition at CYP3A4 (IC_{50} > 30 μM), while ester **5** was more potent CYP3A4 inhibitor (IC_{50} = 0.35 μM) than its parent **1i**. The other two carboxylic acids (**6a–b**) also showed a weak inhibitory activity at CYP3A4 (Table 1). These results demonstrated that a polar group was intolerable in this region of compound **1g** as an *h*GnRH-R antagonist, but an acidic functionality diminished its interaction with the CYP3A4 enzyme. It is worth noting that zwitterion **6c** was only fourfold less potent than amine **1i**, while the carboxylic acid derivative **6a** was over 100-fold less potent than alcohol **1h** at *h*GnRH-R.

Attaching a butyric acid to the basic nitrogen of the potent *h*GnRH-R antagonist **1a** (K_i = 0.64 nM, pK_i = 9.2 ± 0.1, N = 3) resulted in zwitterion **8a** (K_i = 2.2 nM, pK_i = 8.7 ± 0.1, N = 8) with comparable potency. More importantly, **8a** had an IC_{50} value of 36 μM at CYP3A4 (pIC_{50} = 4.4 ± 0.3, N = 3), representing a 360-fold improvement compared to **1a**. In direct comparison with its methyl ester **7a**, which had a K_i of 32 nM at the GnRH receptor and an IC_{50} = 0.51 μM at CYP3A4, the acid functionality of **8a** significantly changed the profile of this class of compounds, which could be associated with the unique physicochemical property of an ampholyte.[7] Compound **8a** had pK_a values of 4.16 for the acid and 7.65 for the amine. Its molecular structure might allow the carboxylic group to interact with the basic nitrogen via folded conformers, therefore, it possesses relatively high lipophilicity at physiological pH (log D = 1.8).

Similar results were also observed for other analogs. Thus, the butyric acid derivative of **1d** (*h*GnRH-R K_i = 1.1 nM, pK_i = 9 ± 0.1, N = 3; CYP3A4 IC_{50} = 0.16 μM, pIC_{50} = 6.8 ± 0.1, N = 3) possessed similar GnRH potency (**8d**, K_i = 3.4 nM, pK_i = 8.5 ± 0.1, N = 9) but much improved CYP3A4 inhibitory activity (IC_{50} = 17 μM, pIC_{50} = 4.8 ± 0.2, N = 5), while its methyl ester **7d** exhibited low affinity at *h*GnRH-R and high CYP3A4 activity.

Several close analogs were also synthesized and studied. The butyric acids **8b–c** and **8e–f** displayed a range of potencies (K_i 3.4–42 nM) at *h*GnRH-R, however, these all showed a low CYP3A4 inhibitory activity

Table 2. SAR of uracil derivatives with or without a butyric acid



1, 7 and 8

Compound	Y	R	R′	GnRH K_i (nM)	CYP3A4 IC_{50} (μM)
1a	CF_3	2-F,3-MeO	H	0.64	0.10
7a			$(CH_2)_3COOMe$	32	0.51
8a			$(CH_2)_3COOH$	2.2	36
1b	F	2-F,3-MeO	H	5.3	0.60
8b			$(CH_2)_3COOH$	22	33
1c	$MeSO_2$	2-F,3-MeO	H	0.9	0.38
8c			$(CH_2)_3COOH$	3.4	>50
1d	CF_3	2-Cl	H	1.1	0.16
7d			$(CH_2)_3COOMe$	66	0.13
8d			$(CH_2)_3COOH$	3.4	17
1e	F	2-Cl	H	6.0	0.44
8e			$(CH_2)_3COOH$	42	20
1f	$MeSO_2$	2-Cl	H	1.3	0.18
8f			$(CH_2)_3COOH$	3.6	48

($IC_{50} \geqslant 20$ μM, Table 2). These results indicate that the incorporation of a butyric acid into amines **1** reduces the binding affinity of these compounds by two- to four-fold at *h*GnRH-R, and CYP3A4 inhibition by >50-fold.

The functional *h*GnRH-R antagonism of select potent compounds was demonstrated in a calcium flux assay.[8] Thus, **8a** and **8d** possessed IC_{50} values of 4.9 and 11 nM, respectively. In contrast, **6a** with a low binding affinity was much less active in this assay (IC_{50} = 7.9 μM).

In addition to their low CYP3A4 inhibitory activity, compounds **8** also exhibited good metabolic stability based on in vitro incubation with human liver microsomes.[9] The predicted scaled systemic clearance was 10, 9.5, and 7.1 mL/min kg, respectively, for **8a**, **8d**, and **8f**.

Due to their desirable physicochemical properties (measured log *D* at pH of 7.4 was 1.8 and 2.1, respectively, for **8a** and **8d**) and in vitro pharmacological and metabolic profiles, **8a** and **8d** were further studied in Sprague–Dawley rats and Cynomolgus monkeys for their pharmacokinetic profiles (Table 3). After a 10 mg/kg intravenous injection to rats, **8d** displayed a moderate plasma clearance (CL = 37.6 mL/min kg) and a low volume of distribution (V_d = 1.3 L/kg), resulting in a short half-life ($t_{1/2}$) of 0.4 h. The oral exposure of this compound in rats was low, and the area under curve (AUC) was only 132 ng/mL h after a 10 mg/kg oral administration, which gave an oral bioavailability of 3%. In monkeys, **8d** had a low CL of 7.9 mL/min h and a moderate V_d of 4.9 L/kg, which resulted in a long $t_{1/2}$ of 7.5 h. After the oral administration of 10 mg/kg **8d** to monkeys, a maximal concentration (C_{max}) of 7.9 μg/mL was attained at 0.63 h (T_{max}) and the AUC was 11.1 μg/mL h. The monkey oral bioavailability was 52.7%.

Similarly, **8a** exhibited a high CL of 64.1 mL/min kg and poor oral exposure in rats (Table 3). In monkeys, **8a** had a moderate CL of 20.9 mL/min kg and a low V_d of 1.8 L/kg. Its $t_{1/2}$ of 1 h was much shorter than that of **8d**. The higher plasma clearance of **8a** than that of **8d** could be associated with *O*-demethylation of **8a**, which was identified as a major metabolite from in vitro studies. An oral dose of **8a** at 10 mg/kg gave an AUC of 1.9 μg/mL h, resulting in an oral bioavailability of 22.6% in monkeys.

Table 3. Pharmacokinetic parameters of compounds **8a** and **8d** after an intravenous or oral dose to rats and monkeys (10 mg/kg, N = 3 for each point)

Compound	8a		8d	
	Rat	Monkey	Rat	Monkey
CL (mL/min kg)	64.1	20.9	37.6	7.9
V_d (L/kg)	1.7	1.8	1.3	4.9
$t_{1/2}$ (h)	0.3	1	0.4	7.5
C_{max} (ng/mL)	77	2039	149	7874
T_{max} (h)	0.25	0.5	0.25	0.63
po AUC (ng/mL h)	63.3	1904	131.8	11,097
F (%)	2.4	22.6	3	52.7

Both **8a** and **8d** are highly selective for *h*GnRH-R, compared to rat and macaque GnRH receptors, as has been described for several other classes of non-peptide GnRH antagonists. The affinity for the rat receptor was 11.2 and 12.5 μM, respectively. Thus, pharmacodynamic responses in the rat were not evaluated. Affinity for the macaque receptor was better (81 and 100 nM, respectively) than for the rat, but greatly reduced compared to the human receptor (~60-fold). This species selectivity is greater than that reported for another uracil GnRH-R antagonist, NBI-42902 (~12- to 17-fold depending on the assay).[10] Accordingly, when compared in the castrate macaque, despite improved plasma exposure of **8b**, the degree of LH suppression observed was substantially less than that observed for an equivalent dose of NBI-42902.[11] These data will be discussed in detail elsewhere.

The precise nature of the receptor–ligand interactions which enable the mutual satisfaction of both CYP3A4 and GnRH-R affinity design objectives is less clear. Based on our previous mutagenesis and molecular modeling of uracil based GnRH-R antagonists,[12] several basic residues in the receptor are potential counter-ions for the acid moiety in the non-peptide ligands discussed here. In addition, polar residues from the extracellular domains which have not been modeled may also be in proximity to the ligand based on the involvement of this region in trapping insurmountable GnRH-R antagonists.[4] However, initial mutagenesis experiments failed to identify a potential basic residue acting as a counter-ion to the acids in compounds such as **8a** and **8d**. Further, because basic groups attached by alkyl linkers analogous to the butyric acid group here are also consistent with high *h*GnRH-R binding affinity (unpublished), we hypothesize that these charged groups may not be engaged in specific receptor interactions, but rather simply remain solvent accessible.

In conclusion, a series of uracils were synthesized as *h*GnRH-R antagonists. Attempts to incorporate an acidic moiety at the 5-phenyl ring of uracil **1g** failed to generate potent compounds at *h*GnRH-R, but provided valuable information for lowering CYP3A4 inhibition. Incorporating a butyric acid into the amino group of **1** resulted in several potent *h*GnRH-R antagonists, such as **8a** and **8d**, with much improved CYP3A4 profiles. **8d** was also found to have a long half-life and good oral bioavailability in monkeys. Thus, we have demonstrated that incorporating a carboxylic acid at an appropriate position of potent *h*GnRH-R antagonists greatly reduces their CYP3A4 inhibitory activity while maintains their potency at the target receptor.

References and notes

1. Zlokarnik, G.; Grootenhuis, P. D.; Watson, J. B. *Drug Discovery Today* **2005**, *10*, 1443.
2. Riley, R. J.; parker, A. J.; Trigg, S.; Manners, C. N. *Pharm. Res.* **2001**, *18*, 652.

3. Guo, Z.; Chen, Y.; Huang, C. Q.; Gross, T. D.; Pontillo, J.; Rowbottom, M. W.; Saunders, J.; Struthers, S.; Tucci, F. C.; Xie, Q.; Wade, W.; Zhu, Y. F.; Wu, D.; Chen, C. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2519.
4. Kohout, T. A.; Xie, Q.; Reijmers, S.; Finn, K. J.; Guo, Z.; Zhu, Y. F.; Struthers, R. S. *Mol. Pharmacol.* **2007**, *72*, 238.
5. Guo, Z.; Zhu, Y. F.; Gross, T. D.; Tucci, F. C.; Gao, Y.; Moorjani, M.; Connors, P. J., Jr.; Rowbottom, M. W.; Chen, Y.; Struthers, R. S.; Xie, Q.; Saunders, J.; Reinhart, G.; Chen, T. K.; Bonneville, A. L.; Chen, C. *J. Med. Chem.* **2004**, *47*, 1259.
6. Emoto, C.; Iwasaki, K. *Xenobiotica* **2006**, *36*, 219.
7. Pagliara, A.; Carrupt, P-A. P.-A.; Caron, G.; Gaillard, P.; Testa, B. *Chem. Rev.* **1997**, *97*, 3385.
8. Calcium flux assay: Zhou, W.; Rodic, V.; Kitanovic, S.; Flanagan, C. A.; Chi, L.; Weinstein, H.; Maayani, S.; Millar, R. P.; Sealfon, S. C. *J. Biol. Chem.* **1995**, *270*, 18853, Also see Ref. 11.
9. For assay conditions, see Ref. 5.
10. Tucci, F. C.; Zhu, Y. F.; Struthers, R. S.; Guo, Z.; Gross, T. D.; Rowbottom, M. W.; Acevedo, O.; Gao, Y.; Saunders, J.; Xie, Q.; Reinhart, G. J.; Liu, X. J.; Ling, N.; Bonneville, A. K.; Chen, T.; Bozigian, H.; Chen, C. *J. Med. Chem.* **2005**, *48*, 1169.
11. Struthers, R. S.; Xie, Q.; Sullivan, S. K.; Reinhart, G. J.; Kohout, T. A.; Zhu, Y. F.; Chen, C.; Liu, X. J.; Ling, N.; Yang, W.; Maki, R. A.; Bonneville, A. K.; Chen, T. K.; Bozigian, H. P. *Endocrinology* **2007**, *148*, 857.
12. Sullivan, S. K.; Hoare, S. R.; Fleck, B. A.; Zhu, Y. F.; Heise, C. E.; Struthers, R. S.; Crowe, P. D. *Biochem. Pharmacol.* **2006**, *72*, 838.