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Zwitterionic uracil derivatives as potent GnRH receptor antagonists with improved pharmaceutical properties

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ABSTRACT

A novel series of potent zwitterionic uracil GnRH antagonists were discovered that showed reduced liability for CYP3A4 enzyme inhibition.

The potential of non-peptide Gonadotropin-releasing hormone (GnRH) receptor antagonists serving as novel therapeutics for hormone-dependent disease states such as prostate cancer, endometriosis, and benign prostate hyperplasia has led to discovery of a wide range of small molecule antagonists.[1,2] We have reported that uracil-based analogs are potent GnRH receptor antagonists based on in vitro and in vivo characterizations.[3] However, CYP3A4 inhibition was a common issue for early uracil analogs that contained a basic amine such as **1** in Figure 1. Since inhibition of CYP3A4 enzyme is well known to potentially induce drug–drug interactions, elimination of such undesired property from this class of molecules was clearly necessary. Recently, we have shown[4] that addition of an acid can drastically reduce the possibility of this class of molecules to inhibit CYP3A4 enzyme activity regardless of the location of the acid. However, the GnRH receptor-binding affinity was heavily dependent on the exact location of this acidic functional group. For example, the acid linked to the phenyl group at the right hand side of the molecule (**2b**) diminishes the GnRH receptor-binding affinity compared to its ester precursor (**2a**), yet the acid attached to the amine group through a propylene chain (**3b**) is highly potent GnRH receptor binder. As a matter of fact, such combination of the amino and acid functionalities offered similar potency to its amine precursor **1**, yet without the CYP3A4 liability. Interestingly, such zwitterionic molecules show good oral bioavailability in cynomolgus monkeys, albeit relatively poor exposure in rats.[4] To further expand the SAR on zwitterionic uracils, we report here a novel way of linking an acid group to the 5-phenyl uracils, which generated a series of novel and potent zwitterionic molecules without inhibition of CYP3A4 enzyme.

The initial syntheses of such molecules are outlined in Scheme 1. The starting compounds (**4a**–**d**)[3,4] were first treated with BBr_3 to remove the methyl group; subsequently, the amino group was protected using Boc_2O to give compounds **5a**–**d**. Alkylation with $Br(CH_2)_nCO_2Et$, followed by hydrolysis of the ethyl ester and removal of the Boc protecting group, yielded the desired zwitterionic compounds **6a**, **6b**, **7a**–**c**, **8a**–**c**, **8e**, and **9a**. Compound **8d** was prepared alternatively according to Scheme 2 where **5c** was first alkylated with 3-bromopropanol, followed by the oxidation of the hydroxyl group to the corresponding carboxylic acid functionality and then removal of the Boc-protecting group. These compounds were assayed against the human GnRH receptor binding, IP_3 function, and CYP3A4 inhibition.[6] The results are summarized in Table 1. Our previous SAR has indicated that polar group cannot be tolerant around the 3-methoxylphenyl region at 5-uracil, thus our campaign to introduce the acid functionality on 3-methoxyphenyl

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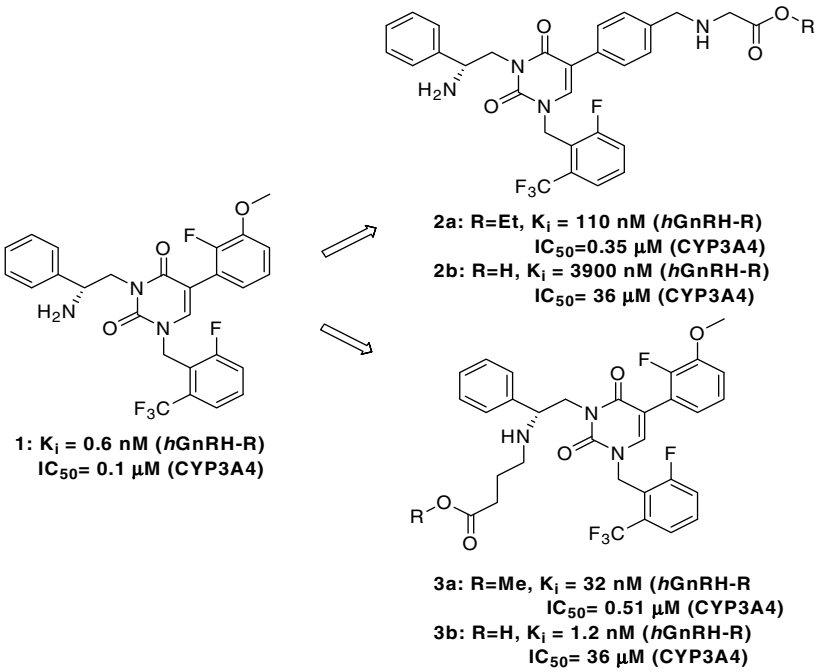
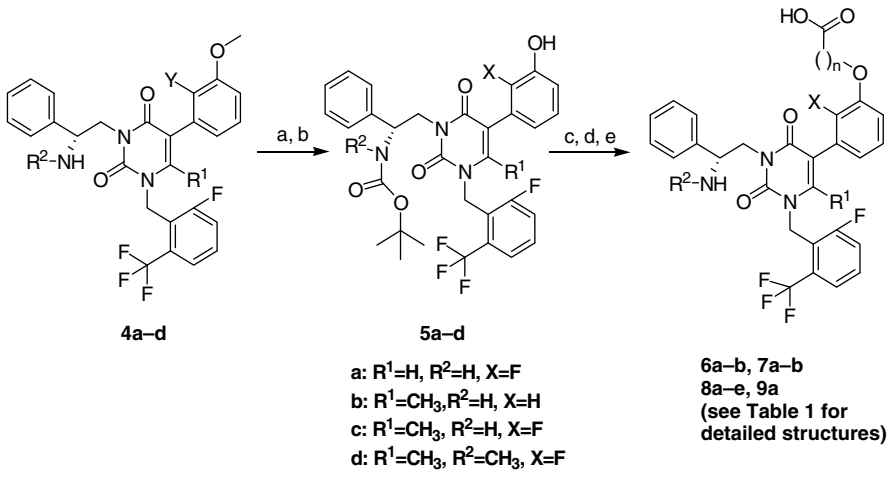
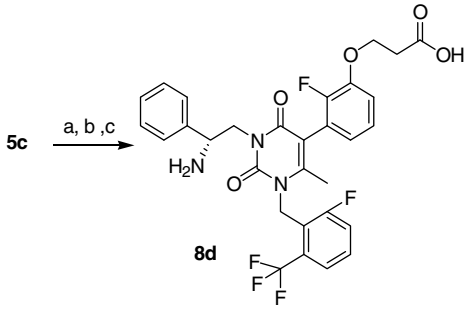


Figure 1. Structures and biological activities of previously reported compounds **1**–**3**.



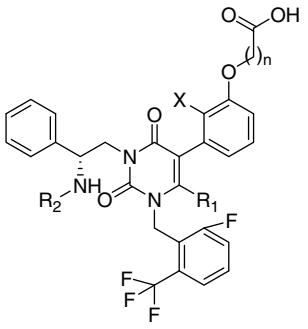
Scheme 1. Reagents and condition: (a) BBr_3, DCM, −78 °C; (b) Boc_2O, DCM, Et_3N; (c) K_2CO_3, DMF, $Br(CH_2)_nCO_2Et$; (d) LiOH, THF/water; (e) TFA/DCM.



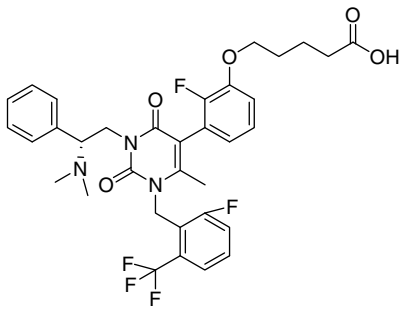
Scheme 2. Reagents and condition: (a) K_2CO_3, DMF, $BrCH_2CH_2CH_2OH$, 50 °C; (b) cat. $RuCl_3$, $NaIO_4$, DCM, MeCN, H_2O; (c) TFA/DCM.

position of compound **1** initialized with a long alkyl-acid such as pentanoic acid (**6a**), which yielded moderate but encouraging activity (K_i = 34 nM); the longer acid **6b** did not improve the activity. Both compounds, as we expected, did not exhibit significant CYP3A4 inhibition. To search for an improvement on GnRH activity, we turned our attention to modify on more potent analogs (**4b**–**d**). Indeed, compound **7a**, 6-methyluracil analog based on **4b**, was much more potent (K_i = 2.1 nM) than that of the non-methyl analog **6a**. However, shortening the chain length (**7b** and **7c**) decreased the potency slightly. Historically, addition of a fluoro group to the 3-methoxyphenyl ring of **4b** further enhances the GnRH activity. Therefore, compounds **8a**–**e** were prepared accordingly. Enhancement of the potency by fluoro group was not clearly observed in the binding assay, but was well displayed in the functional assay which measures the ability of a compound to inhibit GnRH-stimulated [^{3}H] inositol phosphate hydrolysis. Overall, fluoro analogs were about 5–10 times more potent than the corresponding non-fluoro analogs (such as **8a** and **8b** vs **7a** and **7b**). Because of their low possibility of CYP3A4 inhibition and potent GnRH antagonistic activity, pharmacokinetic studies of several

Table 1
SAR of the uracil ziwitterionic molecules



6a-9a



9b

Compound	R_1	R_2	X	*n*	K_i[a] (nM)	IC_{50}[b] (nM)	CYP3A4 IC_{50}[d] (μM)
6a	H	H	F	4	34	n.d.[c]	36
6b	H	H	F	5	33	n.d.	25
7a	CH_3	H	H	5	2.1; *1.1*	2.8	n.d.
7b	CH_3	H	H	4	4.1	26	22
7c	CH_3	H	H	3	*7.5*	23	10
8a	CH_3	H	F	5	1.3; *1.2*	0.6	8.2
8b	CH_3	H	F	4	1.2; *1.0*	2.6	28
8c	CH_3	H	F	3	*4.7*	n.d.	21%[e]
8d	CH_3	H	F	2	*10.1*	n.d.	n.d.
8e	CH_3	H	F	1	*52*	n.d.	16%[e]
9a	CH_3	CH_3	F	4	1.2	n.d.	13
9b	—	—	—	—	15	n.d.	42

[a] K_i values in italic were obtained from the binding assay in competition with [^{125}I]Tyr[5], D-Leu[6], *N*MeLeu[7], and Pro-*N*-Et-GnRH using cloned human GnRH receptor that was transfected on RBL cells, while others were obtained from HEK cells. The data from these two binding assays correlate well.
[b] Inhibition of GnRH-stimulated [^{3}H]inositol phosphate hydrolysis from human GnRH receptors stably transfected on RBL whole cells.
[c] n.d., not determined.
[d] Microtiter plate-based fluorimetric assay using recombinant CYP3A4 with 7-benzyloxy-4-(trifluoromethyl)coumarin (BFC)as the substrate.
[e] Percentage of inhibition at 200 μM concentration.

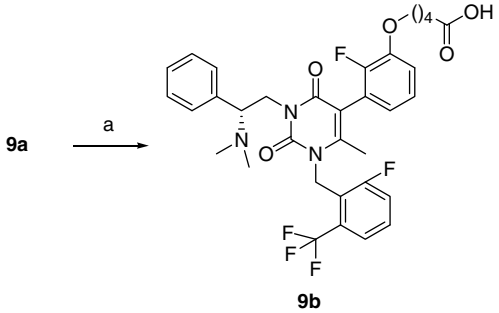
compounds were conducted in rats (Table 2).[7] The results indicated that this class of molecules possessed low bioavailability in rats, which was similar to the first reported class of zwitterionic uracils such as **3b**. But the terminal half-life of each compound tested in this class was notably improved over **3b**. The poor oral bioavailability could be attributed to the possible low permeability, as Caco-2 cell assay demonstrated that these compounds were poorly permeable (e.g., $P_{app\ (\mathbf{a}-\mathbf{b})} = 0.1 \times 10^{-6}$ cm/s for both **7b** and **8b**). Since reducing hydrogen bond donors could improve permeability, compounds **9a** and **9b** were designed and synthesized to aim for maintaining the target potency with reducing hydrogen bond donors. Synthesis of **9a** was described in Scheme 1, while **9b** was accomplished by a simple methylation from **9a** (Scheme 3). Indeed, with addition of a methyl group on the basic amine, the permeability improved in Caco-2 data ($P_{app(\mathbf{a}-\mathbf{b})} = 1.2$ and 17×10^{-6} cm/s, respectively, for **9a** and **9b**). Unfortunately, dimethylated **9b** with better permeability was only moderately potent (K_i = 15 nM), while mono-methylated **9a** maintained the high potency in GnRH binding assay. Thus, **9a** was selected for pharmacokinetic study in rats which demonstrated an improved oral bioavailability (F = 13%) in rats over the primarily amine analog **8b** (F = 6.6%).

Metabolism profiling of **9a** in human hepatocytes implicated that it partially produced a β-oxidative metabolite **10** from the *O*-alkyl acid side chain as shown in Figure 2, which probably mimics the fatty acid metabolism pathway.[8] Since such unusual metabolism pathway gives rise to a speculative safety concern, an effort to block such oxidation was undertaken. Our strategy was to use heteroatom replacement and introduction of *gem*-dimethyl group on one of the methylene group. The syntheses of these analogs are described in Scheme 4. For nitrogen insertion, **5d** reacted with 1,2-bromoethane and 1,3-dibromopropane to yield **11a** and **11b**,

Table 2
Pharmacokinetics of the selected zwitterionic uracils in rats

Compound	Pharmacokinetics[a]		
	F (%)	Cl (iv) (ml/min. kg)	$t_{1/2}$ (iv) (h)
3b	2.4	64	0.3
7a	4.8	36	4.9
7b	1.1	7.5	10
8a	3.7	21	2.9
8b	6.6	14	6.6
9a	13	50	5.1

[a] pharmacokinetic studies in male Sprague–Dawley rats at 10 mg/kg (po) and 2.5 mg/kg (iv) except for **3b**, where used 10 mg/kg doses were given orally and intravenously.



9a → a → **9b**

Scheme 3. Reagents: (a) formaldehyde in water, $NaBH_3CN$, DCM.

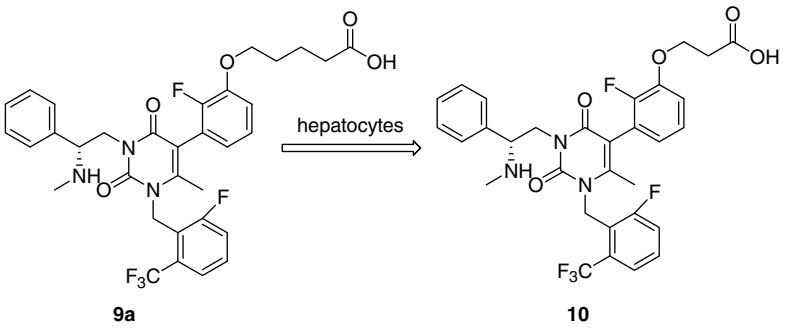
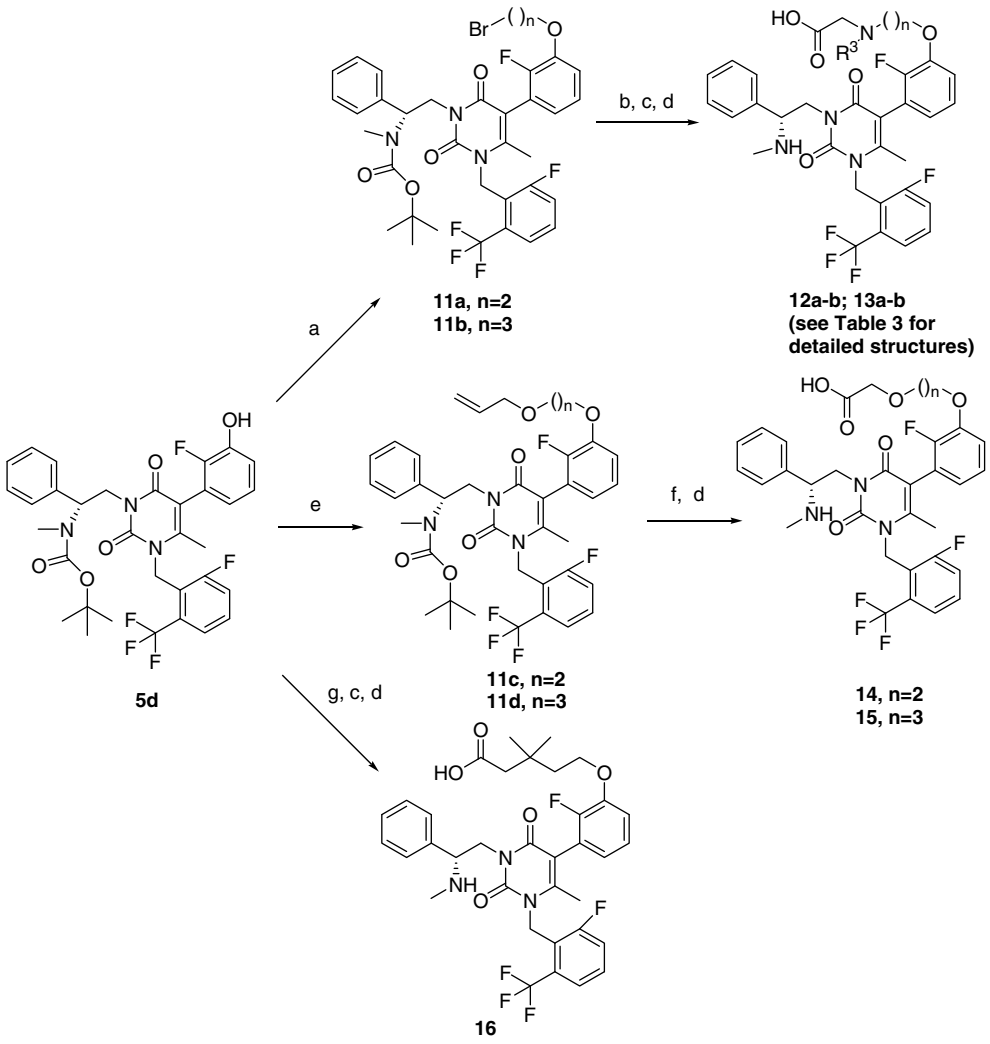


Figure 2. β-Oxidation metabolism of **9a** in human hepatocytes.

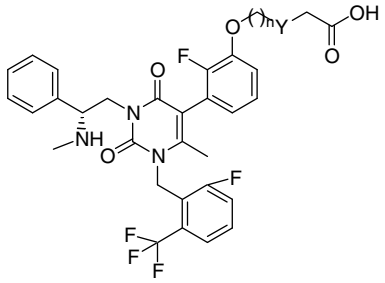


Scheme 4. Reagents and condition: (a) K_2CO_3, $Br(CH_2)_nBr$, DMF; (b) $R^3NHCH_2CO_2Et$, Et_3N, DCM; (c) LiOH, THF/H_2O; (d) 50% TFA in DCM; (e) $CH_2{=}CHCH_2O(CH_2)_nOH$, Ph_3P, *t*-$BuO_2CN{=}NCO_2Bu$-*t*, THF; (f) cat. $RuCl_3$, $NaIO_4$; (g) $BrCH_2CH_2CMe_2CH_2CO_2Et$, K_2CO_3, DMF.

respectively. The bromo groups on **11a and 11b** were then replaced with amino esters, followed by hydrolysis of the esters and removal of the Boc protecting group to afford the desired products **12a** and **12b**, and **13a** and **13b**. Compound **5d** was also alkylated with 2-allyloxy-ethanol and 3-allyloxy-propan-1-ol under Mitsunobu condition to afford **11c** and **11d**. Oxidative cleavage of the double bonds on **11c** and **11d** followed by removal of the Boc-protecting group yielded the final acids **14** and **15**. For the gem-dimethyl analog, **5d** was alkylated first with $BrCH_2CH_2C(CH_3)_2CH_2CO_2Et$,[9] followed by de-protection and hydrolysis to produce **16**. It turned out that all modifications on the alkyl side chain reduced the binding affinity to the GnRH receptor as the data summarized in Table 3.

In summary, attachment of an acid functionality to 5-phenyluracil yielded potent zwitterionic GnRH receptor antagonists that showed drastically reduced CYP3A4 inhibition. An adequate length between the core and the acid was required only for the targeted activity, not for blocking CYP3A4 interaction. Optimal length

Table 3
SAR of the zwitterionic uracils with modification on acid side chain



Compound	*n*	Y	K_i[a] (nM)	CYP3A4 IC_{50}[b] (μM)
12a	2	NH	27	n.d.[c]
12b	2	N–CH_3	98	n.d.
13a	3	NH	155	33
13b	3	N–CH_3	35	n.d.
14	2	O	9.3	10
15	3	O	38	22
16	2	$C(CH_3)_2$	29	22

[a] K_i values were obtained from the binding assay in competition with [^{125}I]Tyr^5, D-Leu^6, *N*MeLeu^7, Pro-*N*-Et-GnRH using cloned human GnRH receptor transfected on RBL cell line.
[b] Microtiter plate-based fluorimetric assay using recombinant CYP3A4 with 7-benzyloxy-4-(trifluoromethyl)coumarin (BFC) as the substrate.
[c] n.d., not determined.

between the 5-phenyl on the core and the acid group is –$O(CH_2)_4$– and –$O(CH_2)_5$–. Oral bioavailability of such zwitterion molecules was improved through a simple mono-methylation on the basic amine moiety. Despite the flexible nature of the *O*-alkyl acid side chain, modifications aiming for elimination of the unusual β-oxidation metabolism pathway led to the reduction of potency for the molecules to bind to the GnRH receptor.

Acknowledgments

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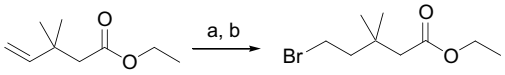

a) BH_3, THF; b) HBr

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