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Structure–activity relationships of 1,3,5-triazine-2,4,6-triones as human gonadotropin-releasing hormone receptor antagonists

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Abstract—SAR studies of 1,3,5-triazine-2,4,6-triones as human gonadotropin-releasing hormone receptor antagonists resulted in potent compounds. The best compound from the series had a binding affinity of 2 nM.

Gonadotropin-releasing hormone (GnRH) is a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) produced in and secreted by the hypothalamus in a pulsatile manner, which interacts with specific GnRH receptors within the anterior pituitary, releasing both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from this gland.[1,2] FSH and LH, in turn, stimulate the ovaries and testes to produce gonadal steroids that act on the reproductive organs. Several reproductive disease conditions such as endometriosis, uterine fibroids and prostate cancer are caused by overstimulation of the reproductive organs by the gonadal steroids, and thus can be treated by suppression of the pituitary–gonadal axis. This can be clinically achieved via activation or inhibition of the GnRH receptor and a number of peptide agonists and antagonists are currently approved for treating these disorders as represented by leuprorelin® and Cetrotide™,[1] respectively.[3,4]

However, treatment with peptide agonists or antagonists requires parenteral administration due to their poor oral bioavailability. By contrast, small molecule GnRH antagonists offer the potential of oral administration and therefore could gain wider acceptance from patients given the enhanced flexibility that oral dosaging offers. Thus, intensive efforts have been initiated in the development of small molecule GnRH antagonists by many laboratories.[5–8] We have previously reported a class of uracils as potent human gonadotropin-releasing hormone (*h*GnRH) receptor antagonists exemplified by (*R*)-1-(2,6-difluorobenzyl)-5-(3-methoxyphenyl)-3-{2-[*N*-methyl-*N*-(2-pyridyl)methyl]aminopropyl}-6-methyluracil **1**[9,10] (Fig. 1). In a recent letter,[11] we reported the development of a novel and convenient synthesis of 1,3,5-triazine-2,4,6-triones, and initial SAR studies around this monocyclic nucleus as GnRH antagonists. Here, we report the SAR in other regions of the core, at the left-hand side represented by N-3 substitution (see structure **2**, Fig. 1) and the 'southern' part of the 1,3,5-triazine-2,4,6-trione core (N-1).

The 1,3,5-triazine-2,4,6-trione core structure was synthesized using a one-pot condensation procedure as

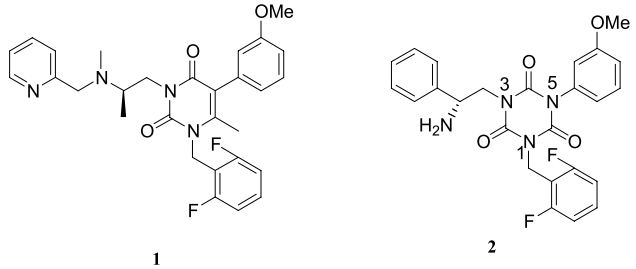

Figure 1. Structures of small molecule GnRH antagonists.

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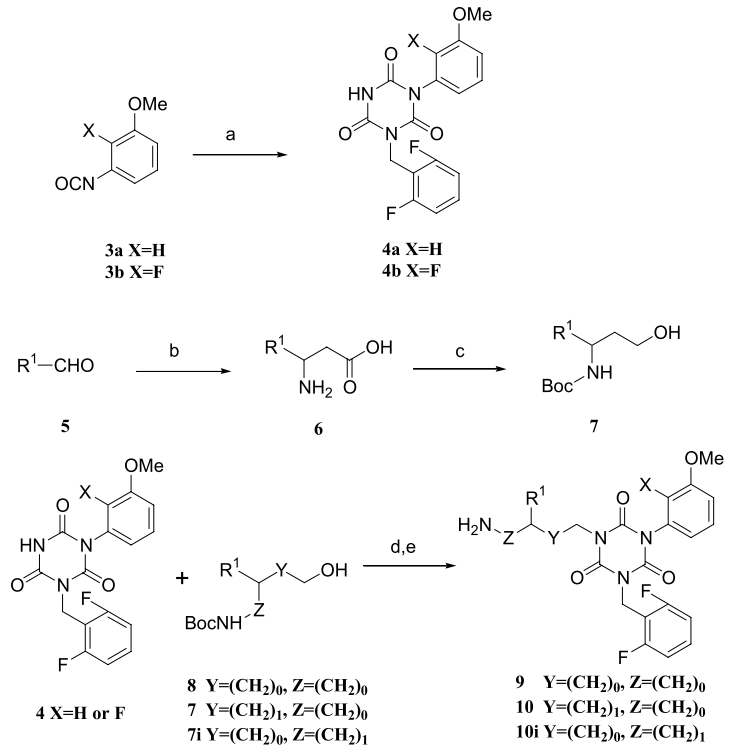
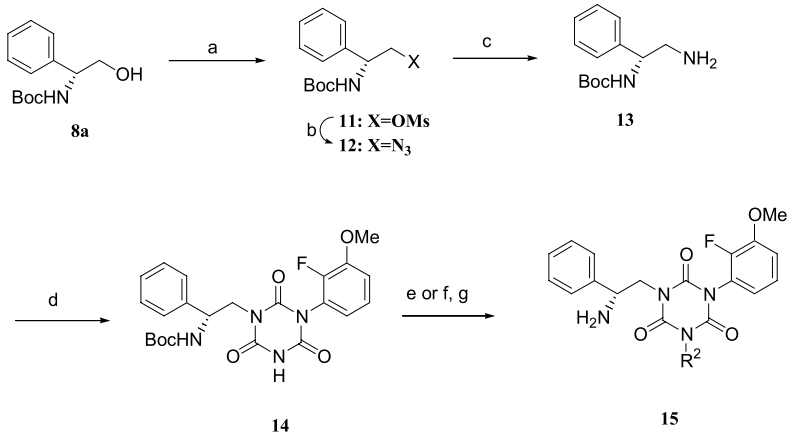
described in our previous paper.[11] In our effort to optimize this series of compounds, we first utilized readily available α- and β-amino alcohols to attach substituents at the 3-position via the Mitsunobu reaction, while keeping constant the 3-methoxyphenyl or 2-fluoro-3-methoxyphenyl group at the 5-position and the 2,6-difluorobenzyl group at the 1-position. Compounds **9** and **10** were synthesized according to the procedure depicted in Scheme 1. Thus, 3-methoxyphenyl isocyanate (**3a**) was treated with 1 equiv of 2,6-difluorobenzylamine in dichloromethane to form a urea intermediate, to which 1.5 equiv of chlorocarbonyl isocyanate was subsequently added to generate the disubstituted triazinetrione compound **4a** in 87% yield. Compound **4b** was prepared in a similar manner from 2-fluoro-3-methoxyphenyl isocyanate prepared from 2-fluoro-3-methoxybenzoic acid via a Curtis reaction.[12] Boc-protected α-amino alcohols that are unavailable from commercial sources were prepared from the corresponding α-amino acids by a lithium aluminum hydride (LAH) reduction, followed by Boc protection in a one-pot procedure. β-Amino acids **6** were readily prepared from aldehydes

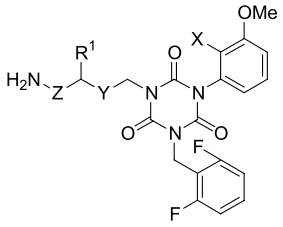


Scheme 1. Reagents and conditions: (a) (1) 2,6-difluorobenzylamine, dichloromethane, 2 h; (2) ClCONCO, DCM, rt, 12 h; (b) malonic acid, ammonium acetate, EtOH, reflux, 16 h; (c) LAH/THF, then Boc_2O/DCM; (d) PPh_3, di-*t*-butyl-azodicarboxylate, THF, 5 h; (e) TFA/CH_2Cl_2 (1:1), 1 h.



Scheme 2. Reagents and conditions: (a) MsCl, NEt_3, dichloromethane, 2 h; (b) NaN_3, DMF, 80 °C, 8 h; (c) $LiAlH_4$, THF, 10 h; (d) (1) 2-fluoro-3-methoxyphenyl isocyanate, dichloromethane, 2 h; (2) ClCONCO, DCM, room temperature, 12 h; (e) R^2OH, PPh_3, DEAD, THF, 5 h; (f) alkyl halide, K_2CO_3, DMF, 12 h; (g) TFA, dichloromethane, 1 h.

Table 1. Binding affinities of compounds **2**, **9**, and **10** on the hGnRH receptor[15]



9 Y=$(CH_2)_0$, Z=$(CH_2)_0$
10 Y=$(CH_2)_1$, Z=$(CH_2)_0$
10i Y=$(CH_2)_0$, Z=$(CH_2)_1$

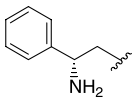
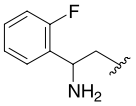
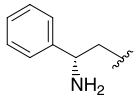
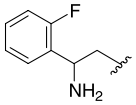
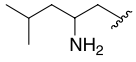
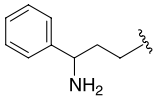
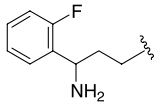
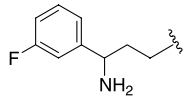
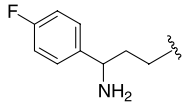
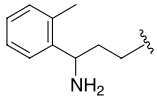
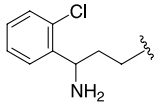
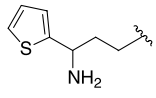
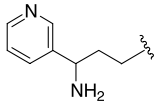
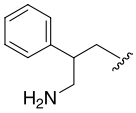
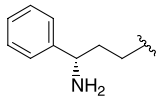
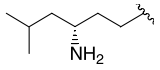
Compound	X	H_2N–Z–CH(R^1)–Y–CH_2–	Chirality	K_i (nM)
2	H		*R*	37
9a	H		*RS*	120
9b	F		*R*	9
9c	F		*RS*	20
9d	F		*RS*	620
10a	H		*RS*	70
10b	H		*RS*	800
10c	H		*RS*	66
10d	H		*RS*	83
10e	H		*RS*	75
10f	H		*RS*	180
10g	H		*RS*	27
10h	H		*RS*	580

Table 1 (*continued*)

Compound	X	H_2N–Z–CH(R^1)–Y–CH_2–	Chirality	K_i (nM)
10i	H		*RS*	28
10j	F		*S*	24
10k	F		*R*	1500

5, via reaction with malonic acid and ammonium acetate in refluxing ethanol in 40–80% yields.[13] These intermediates were converted to the β-amino alcohols **7** by LAH reduction, followed by Boc-protection in THF. These alcohols were then coupled with 1,3,5-triazine-2,4,6-triones (**4**) using Mitsunobu conditions, followed by deprotection of the Boc-group with TFA in dichloromethane (1:1, v/v) to afford the primary amines **9** and **10**, respectively, in overall 70–90% isolated yields.

We were also particularly interested in varying the substituent at the 1-position of the 1,3,5-triazine-2,4,6-triones. An alternative synthetic approach (Scheme 2) was developed to explore the SAR at this position while keeping the (*R*)-2-amino-2-phenylethyl group at the 3-position and the 2-fluoro-3-methoxyphenyl group at the 5-position constant. Thus, (*R*)-*N*-Boc-phenylglycinol **8a** was treated with methanesulfonyl chloride in dichloromethane, followed by replacement with sodium azide in DMF at 80 °C to yield the azido compound **12** in 82% yield. The azido intermediate was then reduced by lithium aluminum hydride in THF to give the Boc-protected diamino compound **13** in 96% yield. Subsequent urea formation with 2-fluoro-3-methoxyphenyl isocyanate, followed by a cyclization with chlorocarbonyl isocyanate, gave the triazine-trione **14** in 65% yield. Alkylation of **14** with alkyl halides in the presence of potassium carbonate in DMF, followed by Boc-deprotection, gave the corresponding tri-substituted triazine-triones **15**, which were isolated in 30–90% yields. Alkyl alcohols could also be coupled to this position under standard Mitusnobu conditions. Over 80 triazine-triones were prepared using the chemistry described and a wide variety of substitutions were explored. A selection of these compounds is listed in Table 2.

All synthesized compounds were evaluated for their ability to inhibit des-Gly^{10}[^{125}I-Tyr,[5] DLeu,[6] NMeLeu,[7] Pro^{10}-NEt]-GnRH radioligand binding to the cloned hGnRH receptor stably expressed in HEK293 cells using a 96-well filtration assay format as previously reported.[14]

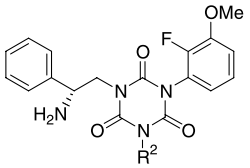
The binding affinities of **9** and **10** for the hGnRH receptor are summarized in Table 1. A 2-fluoro substituent at the 3-methoxy phenyl group increased binding affinity 4-fold, and **9b** was the most potent compound in this subseries (K_i = 9 nM). We speculate that the 2-fluoro group,

being slightly larger than a proton, could impede the free rotation of the 5-phenyl group and thus force the phenyl ring into a perpendicular conformation with respect to the triazine-trione core, which in turn, may be the preferred orientation for interaction with the GnRH receptor. Replacing the phenyl ring of the 3-side chain in **9b** with an isobutyl group resulted in significant loss of affinity as in **9d** (K_i = 620 nM). Analogs derived from β-amino alcohols **10** demonstrated interesting SAR. The 3-amino-3-phenylpropyl analog **10a** (racemic mixture) exhibited similar binding affinity as **2**. SAR on the phenyl ring of the 3-amino-3-phenyl-propyl group showed that the substituents and position of substitution had only limited impact on the binding affinity. As for the heteroaromatic derivatives, the slightly electron-rich thiophene compound has a K_i of 27 nM (**10g**), while the 3-pyridyl analog exhibited poor binding affinity (K_i = 580 nM). Interestingly, when the phenyl ring was moved away from the amino group, the corresponding compound **10i** was slightly more potent than **10a**. The fact that **2**, **10a**, and **10i** had similar binding affinities indicates that both the amino and phenyl groups have certain degree of freedom for optimal binding interaction with the receptor.

Results from the next study clearly indicate that the 'southern' substituent of the triazine-trione core **15** participates in a key binding interaction with the hGnRH receptor. Alkyl substituents on the core **15** as illustrated by **15a** (Table 2) resulted in compounds with low affinity for the receptor. For compounds containing a substituted phenyl ring, SAR studies indicate that an electron-deficient ring was preferred. Compound **15b** with an electron-donating 2-methoxy group was four times less potent than the 2-methyl analog (**15c**, K_i = 29 nM). Compound **15g** with the 2-(trifluoromethoxy)benzyl group was approximately 6-fold more potent than **15b**. Interestingly, the addition of a second substituent at position 6 of the 2-fluoro-benzyl ring further improved binding affinity (**15h**–**15j**). Compound **15j** proved to be the most potent 1,3,5-triazine-2,4,6-trione from this study (K_i = 2 nM). Introduction of a methyl group at the benzylic position (**15k**, K_i = 630 nM) resulted in significant loss of affinity, presumably due to increased steric hindrance which forces the 2,6-difluorobenzyl ring out of the optimal binding configuration. Moreover, bulky 2,6-disubstitutions on the benzyl ring were disfavored, thus, **15l** and **15m** had decreased binding affinity than **15d**. This result again indicates that the relative position of the benzyl ring is very important for receptor binding.

Functional activity of a few selected compounds for the hGnRH receptor was determined by inhibition of GnRH stimulated inositol phosphate accumulation in RBL cells.[16] The results shown in Table 3 indicate that they are all potent functional antagonists. For example, compound **15j** exhibited a K_i value of 2 nM on hGnRH receptor binding, and an IC_{50} value of 33 nM in the inositol phosphate accumulation assay. These compounds also displayed species difference in their binding to the GnRH receptors.[9] They exhibited reduced binding affinity on the monkey GnRH receptor and much lower

Table 2. Binding affinities of *N*-1-alkyl compounds **15** on the hGnRH receptor[15]



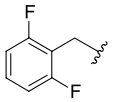
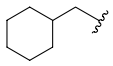
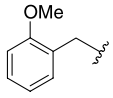
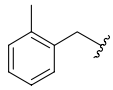
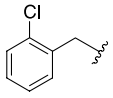
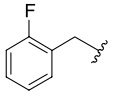
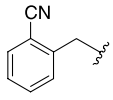
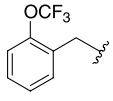
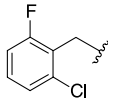
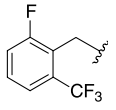
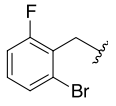
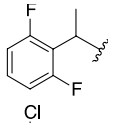
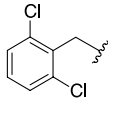
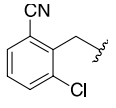
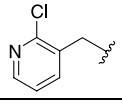
Compound	R^2	K_i (nM)
9b		9
15a		>10,000
15b		120
15c		29
15d		20
15e		11
15f		13
15g		18
15h		5
15i		3
15j		2
15k		630
15l		36
15m		73
15n		66

Table 3. Data from hGnRH receptor functional assay, binding affinities to the rats and monkey GnRH receptors, human liver microsomes (HLM) stability and CaCo-2 permeability determination for selected compounds **15**[15]

Compound	K_i (nM)			IC_{50} (nM)	Cl (HLM) (mL/min/kg)	Caco-2 permeability ($P_{app} \times 10^{-6}$, cm/s)
	Human	Monkey	Rat			
15f	13	86	13,000	190	249	25.6
15g	18	240	9,400	480	146	6.2
15j	2	24	2,300	33	158	13.9

affinity on the rat GnRH receptor. For example, compound **15j** exhibited 10- and 1000-fold lower binding affinity on the monkey and rat GnRH receptors, respectively (K_i = 24 nM and 2.3 μM), when compared to the hGnRH receptor (K_i = 2 nM).

Selected compounds from these novel triazine-triones were also assayed for cell permeability in Caco-2 cells and metabolic stability in human liver microsomes (HLM). The apparent permeability (P_{app}) of **15f**, **15g**, and **15j** was determined across Caco-2 cell monolayers in both the apical (AP) to basolateral (BL) and BL to AP directions. These three compounds demonstrated medium (**15g**, $P_{app\ a>b}$ 6.2×10^{-6} cm/s) to very good (**15f**, $P_{app\ a>b}$ 25.6×10^{-6} cm/s) P_{app} values in this assay. Incubation of **15g** and **15j** with HLM resulted in an intrinsic clearance of 146 and 158 mL/min/kg, respectively (Table 3). From this assay, these compounds proved to be much more metabolically stable than **1**.[9]

In conclusion, we have prepared a series of novel 1,3,5-triazine-2,4,6-triones as potent antagonists of the hGnRH receptor. SAR study at the left-hand side demonstrated that branched primary amines, such as the substituted 2-aminoethyl- or 3-aminopropyl groups were a key feature for high binding affinity. SAR study around the bottom of the 1,3,5-triazine-2,4,6-trione core led to the discovery of analogs with better binding affinity than the 2,6-difluorobenzyl compounds.

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15. On each assay plate a standard antagonist of comparable affinity to those being tested was included as a control for plate-to-plate variability. Overall, K_i values were highly reproducible with an average standard deviation of 45% for replicate K_i determinations. Key compounds were assayed in three to eight independent experiments.
16. Functional activity of the compounds for the hGnRH receptor was determined by inhibition of GnRH stimulated inositol phosphate accumulation. RBL cells stably expressing the full-length hGnRH receptor were plated onto 96 well plates (Corning) at 100,000 cells per well in 100 μL of inositol free medium (inositol free DMEM supplemented with 10 % dialyzed fetal bovine serum, 2 mM L-glutamine, 10 mM HEPES, 50 μg/mL penicillin/streptomycin, and 0.5 mg/ml G418) containing 1 μCi/mL of [^{3}H]myo-inositol and grown overnight. The following day, cells were washed twice with buffer I (140 mM NaCl, 4 mM KCl, 20 mM HEPES, 0.1% BSA, 8.3 mM D-glucose, 1 mM $MgCl_2$, and 1 mM $CaCl_2$, pH 7.4). The appropriate dilutions of antagonists were prepared in 4 nM GnRH diluted in buffer I containing 10 mM LiCl. Simultaneous agonist/antagonist stimulation was carried out in the presence of LiCl for 1 h at 37 °C. After stimulation, the medium was removed and 200 μL of 10 mM formic acid was added to each well and incubated at 4 °C for 30 min to extract the inositol phosphates from

the cells. The samples were transferred to 96-well Whatman GF/C Unifilter Microplates (Whatman) packed with 1:10 w/v AG-1X8 resin in dH_2O (Bio-Rad Laboratories). The plates were centrifuged at a speed of 1500 rpm for 3 min and the flow through was removed. The resins were washed once with 200 μL dH_2O and then once with 200 μL of 60 mM ammonium formate and 5 mM sodium tetraborate. IP, IP_2, and IP_3 were eluted with 50 μL of 1 M ammonium formate and 0.1 M formic acid into solid scintillant coated, 96-well microplates (Perkin-Elmer) and after drying the plates were counted in a TopCount NXT (Perkin-Elmer). IC_{50} values for the inhibition of GnRH stimulated inositol phosphate accumulation were calculated using the Prism software package (GraphPad Software) with a 'sigmoidal dose–response (variable slope)' option for curve fitting.