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Synthesis of aryl-1,2,4-triazine-3,5-diones as antagonists of the gonadotropin-releasing hormone receptor

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Abstract—Several efficient synthetic routes for 2-, 4-, and 6-aryl-1,2,4-triazine-3,5-diones were developed. Derivatives were synthesized and studied as gonadotropin-releasing hormone antagonists in an effort to understand structure–activity relationships of the monocyclic compounds.

In our efforts to identify orally active antagonists of the gonadotropin-releasing hormone receptor (GnRH-R) for possible treatment of sex hormone related diseases, such as endometriosis, uterine fibroids, and prostate cancer,[1] we have discovered a series of 6-methyluracils (6-methylpyrimidin-2,4-diones) exemplified by **1d** having low-nanomolar binding affinity.[2] We have also concluded that the 4-oxo moiety of the uracil ring is critical for high-affinity binding based on a large difference in potency between two regioisomers.[3] Based on an internal receptor modeling study, the oxo functionality is speculated to have a hydrogen-bonding interaction with the GnRH receptor. Another important feature of **1** is the 6-methyl group, which is optimal at this position for receptor binding. Thus, the non-methyl uracil **3a** (K_i = 5.3 nM; Fig. 1)[4] is about 10-fold less potent than **1d** (K_i = 0.56 nM), and its 6-ethyl analogue **3b** (K_i = 50 nM) is almost 100-fold less active than **1d**. Although the 5-(2-fluoro-3-methoxyphenyl) group in both **1d** and **3a** has an orthogonal conformation relative to the uracil core in their crystal structures, **1d** can exist as two atropisomers, as evidenced by two sets of signals in NMR spectra, while **3a** does not. This suggests that the rotation about the carbon–carbon bond connecting the 5-phenyl ring of **1d** is slower than that in **3a**. The different rotational barrier could contribute to the discrepancy in potency between **1d** and **3a**, and the bulky ethyl group could be the cause of the large loss of potency in **3b**. To further understand the role of the 6-methyl group, as well as the 2-oxo moiety of **1**, we synthesized several phenyl substituted 1,2,4-triazine-3,5-diones (6-azauracils) and studied them in the GnRH binding assay. Herein, we report the development of synthetic routes and structure–activity relationships of these compounds.

The synthesis of 2-aryl-6-methyl-1,2,4-triazine-3,5-dione, described in a patent publication, involves cyclization of 5,5-dimethyl-2-phenyl-[1,2,4]triazolidin-3-one with pyruvic acid.[5] To apply this reaction to the synthesis of the 6-benzyl analogue (Scheme 1), 2,6-difluorophenylpyruvic acid **9** was synthesized from the corresponding benzaldehyde **8** according to a known protocol.[6] Cyclization of **9** with 2-aryl-5,5-dimethyltriazolidinone under acidic conditions (concd H_2SO_4/dioxane/reflux) afforded the 6-(2,6-difluorobenzyl)-2-aryl-1,2,4-triazine-3,5-dione **10** in 32% yield. Alkylation of triazinedione **10** by the Mitsunobu reaction[7] with *N*-Boc protected phenylalaninol (**11**, PPh_3/DEAD/THF/rt), followed by *N*-Boc deprotection with trifluoroacetic acid, gave the final products **4** (43% for **4a**, 42% for **4b**). The preparation of 4-aryl-1,2,4-triazine-3,5-diones **5** started from the benzylpyruvic acid **9** (Scheme 2). Cyclization of **9** with *N*-aryl thiosemicarbazide[8] (prepared from aryl isothiocyanate and hydrazine) in refluxing hydrochloric acid afforded the 5-thioxo-1,2,4-triazin-3-one **12** in 93% yield, which was converted to the corresponding triazinedione **13** under basic conditions (KOH/MeOH/rt, then aq HCl, 86%). Mitsunobu reaction of **13** with phenylalaninol **11**

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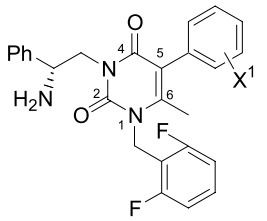
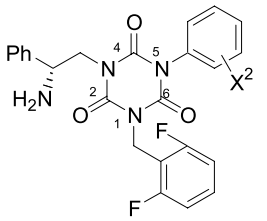
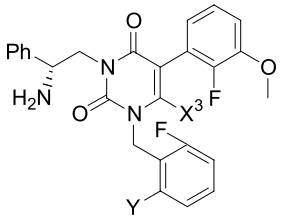


1a: X^1 = H, K_i = 15 nM;
1b: X^1 = 2-F, K_i = 5.6 nM
1c: X^1 = 3-MeO, K_i = 6.5 nM
1d: X^1 = 2-F,3-MeO, K_i = 0.56 nM

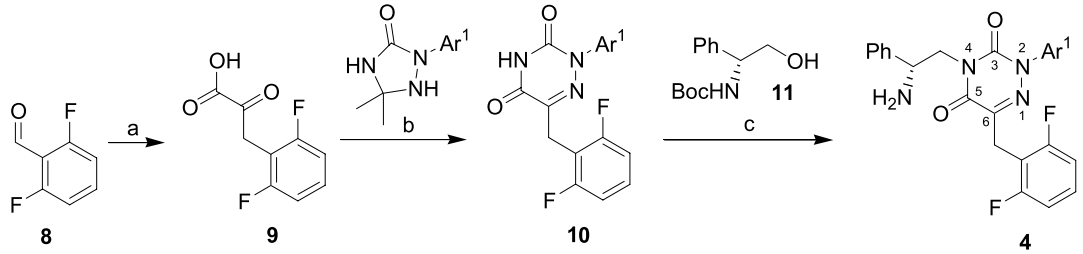


2a: X^2 = H, K_i = 1,100 nM;
2b: X^2 = 2-F, K_i = 89 nM
2c: X^2 = 3-MeO, K_i = 37 nM

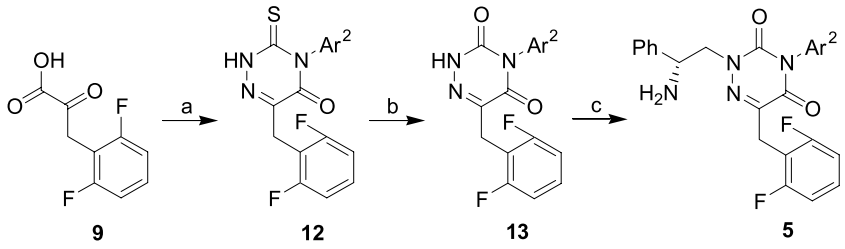


3a: X^3 = H, Y = F, K_i = 5.3 nM;
3b: X^3 = Et, Y = F, K_i = 50 nM
3c: X^3 = H, Y = CF_3, K_i = 0.64 nM

Figure 1. Some monocyclic GnRH antagonists.



Scheme 1. Reagents and conditions: (a) i. $AcNHCH_2COOH/NaOAc/Ac_2O$; ii. NaOH, then aq HCl, 59%; (b) H_2SO_4 (cat.)/dioxane/reflux, 16 h, 32%; (c) i. **11**/PPh_3/DEAD/THF/rt, 11 h; ii. TFA/CH_2Cl_2/rt, 0.5 h.



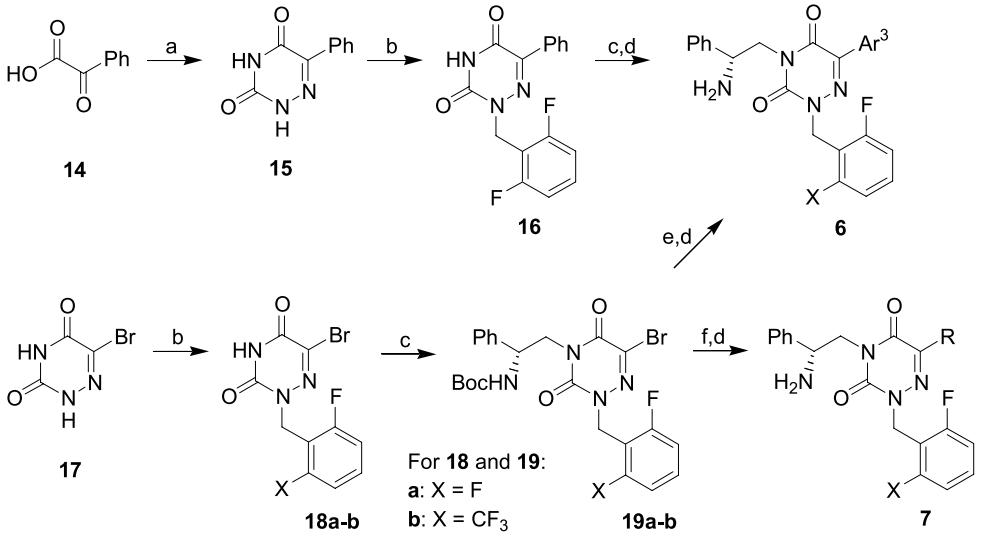
Scheme 2. Reagents and conditions: (a) $Ar^2NHC(=S)NHNH_2$/aq HCl/reflux, 16 h, 93%; (b) aq KOH/MeOH/rt, 0.5 h, then aq HCl, 86%; (c) i. **11**/PPh_3/DEAD/THF/rt, 16 h; ii. TFA/CH_2Cl_2/rt, 0.5 h.

successfully installed the side chain at the 2-position of the core, and the final products **5** were obtained after *N*-Boc deprotection with TFA (65% for **5a**, 52% for **5b**).

The 6-phenyl-1,2,4-triazine-3,5-dione (5-phenyl-6-aza-uracil) **15** was obtained from reaction of benzoylformic acid, which can be readily prepared by oxidation of acetophenone,[9] with semicarbazide to form a hydrazone, which was then cyclized under basic conditions at elevated temperature ($NaOEt/HOCH_2CH_2OH$/reflux, 75%).[10] Alkylation of **15** at the 2-position was accomplished using a known protocol with 2,6-difluorobenzyl bromide promoted by *N*,*O*-bis(trimethylsilyl)acetamide (MeCN/80 °C) to give **16** in 88% yield.[11] The aminoalkyl side chain was introduced via a Mitsunobu reaction (**11**/PPh_3/DEAD/THF/rt) to provide the products **6** after TFA deprotection (Scheme 3).

Alternatively, benzylation ($ArCH_2Br$/BSA/MeCN/80 °C) of the 6-bromo-1,2,4-triazine-3,5-dione **17**[12] afforded **18a**,**b**, which were alkylated with phenylalaninol under Mitsunobu conditions to provide the bromo compounds **19a**,**b**. Suzuki reaction[13] of **19a**,**b** with a variety of arylboronic acids [$Pd(PPh_3)_4/Ba(OH)_2$/DME/C_6H_6/EtOH/reflux] gave the final products **6** after deprotection. Replacement of the 6-bromo group of **19a** with a nucleophile such as phenol or alkylamine[14] (Py/EtOH/reflux) provided compounds **7** after *N*-Boc removal. All final compounds were purified and characterized by an HPLC–MS system.[15]

All of the synthesized compounds were tested in the GnRH binding assay as previously described.[2] In comparison with the *s*-triazinetriones **2**,[3] and uracils **3**,[4] or 6-methyluracils **1**,[2] the 2-phenyl-1,2,4-triazine-3,5-dione **4a** had a K_i of 2.3 μM, which was slightly less potent than the triazine analogue **2a** (K_i = 1.1 μM), but much less active than the 6-methyluracil **1a** (K_i = 15 nM). The 2-fluoro analogue **4b** (K_i = 580 nM), however, was much less potent than the corresponding triazine **2b** (K_i = 89 nM) and 6-methyluracil **1b** (K_i = 5.6 nM), suggesting the core structure of **4** possesses an unfavored



Scheme 3. Reagents and conditions: (a) i. $NH_2C(=O)NHNH_2$/EtOH/H_2O/rt, 16 h, 88%; ii. NaOEt/$(CH_2OH)_2$/reflux, 15 h, 85%; (b) BSA/MeCN/80 °C, 3 h; then (2,6-F)$C_6H_3CH_2Br$/80 °C, 15 h, 74–88%; (c) **11**/PPh_3/DEAD/THF/rt, 11 h; (d) TFA/CH_2Cl_2/rt, 0.5 h; (e) $Ar^3B(OH)_2$/$Pd(PPh_3)_4$/aq $Ba(OH)_2$/DME/C_6H_6/EtOH/90 °C, 5 h, 52% for **6s**; (f) RH/Py/EtOH/reflux, 15 h.

conformation for receptor binding. The binding affinity of the 4-phenyl-1,2,4-triazine-3,5-dione **5a** (K_i = 450 nM) was slightly better than that of the triazine **2a** and 2-phenyltriazinedione **4a**. Similarly, the 4-(3-methoxylphenyl)triazinedione **5b** (K_i = 110 nM) was only about 3-fold less potent than the triazine **2c** (K_i = 37 nM), although it was still much less active than the 6-methyluracil **1c**. The data summarized in Table 1 suggest that the 6-oxo moiety is more important for receptor binding than the 2-oxo moiety of the triazinetrione **2**. Apparently, both 2- and 4-aryl-1,2,4-triazine-3,5-diones are less desirable templates than either the triazinetriones **2** or 6-methyluracils **1** as GnRH antagonists.

Next, we conducted a more detailed study on a series of 1,2,4-triazine-3,5-diones with a 6-aryl ring (**6**, Table 2), since this template (5-aryl-6-azauracils) is structurally closest to the 5-aryluracils **3.** Further, the described synthesis allowed a rapid survey of substitution effects at the 6-position. The 6-phenyl triazinedione **6a** (K_i = 1.6 μM) possessed binding affinity similar to the 2-phenyl analogue **4a.** As an orthogonal relation between the 6-phenyl ring and the 1,2,4-triazine-3,5-dione core is expected to be critical for high affinity, we surveyed a series of ortho-substituted phenyl and bicyclic aromatic analogues. 2-Chloro (**6b**) or 2,4-dichloro (**6c**) substitution had minimal effect on potency, despite the fact that incorporation of a 2-chloro in the 5-phenyl of uracil **3** increases potency 6-fold.[4] The 2-methyl substitution (**6d**), including the 2,3- or 2,5-dimethyl analogues (**6e**,**f**), also had little impact on the binding. The more bulky 2,4,6-trimethyl (**6g**, K_i = 11 μM), ethyl (**6h**, K_i = 8.4 μM), and methoxy derivatives (**6k**, K_i = 8.7 μM) also displayed reduced potency.

The basic dimethylaminomethyl **6i** was inactive, and the strongly electron-withdrawing trifluoromethyl compound (**6j**, K_i = 1.3 μM) possessed an almost identical K_i value to the 2-methyl analogue **6d**, suggesting no clear

Table 1. Binding affinity of 2- and 4-aryl-1,2,4-triazine-3,5-diones **4** and **5**

Compound	Ar^1 or Ar^2	K_i (nM)
4a	Ph–	2300
4b	2-FC_6H_4–	580
5a	Ph–	450
5b	3-$MeOC_6H_4$–	110

Table 2. SAR of 6-substituted 1,2,4-triazine-3,5-diones **6** and **7** at GnRH-R

Compound	X	Ar^3 or R	K_i (nM)
6a	F	Ph–	1600
6b	F	2-ClC_6H_4–	850
6c	F	2,4-$Cl_2C_6H_3$–	2600
6d	F	2-MeC_6H_4–	1200
6e	F	2,3-$Me_2C_6H_3$–	1000
6f	F	2,5-$Me_2C_6H_3$–	4700
6g	F	2,4,6-$Me_3C_6H_2$–	11,000
6h	F	2-EtC_6H_4–	8400
6i	F	2-$(Me_2NCH_2)C_6H_4$–	>10,000
6j	F	2-$CF_3C_6H_4$–	1300
6k	F	2-$MeOC_6H_4$–	8700
6l	F	2,4-$MeO_2C_6H_3$–	5200
6m	F	2-MeO-5-FC_6H_3–	>10,000
6n	F	2-MeO-5-ClC_6H_3–	>10,000
6o	F	2-MeO-5-MeC_6H_3–	>10,000
6p	F	2,5-$MeO_2C_6H_3$–	>10,000
6q	F	2-MeO-5-iPrC_6H_3–	>10,000
6r	F	2-$EtOC_6H_4$–	>10,000
6s	F	2-F-3-$MeOC_6H_3$–	92
6t	F	8-Quinolinyl–	>10,000
6u	F	1-Naphthyl–	4300
6v	F	3-Benzothienyl–	>10,000
6w	CF_3	2-F-3-$MeOC_6H_3$–	13
6x	CF_3	2-Cl-3-$MeOC_6H_3$–	2.3
7a	F	PhO–	>10,000
7b	F	PhS–	>10,000
7c	F	1-Morpholinyl–	>10,000

electronic preference for this phenyl ring. Any 2-methoxyphenyl compound with an additional substituent on the phenyl ring (**6m**–**6g**) showed no competitive binding, except the 2,4-dimethoxy analogue **6l** (K_i = 5.2 μM). The ethoxy **6r**, as well as the 8-quinolinyl **6t** and the 3-benzothienyl compound **6v**, also displayed no binding activity, while the 1-naphthyl derivative exhibited poor affinity (**6u**, K_i = 4.3 μM). As predicted, the 2-fluoro-3-methoxyphenyl compound **6s** (K_i = 92 nM) displayed an almost 20-fold increase in binding affinity over the phenyl derivative **6a**. Although these results are parallel to those of the 6-methyluracils **1a** (K_i = 15 nM) and **1d** (K_i = 0.56 nM), the 6-azauracil **6s** was about 17-fold less potent than its uracil analogue **3a**. Thus, replacement of the CH moiety of **3a** with the smaller N atom at the 6-position reduced binding affinity.

Previously, we have shown in the non-methyl uracils that a 2-fluoro-6-trifluoromethylbenzyl group at the 1-position (**3c**, K_i = 0.64 nM) possesses about 9-fold increased affinity over the corresponding 2,6-difluorobenzyl analogue (**3a**, K_i = 5.3 nM).[4] In the azauracil series, the 2-fluoro-6-trifluoromethylbenzyl analogue **6w** (K_i = 13 nM) was about 7-fold better than the 2,6-difluorobenzyl analogue **6s**. Interestingly, the 2-chloro-3-methoxyphenyl compound **6x** (K_i = 2.3 nM) showed another 6-fold binding affinity increase over the corresponding 2-fluoro analogue **6w**. These results may suggest that the 3-methoxy group of the 6-phenyl ring in **6** plays a much more important role in receptor interaction than that in **3a** or **3c**. On the basis of our receptor modeling, the counterpart for this interaction could be Asn-212 on transmembrane domain 5 of the human GnRH receptor. This residue is known to be important for hydrogen-bonding interaction with the pyro-glutamine at the 1-position of the GnRH peptide.[16,17]

Finally, 1,2,4-triazine-3,5-diones with 6-phenoxy (**7a**), phenylsulfinyl (**7b**), and 1-morpholinyl group (**7c**) were tested but these compounds showed no affinity in the competitive binding, suggesting that the 6-aryl group of **6** is important for receptor interaction.

In conclusion, several synthetic routes for 2-, 4-, and 6-aryl-1,2,4-triazine-3,5-diones (1-, 3-, and 5-aryl-6-azauracils) were developed for structure–activity relationship study of the monocyclic GnRH antagonists. The successful Mitsunobu reactions enabled us to install a favored (2*R*)-aminophenethyl side chain at both the 2- and 4-positions of the triazinediones. The results from these SAR studies provide further evidence for the role of the 6-methyl group in the uracil **1**, which is important for invoking a conformation that positions two key elements, the 5-phenyl and 1-benzyl groups, for favored interactions with the receptor. **6x** (K_i = 2.3 nM) was identified as a potent GnRH antagonist from this series. The improved potency of this compound was possibly due to an increase in binding interactions of the 6-(2-chloro-3-methoxyphenyl) group with amino acid residues such as Asp-122 on the human GnRH receptor.

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