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Synthesis and Structure–Activity Relationships of Thieno[2,3-*d*]pyrimidine-2,4-dione Derivatives as Potent GnRH Receptor Antagonists

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Abstract—The synthesis and SAR studies of thieno[2,3-*d*]pyrimidine-2,4-diones as human GnRH receptor antagonists to treat reproductive diseases are discussed. It was found that the 2-(2-pyridyl)ethyl group on the 5-aminomethyl functionality of the core structure was a key feature for good receptor binding activity. SAR study of the 6-(4-aminophenyl) group suggests that hydrophobic substituents were preferred. The best compound from this series had binding affinity (K_i) of 0.4 nM to the human GnRH receptor.

Gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone-releasing hormone (LHRH), is a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2), which is produced and secreted by the hypothalamus in a pulsatile manner.[1,2] It acts on the pituitary gland to stimulate the secretion of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These gonadotropines, in turn, act on the reproductive organs, where they participate in the regulation of gonadal steroid production, spermatogenesis in males and follicular development in females. Several reproductive disease conditions such as endometriosis, uterine fibroids and prostate cancer are caused by over stimulation of the reproductive organs by the gonadal steroids, and thus can be treated by suppression of the pituitary–gonad hormonal axis. Currently depot forms of peptidic GnRH agonists, represented by leuprorelin® are used to treat such conditions through a receptor down-regulation mechanism to suppress gonadal steroid production.[3] However, recent clinical evidence has shown that peptidic GnRH antagonists can act immediately at the receptor to lower steroid levels and therefore alleviate disease symptoms without the concomitant 'flare effect', which is exhibited by the peptide agonists due to their initial overstimulation of the GnRH receptor.[4] In the clinic, it has been shown that peptidic GnRH antagonists can directly lower gonadal sex hormone levels to alleviate disease symptoms without the concomitant flare effect. Nevertheless, whether the patient is treated with the peptide agonists or antagonists, both regimens still require parenteral administration, typically in depot form 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. In response to this need, intensive efforts have been initiated in the development of small molecule GnRH antagonists by many laboratories. Compound **1** (T-98475) and its analogues are the first small molecules reported[5] to have high affinity on the human GnRH receptor (IC_{50} 0.2 nM) albeit with reduced binding affinity for the rat receptor (IC_{50} 60 nM).[5] In addition, a series of papers on quinolones and tryptamines as potent GnRH antagonists were recently published. For example, quinolone **2** possesses high binding affinity (IC_{50} 0.4 nM) for the human GnRH receptor as well as the rat GnRH receptor (IC_{50} 4 nM).[6] Indole derivatives such as **3** were reported to be potent and orally bioavailable

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GnRH antagonists.[7] Most recently, TAK-013 (IC_{50} 0.2 nM) and its analogues were reported as potent and orally active hGnRH antagonists.[8]

We have previously described the identification of 7-phenylpyrrolo[1,2-*a*]pyrimid-4-ones **5** and **6**[9] (Fig. 1), and more recently, 2-phenylimidazolo[1,2-*a*]pyrimidines **7**[10] as potent small molecule GnRH receptor antagonists. SAR studies on these series showed that a basic side-chain is very important for strong receptor-binding activity and the phenyl ring is a good substitution on the right side of the molecules. In our efforts to produce orally active small molecules with good overall PK profile, we were interested in understanding the SAR on other bicyclic series as well. In this letter we wish to report the synthesis and SAR study of thieno[2,3-*d*]pyrimidine-2,4-dione compounds as potent small molecule GnRH antagonists.

Although TAK-013 is a highly potent GnRH antagonist, it is highly lipophilic with high molecular weight, and almost insoluble in water. A quick calculation with ACD software[11] suggests the logD of this compound is about 6. The measured logD value is >4 due to high partition in octanol. In this paper we report our results of screening polar side chain in the efforts to discover more water-soluble analogues of TAK-013. Based on our early success on the 2-pyridylethylamine side chain for several potent classes of GnRH antagonist, we also like to utilize it to replace the *N*-benzyl-*N*-methylamino side chain. The slightly basic pyridine ring should help to reduce the lipophilicity therefore increase water solubility (the pK_a value of 2-picoline is 5.94).[12]

The thieno[2,3-*d*]pyrimidine-2,4-dione core was synthesized according to a procedure described in Scheme 1. 4-Nitrophenylacetone (**8**) and ethyl cyanoacetate were treated with sulfur in ethanol to form 2-amino-4-methyl-5-(4-nitrophenyl)thiophene-3-carboxylic acid ethyl ester **9**.[13] Next, **9** and phenylisocyanate were heated with pyridine in toluene at 100 °C for 4 h to form a urea intermediate, which was treated with NaOMe/MeOH in refluxing acetonitrile to give 3-phenyl-thienopyrimidine-2,4-dione **10**.[14] **10** was alkylated at N1 position by 2,6-difluorobenzylbromide with K_2CO_3 in DMF to give **11**.[15] The 5-methyl group of **11** was then brominated with NBS/AIBN in carbon tetrachloride to yield **12**. The resulting bromomethyl compound **12** was treated with *N*-methylbenzylamine in acetonitrile to give compounds **13a** as the desired products (Scheme 1).

To functionalize the nitro group on the 6-(4-nitrophenyl) moiety for additional modification, 5-(*N*-benzyl-*N*-methylaminomethyl)-1-(2,6-difluorobenzyl)-6-(4-nitrophenyl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4-dione (**13a**) was hydrogenated with Pd-C in ethanol to yield the 6-(4-aminophenyl) analogue **14** (Scheme 2). The aniline (**14**) was treated under reductive alkylation conditions with aldehydes (RCHO) in dichloroethane, followed by treatment with borane-pyridine, to give the desired compounds **15**. For reductive alkylation with the more hindered substrate, acetone, $NaBH(OAc)_3$ was used as the reducing reagent. When R^3 was a methyl group, the second *N*-methylation was achieved by reductive alkylation of **15a** with another molecule of formaldehyde to afford the final product **16**. Amide formation of **14** was performed by treatment with acetic anhydride or

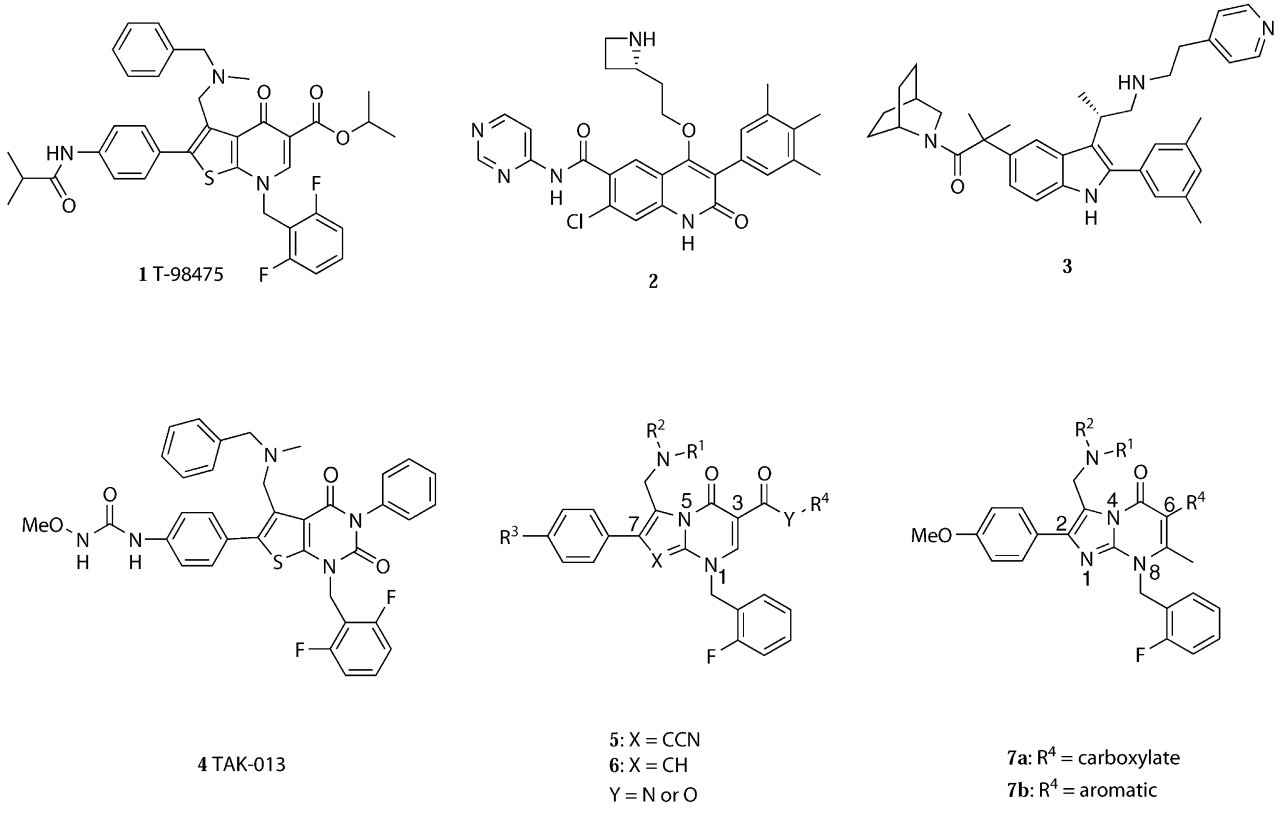
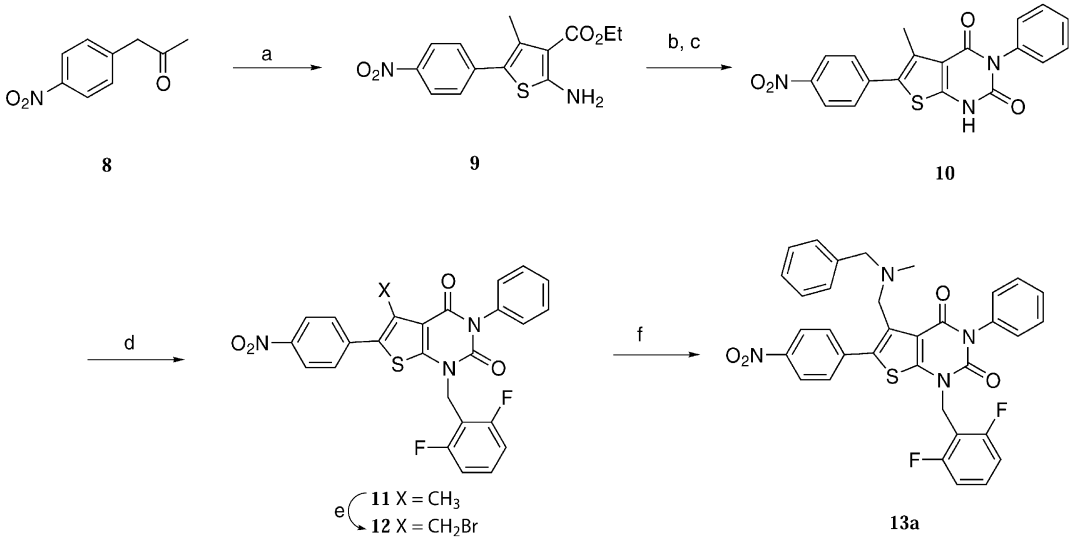


Figure 1. Small molecule GnRH antagonists.

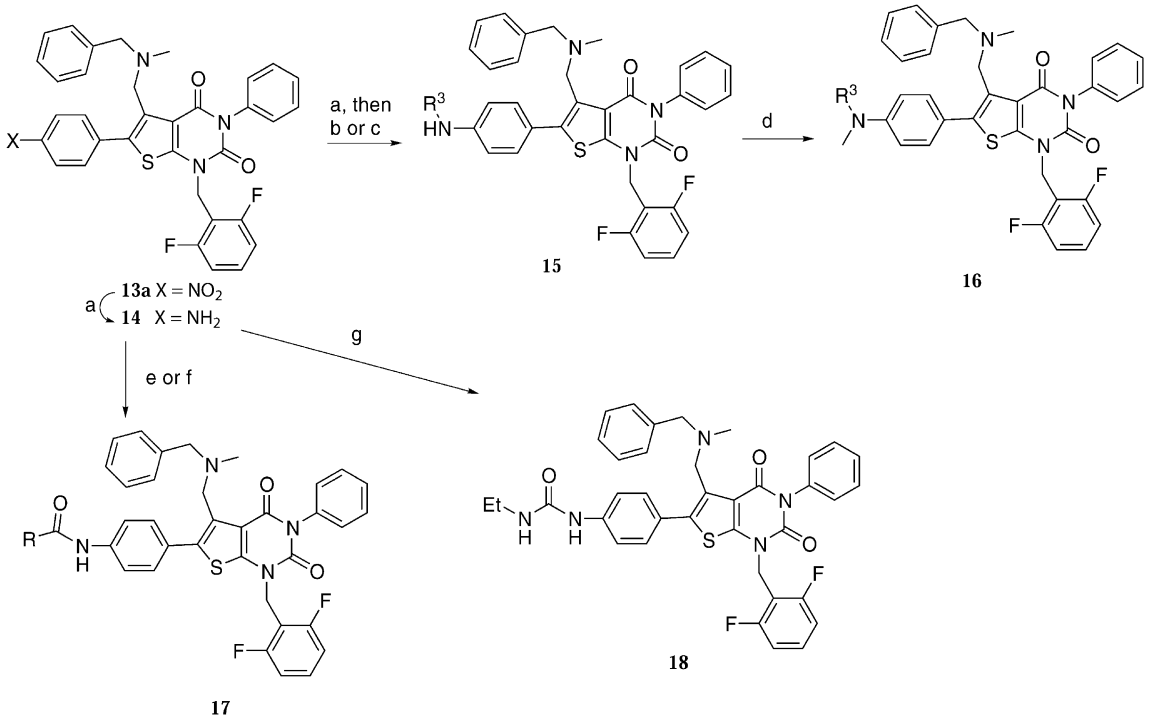
trifluoroacetic anhydride to yield **17a** and **17b**. Alternatively, amides **17c–d** were prepared from the corresponding acid chlorides with diisopropylethylamine in dichloromethane. Urea functionality was introduced into the molecule by treatment of **14** with ethyl isocyanate to yield **18**. (Scheme 2) To introduce an extra basic amino group into the core structure, aniline **14** was coupled to *N*-Boc-protected amino acids using HBTU as the coupling reagent in DMF. Deprotection of the amino-Boc group with TFA yielded the desired products **19–20** (Scheme 3).

To explore the SARs on the 5-methyl position of the thieno[2,3-*d*]pyrimidine-2,4-dione core structure, the 5-methyl group of **11** was then brominated; and the resulting bromomethyl compound **12** was treated with a variety of amines (R_1R_2NH) in acetonitrile to give compounds **13(b–i)** as the desired products (Scheme 4).

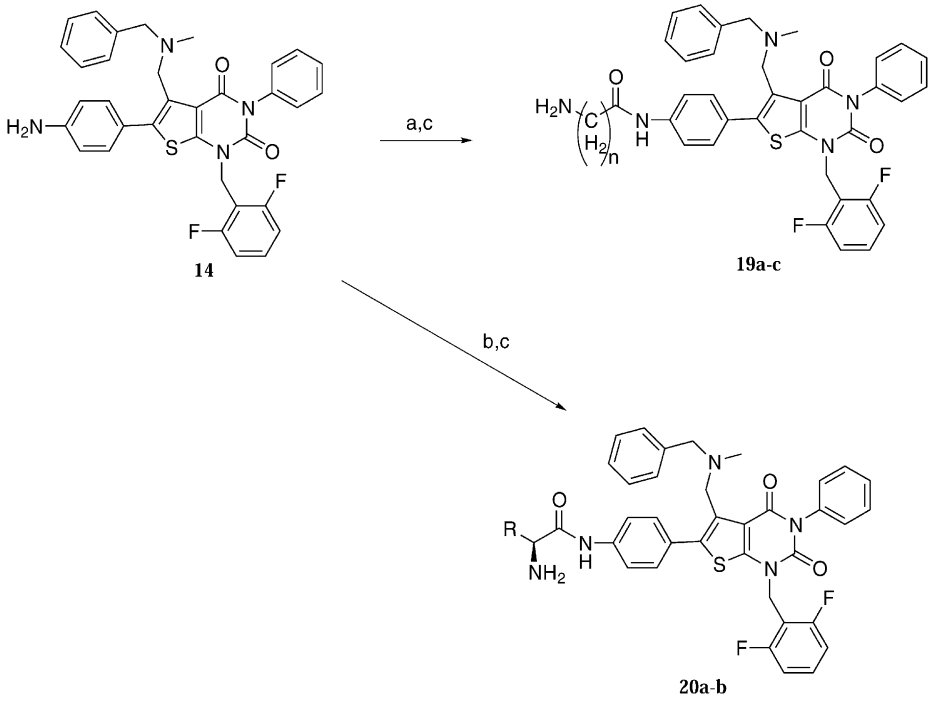
All synthesized compounds were evaluated for their ability to compete for des-Gly^{10}[^{125}I-Tyr^5,DLeu^6,NMe-Leu^7,Pro^9-NEt]-GnRH radioligand binding to the



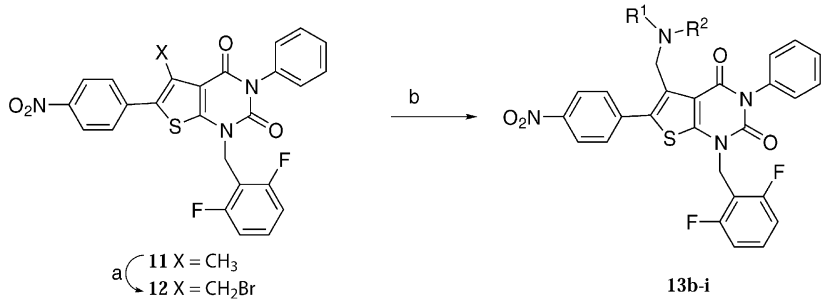
Scheme 1. Reagents and conditions: (a) $NCCH_2COOEt$, S, $BuNH_2$, EtOH; (b) PhNCO, pyridine, toluene; (c) NaOMe, MeOH, ACN; (d) 2,6-difluorobenzyl bromide, K_2CO_3, DMF; (e) NBS, AIBN, CCl_4; (f) $PhCH_2NHMe$, ACN.



Scheme 2. Reagents and conditions: (a) H_2, Pd/C, EtOH; (b) RCHO, BH_3/pyridine, dichloroethane; (c) acetone, $NaBH(OAc)_3$, dichloroethane; (d) CH_2O, BH_3/pyridine, dichloroethane; (e) $(RCO)_2O$, iPr$_2$EtNH, CH_2Cl_2; (f) RCOCl, iPr$_2$EtNH, CH_2Cl_2; (g) EtNCO, iPr$_2$EtNH, CH_2Cl_2.



Scheme 3. Reagents and conditions: (a) BocNH$(CH_2)_n$COOH, $n=1$–3, HBTU, NEt_3, DMF; (b) *N*-*t*-Boc-L-alanine or *N*-*t*-Boc-L-leucine, HBTU, NEt_3, DMF; (c) TFA/CH_2Cl_2 (1:1).



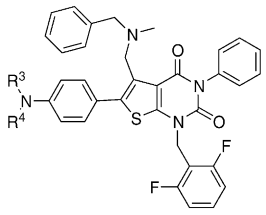
Scheme 4. Reagents and conditions: (a) NBS, AIBN, CCl_4; (b) R^1R^2NH, ACN.

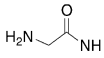
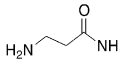
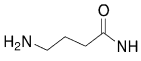
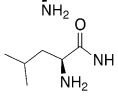
cloned human GnRH receptor stably expressed in HEK293 cells using a 96-well filtration assay format.[16]

The impact of the substitutions on the 6-(4-aminophenyl) group was examined and receptor binding data for these compounds **14**–**20** are reported in Table 1. Reduction of the nitro group in **13a** to the amino analogue **14** improved the binding potency by 5-fold. Alkylation of the amino group with small alkyl groups, such as ethyl **15b** slightly increased its affinity. However, incorporation of dimethyl (**16**) or isopropyl group (**15d**) on the nitrogen decreased the affinity. Interestingly, with amide bond linkage, **17** showed good potency. Thus, acetamide **17a** was 9 times better than **14**, although the trifluoroacetyl analogue **17b** was less active. These data suggest that the NH might be involved in hydrogen bonding with the GnRH receptor. Propionyl amide **17c** was the most potent compound in this class. Not surprisingly, the ethyl urea **18** showed the best potency in this modification. All these results agree with the hypothesis that this aniline hydrogen plays an important role as a hydrogen-bond donor in hydrogen bonding with the GnRH receptor. Base on the receptor modeling proposed by Takeda scientists, the counterpart of this hydrogen bonding on the receptor is Asn102 on top of the helix II.[4,8] Interestingly there is an aspartic acid residue Asp98 one-turn below Asn102, which may interact with any basic group from the small molecule. Based on this theory we attempted to introduce a basic function group to improve the physicochemical properties of these compounds. Thus, coupling of various amino acids with the aniline **14** resulted in compounds **19a**–**c** and **20a**–**b**. Surprisingly, all these amides were much less active than those that did not bear a basic group, although compounds **20a**–**b** still showed modest activity (43 and 58 nM, respectively).

The effect of substituents on the aminomethyl group at the 5-position of **13** on hGnRH receptor binding affinity are summarized in Table 2. Although TAK-013 is a highly potent GnRH antagonist, it may suffer from high lipophilicity due to low pK_a of the benzylamine (calculated value for logP and pK_a were 6.5 and 5.0, respectively). One of our objectives for this study is to improve physicochemical properties of this series of compounds by substituting the lipophilic *N*-benzyl moiety with a

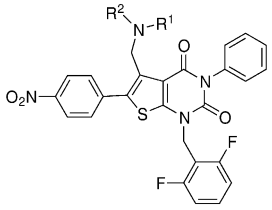
Table 1. Binding affinities of compounds **14**–**20** on the hGnRH receptor[17]

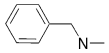
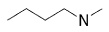
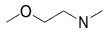
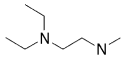
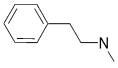
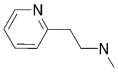
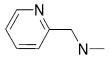


Compd	R^3NR^4	K_i (nM)
14	NH_2	36
15b		11
15c		17
15d		88
16		61
17a		4.1
17b		16
17c		0.8
17d		2.9
18		0.4
19a		150
19b		360
19c		300
20a		43
20b		58

smaller or more polar group. Replacement of the benzyl group of **13a** with a butyl group made no obvious difference in affinity while the dimethylamino analogue was over 3-fold less active. As expected, introduction of a polar methoxy or diethylamino substituent at the β-position from the nitrogen (**13d** and **13e)** slightly improved binding affinity, although the cyclic 1-methylpiperidine **13f** was less active. When the benzyl moiety was replaced with a 2-pyridylmethyl group, compound **13i** was 20-fold better in binding affinity ($K_i = 9$ nM). Even more, when a 2-(2-pyridyl)ethyl was used, the resulting compound **13h** showed subnanomolar binding affinity ($K_i = 0.6$ nM), which is 300-fold better than **13a**.

Table 2. Binding affinities of compounds **13a**–**i** on the hGnRH receptor[17]



Compd	R^1R^2N	K_i (nM)
13a		180
13b		640
13c		270
13d		170
13e		76
13f		460
13g		170
13h		0.6
13i		9

By contrast, the phenethyl analogue **13g** had similar activity as **13a**.

One explanation for these results is that the substituted methylamine had interaction with Asp302 on top of Helix VII of the GnRH receptor.[4,8] The extra heteroatom O and N offered hydrogen-bonding capability with this residue, and thus improved the interaction with the receptor. In addition, the pyridine ring may mimic the phenyl group, which may interact with the receptor through π–π stacking. The residue for this interaction has not been identified from receptor modeling.

Although the incorporation of a weak basic moiety on the left side of the molecules resulted in loss of binding affinity, the replacement of the *N*-benzyl moiety with the 2-pyridylethyl group did increase 300-fold binding affinity on the GnRH receptor. While the pyridine derivative **13h** ($K_i = 0.6$ nM) exhibited comparable activity with TAK-013, it was less lipophilic than TAK-013 (the calculated logD value is 4.81, which is one log unit lower than TAK-013, and the measured value is 3.3). This discovery also enables the possibility of further modification towards more water-soluble analogues of TAK-013.[18]

In conclusion, a series of 1-(2,6-difluorobenzyl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4-dione compounds were discovered to have excellent binding affinity to the human GnRH receptor. Hydrophobic substituents on the aniline nitrogen of the 6-(4-aminophenyl)-thieno[2,3-*d*]pyrimidine-2,4-dione core were preferred. Simple alkyl amides and ureas on the 6-(4-aminophenyl) moiety were very potent human GnRH receptor antagonists, whereas extra basic amines were less favorable. The results of this SAR study suggest that the 2-(2-pyridyl)ethyl group on the 5-aminomethyl functionality of the thieno[2,3-*d*]pyrimidine-2,4-dione core was optimal for the human GnRH receptor binding. This phenomenon may be explained by a predicted receptor model, in which the pyridyl side chain is in close proximity to the aspartic acid-302 on helix VII. The potent compounds discovered from this series may also have favorable physicochemical properties as potential orally active drug candidates.

Acknowledgements

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17. 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 3–8 independent experiments.
18. One can envision that replacing the *N*-benzyl chain of **20a** with the 2-pyridylethyl group will result in compound with good binding affinity as well as good water solubility.