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Small molecule antagonists of the gonadotropin-releasing hormone (GnRH) receptor: Structure–activity relationships of small heterocyclic groups appended to the 2-phenyl-4-piperazinyl-benzimidazole template

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ABSTRACT

A previous report described the serum LH suppression pharmacology of the 2-phenyl-4-piperazinyl-benzimidazole *N*-ethyluracil GnRH receptor antagonist **1** following oral administration in rats. A series of small heterocycles were appended to the 2-(4-*tert*-butylphenyl)-4-piperazinyl-benzimidazole template in place of the *N*-ethyluracil. Two imidazole analogues, **32** and **41**, were shown to possess substantial in vitro potency at the target receptor (hGnRH IC_{50} = 7 and 18 nM, respectively) and aqueous solubility (55 and 100 μg/mL at pH 7.4, respectively). Both compounds had high oral bioavailability in rats and **32** was further examined in an orchidectomized rat model for serum LH suppression based on increased volume of distribution over **41**. Serum LH levels trended lower in orchidectomized rats following oral administration of **32**.

Antagonists of the gonadotropin releasing hormone (GnRH) Receptor have shown positive clinical results in numerous reproductive tissue disorders, such as endometriosis and prostate cancer, due to their ability to inhibit the release of the gonadotropins leuteinizing hormone (LH) and follicle stimulating hormone (FSH).[1] Abarelix and Cetrorelix are peptide antagonists of GnRH currently sold in injectable form due to their poor pharmacokinetic properties and low bioavailability when taken orally.[2] Abarelix is also administered in extended release formulations which lower sex hormones to castration levels in patients, triggering numerous mechanistic side effects. An orally active small molecule GnRH antagonist could offer benefits such as improved compliance, the ability to titrate drug and hormone levels, as well as the flexibility to withdraw the drug relatively quickly when adverse symptoms are seen. Numerous small molecule GnRH antagonists have been reported in the literature.[3] Many of these examples have poor bioavailability and exhibit species selectivity precluding the use of simple in vivo models such as the rat.

We recently reported a potent small molecule antagonist of the GnRH receptor (**1**, Fig. 1) with a 2-phenyl-4-(1-piperazinyl)benzimidazole template that displayed excellent oral bioavailability and elicited plasma suppression of LH after oral administration in rats.[4] However, compound **1** has poor solubility, CYP 3A4 inhibition and poor liver microsome stability (Fig. 1) which were expected to be significant liabilities in drug development. As a result, we set out to mitigate the molecular property issues via structural modification. As shown earlier,[4] the heterocycle linked to the piperazine via a methylene can withstand significant structural variability and retain biological activity. We chose empirical modification of this portion of the molecule with the intention of retaining human and rat GnRH activity, then improving pharmaceutical properties on a suitable lead molecule. In addition, small heterocycles were particularly attractive since molecular weight would not deviate significantly from the lead and may even be reduced, which is an attractive feature for enhancing the pharmaceutical profile.[5]

In an attempt to quickly determine GnRH receptor affinity for new analogues, a diverse array of commercially available heterocyclic aldehydes was reacted in parallel with the 2-phenyl-4-piperazinyl-benzimidazole template **2** under reductive amination conditions (Scheme 1). As shown in Table 1, simple thiophenes, furans, pyrroles and a 2-thiazole analogue showed only weak affinity (Table 1, entries **3**–**10**) for the receptor at 50 nM. The most potent compounds were found to be the 4-imidazoles (**11**–**13**) with substantial binding affinity for the human GnRH receptor. The sole example of a weakly active imidazole analogue in our initial set was the 2-imidazole **14**. Affinity for the rat GnRH receptor, which

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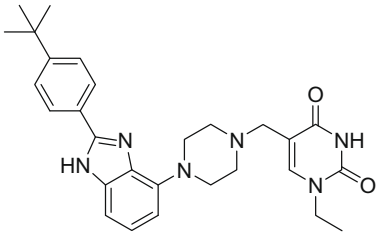
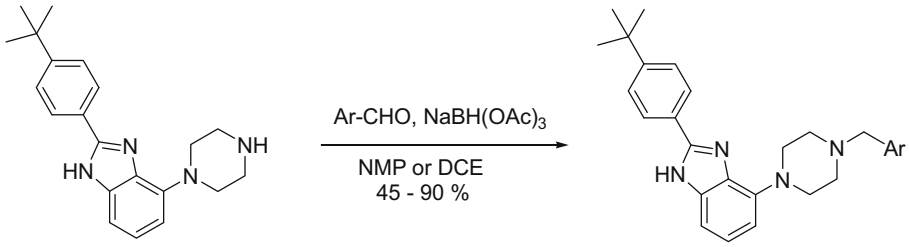


1

hGnRH Binding IC_{50} (nM)	1.7
rGnRH Binding IC_{50} (nM)	18
CYP 3A4 inhib. @ 3 μM (%)	74 (<20)
Solubility @ pH 7.4 (μg/ mL)	11 (>100)
rat liver microsome stability (% remaining after 15 mins @ 1.0 μM)	12 (>80)

Figure 1. Structure, binding data and in vitro pharmaceutical profiling data (with ideal values in parentheses) for an orally active GnRH antagonist lead.



2 **3 - 45**

Scheme 1. Preparation of GnRH antagonists.

was necessary for the evaluation of compounds in early in vivo models, was also substantial in the 4-imidazoles (IC_{50} = 28–290 nM for **11**–**13**, respectively). From this data it was clear that a nitrogen in the heterocyclic ring was required but not necessarily sufficient for affinity to both rat and human receptors.

Further investigation of the SAR surrounding the pendant heterocycle included substituted azoles. A series of alkylated 5-substituted oxazoles showed an increase in GnRH affinity with the introduction of a 2-alkyl group (**15** vs **16**, **17**). Alkylated 4-substituted oxazoles (**18**) were equipotent with the 5-substituted version (**17**). Thiazole compounds substituted in the 5 position were in the same range of activity as the oxazole analogs (**19** vs. **16**). Increasing the 4-alkyl chain length from methyl to ethyl (**19**, **21**) caused a loss in activity, while the 2 position tolerated the change to ethyl (**19**, **20**). A 2-*n*-butyl group in this position (**22**) also caused a loss of activity. In general, oxazole and thiazole affinity for the rat receptor was 10–30 times weaker than it was for the human protein (Table 2). This level of potency was too modest for in vivo consideration so attention was turned to pyrazoles and, as suggested by the data in Table 1, imidazoles as well.

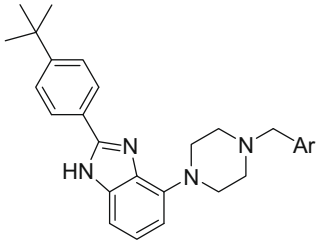
Pyrazoles were more sensitive to ring substitution than the oxazoles and thiazoles. N1-Methyl analogs substituted at the 5-position (Table 3, **25** and **26**) gave the most potent compounds in this series. As in the case of the oxazoles and thiazoles, however, rat GnRH affinity was considerably weaker than affinity for the human receptor. The substituted imidazoles, on the other hand, provided the most potent analogs overall. Alkyl disubstitution at the 1,5- and 1,2-imidazole positions provided potent human GnRH binding agents (IC_{50}'s = 5–46 nM). Affinity for the rat receptor for most of these compounds was only 3–5 times weaker (IC_{50}'s = 24–78 nM) than they were for the human receptor. Alkylation of the nitrogen adjacent to the point of attachment (**36**, **45**) provided the weakest analogs in the series.

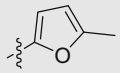
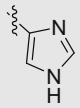
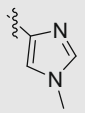
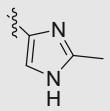
The imidazole analogs that had both human and rat GnRH receptor affinity of 50 nM or less were evaluated in secondary functional assays as well as in vitro pharmaceutical profiling (Table 4). Two compounds were chosen for PK evaluation based on their overall profile. Compounds **32** and **41** showed reasonable potency in functional assays as well as good solubility in buffer (pH 7.4). Their increased solubility over the lead, **1**, is most likely due to decreased crystallinity since the uracil is more polar than the imidazoles. In addition, both compounds were stable in rat liver microsomes ($t_{1/2}$ > 15 min.). Although both compounds appeared to inhibit CYP 3A4 at a level comparable to **1**, they did not inhibit liver enzymes CYP 2D6 and CYP 2C9.

Both compounds **41** and **32** were evaluated in one-day pharmacokinetic assays in orchidectomized rats. Although oral bioavailability was high in both instances, **41** had low volume of distribution. In contrast, **32** had moderate volume of distribution making it likely that it would quickly diffuse to the target tissue (Table 5). As a result, compound **32** was examined in vivo for the ability to lower serum LH levels.

Antagonism of GnRH in vivo leads to a drop in serum LH levels which in turn leads to lower levels of sex hormones. This decrease in serum LH has shown clinical significance in the treatment of sex hormone sensitive conditions. Intact animals have pulsatile patterns of serum LH, making accurate measurements difficult. Orchidectomized rats, however, display elevated, stable levels of serum LH due to the removal of the testosterone mediated feedback loop that controls the central release of GnRH. Two cohorts of orchidectomized rats were treated with compound **32** and vehicle, respectively, via oral gavage. Serum levels of LH were then measured periodically over 24 h. The results of this experiment are shown in Table 6. Hormone levels trended lower in the drug treated group. LH levels at 4 and 6 h, however, were considerably higher than expected for an agent with significant in vitro GnRH antagonist properties and failed to drop LH levels as significantly as compound **1**.[4] We attribute this to residual LH level variability in several animals of the drug treated group which may be due to ineffective castration in some animals.

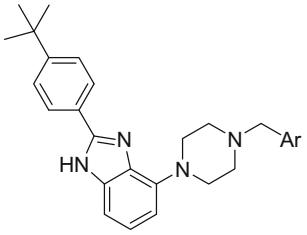
Table 1
GnRH receptor affinity for compounds prepared from commercially available aldehydes

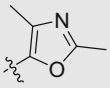
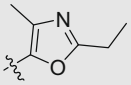
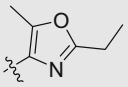
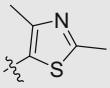
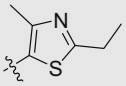
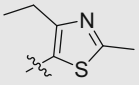
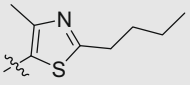
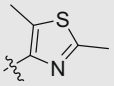


Cmpd number	Ar=	hGnRH IC_{50} (nM) or % inhibition at 50 nM[a]	rGnRH IC_{50} (nM)[a]
3		24%	–
4		6%	–
5		12%	–
6		705	–
7		20%	–
8		7%	–
9		6%	–
10		26%	–
11		21	290
12		33	98
13		7.5	28
14		40%	–

[a] Binding to overexpressed human or rat GnRH receptors in competition with ^{125}I-(D-Trp^6)-GnRH (Ref. 6). Results are given as an average of two independent experiments run in triplicate. The standard deviations for these assays were typically within ±50% of the IC_{50}.

Table 2
GnRH receptor affinity for oxazole and thiazole analogs

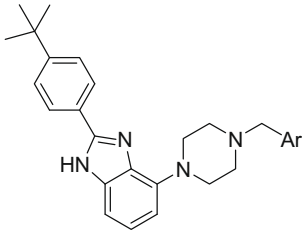


Compd number	Ar	hGnRH IC_{50} (nM) or % inhibition at 50 nM[a]	rGnRH IC_{50} (nM)[a]
15		90	850
16		31	740
17		37	320
18		27	550
19		27	–
20		20	120
21		26%	–
22		4%	–
23		21	–

[a] Binding to overexpressed human or rat GnRH receptors in competition with ^{125}I-(D-Trp^6)-GnRH (Ref. 6). Results are given as an average of two independent experiments run in triplicate. The standard deviations for these assays were typically within ±50% of the IC_{50}.

In conclusion, we used empirical techniques to diversify the appended uracil of lead compound **1**. Systematic replacement of the uracil with smaller heterocycles led to the eventual discovery of imidazoles **32** and **41** with nanomolar affinity for the human and rat receptors. Both compounds had improved liver microsome stability and solubility over the uracil. Inhibition of the CYP 3A4 isozyme, however, remained unchanged from the parent molecule.[8] Oral and iv pharmacokinetics on compound **32** supported an in vivo efficacy trial. Although this compound showed excellent

Table 3
GnRH receptor affinity for pyrazole and imidazole analogs



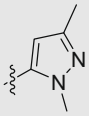
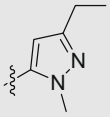
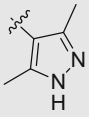
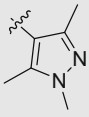
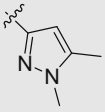
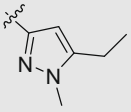
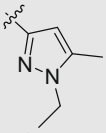
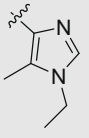
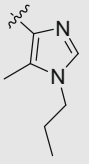
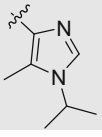
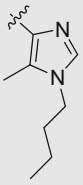
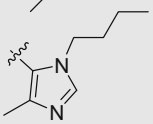
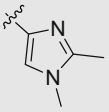
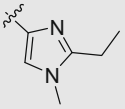
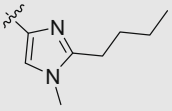
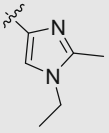
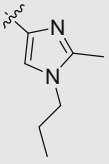
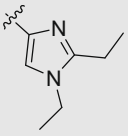
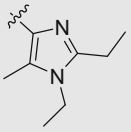
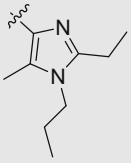
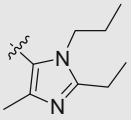
Compd number	Ar	hGnRH IC_{50} (nM) or % inhibition at 50 nM[a]	rGnRH IC_{50} (nM)[a]
24		25%	–
25		23	310
26		22	250
27		68	–
28		38	450
29		83	–
30		43	350
31		32%	–
32		7	24
33		12	47
34		19	61

Table 3 (*continued*)

Compd number	Ar	hGnRH IC_{50} (nM) or % inhibition at 50 nM[a]	rGnRH IC_{50} (nM)[a]
35		46	49
36		67%	–
37		10	34
38		18	78
39		26	58
40		10	51
41		18	41
42		11	69
43		8	49
44		29	57
45		57	120

[a] Binding to overexpressed human or rat GnRH receptors in competition with ^{125}I-(D-Trp^6)-GnRH (Ref. 6). Results are given as an average of two independent experiments run in triplicate. The standard deviations for these assays were typically ±50% of the average or less.

Table 4
In vitro profile and pharmaceutical properties of lead imidazoles

Cmpd number	**1**	**13**	**32**	**35**	**37**	**41**	**43**
hGnRH IC_{50} (nM)[a]	1.7	7.5	7	46	10	18	8
rGnRH IC_{50} (nM)[a]	18	28	24	49	34	41	49
Human inositol phosphate IC_{50} (nM)[b]	4	44	14	71	23	43	13
Rat LH release IC_{50} (nM)[c]	59	250	200	570	300	240	296
Solubility at pH 7.4 (μg/mL)[d]	13	46	>100	3	51	55	25
Rat liver microsome $t_{1/2}$ (min)[d]	5	>30	21	>30	9	25	12
CYP 3A4 (% inhibition at 3 μM)[d]	74	85	73	84	66	85	—
CYP 2D6 (% inhibition at 3 μM)[d]	0	14	14	21	3	13	—
CYP 2C9 (% inhibition at 3 μM)[d]	34	23	27	53	12	19	—

[a] See footnote a in Tables 1–3.
[b] Compound driven IP reduction in whole cells following stimulation with (D-Trp^6)-GnRH (Ref. 7).
[c] Compound driven reduction in LH release from primary rat pituitary cells stimulated with (D-Trp^6)-GnRH (Ref. 4).
[d] Details for pharmaceutical profiling assays can be found in Ref. 9.

Table 5
Single dose pharmacokinetics of compounds **32** and **41** in orchidectomized SD rats

Cmpd number	iv			po		Bioavailability (% F)
	Clearance (mL/min/kg)	Vss (L/kg)	$t_{1/2}$ (h)	AUC (0–α) ng h/mL	$t_{1/2}$ (h)	
41[a]	10	0.2	0.9	3830	2.9	100
32[b]	134	2.7	0.3	3444	3.7	93

[a] iv dose of 1 mg/kg in 20% DMSO/PEG 200; oral dose of 20 mg/kg in 0.4 N HCl/2% TWEEN 80.
[b] iv dose of 1 mg/kg in 20% DMSO/PEG 200; oral dose of 30 mg/kg in PEG 400.

Table 6
Serum LH levels of rats treated with compound **32** (30 mg/kg po, PEG 400)

Time (h)	Compound **32**[a]		Vehicle[a]		Change
	Average LH[b]	SD	Average LH[b]	SD	
0.5	70.3	24.0	70.5	28.0	−0.2
1	50.0	15.9	68.0	24.1	−18
2	46.5	16.9	99.3	48.8	−52.8
4	100.8	50.5	86.3	18.9	+14.5
6	94.5	42.5	125.8	52.2	−32.3
24	90.0	34.8	93.5	24.2	−3.5

[a] $n = 6$ animals per group.
[b] Relative LH levels at $t = 0$ adjusted to 100.

bioavailability and a trend toward pharmacological activity in a rat in vivo assay, it was not as efficacious as **1** in lowering serum LH levels. We attribute this to variable GnRH levels in several animals that may have resulted from inadequate orchidectomy.

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References and notes

1. Moreau, J.-P.; Delavault, P.; Blumberg, J. *Clin. Ther.* **2006**, *28*, 1485.
2. Herbst, K. *Curr. Opin. Pharmacol.* **2003**, *3*, 660–666.
3. Betz, S. F.; Zhu, Y.-F.; Chen, C.; Struthers, R. S. *J. Med. Chem.* **2008**, *51*, 3331.
4. Pelletier, J. C.; Chengalvala, M.; Cottom, J.; Feingold, I.; Garrick, L.; Green, D.; Hauze, D.; Huselton, C.; Jetter, J.; Kao, W.; Kopf, G. S.; Lundquist, J. T., IV; Mann, C.; Mehlmann, J.; Rogers, J.; Shanno, L.; Wrobel, J. *Bioorg. Med. Chem.* **2008**, *16*, 6617.
5. Gleeson, M. P. *J. Med. Chem.* **2008**, *51*, 817.
6. Millar, R. P.; Davidson, J.; Flanagan, C.; Wakefield, I. *Methods Neurosci.* **1995**, *25*, 145.
7. Chengalvala, M.; Kostek, B.; Frail, D. E. J. *Biochem. Biophys. Methods* **1999**, *38*, 163.
8. Hu, Q.; Negri, M.; Jahn-Hoffmann, K.; Zhuang, Y.; Olgen, S.; Bartels, M.; Mueller-Vieira, U.; Lauterbach, T.; Hartmann, R. W. *Bioorg. Med. Chem.* **2008**, *16*, 7715.
9. Di, L.; Kerns, E. H. *Curr. Opin. Drug Disc. Dev.* **2005**, *8*, 495.