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Synthesis and biological evaluation of piperazinyl heterocyclic antagonists of the gonadotropin releasing hormone (GnRH) receptor

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ABSTRACT

Antagonism of the gonadotropin releasing hormone (GnRH) receptor has resulted in positive clinical results in reproductive tissue disorders such as endometriosis and prostate cancer. Following the recent discovery of orally active GnRH antagonists based on a 4-piperazinylbenzimidazole template, we sought to investigate the properties of heterocyclic isosteres of the benzimidazole template. We report here the synthesis and biological activity of eight novel scaffolds, including imidazopyridines, benzothiazoles and benzoxazoles. The 2-(4-*tert*-butylphenyl)-8-(piperazin-1-yl)imidazo[1,2-*a*]pyridine ring system was shown to have nanomolar binding potency at the human and rat GnRH receptors as well as functional antagonism in vitro. Additional structure–activity relationships within this series are reported along with a pharmacokinetic comparison to the benzimidazole-based lead molecule.

Gonadotropin releasing hormone (GnRH) is a decapeptide synthesized in the hypothalamus and released into the hypophyseal circulation in a pulsating fashion, where it travels to the anterior pituitary gland.[1–3] GnRH acts to stimulate the G-protein coupled GnRH receptor leading to the downstream release of follicle stimulating hormone (FSH) and leuteinizing hormone (LH). The release of these hormones leads to gametogenesis and the synthesis of sex steroids. Consequently, antagonism of the GnRH receptor and the resulting inhibition of sex steroid synthesis have shown clinical promise in the treatment of hormone-dependent disorders such as endometriosis and prostate cancer.[4,5]

We have recently reported the elaboration and structure–activity relationships of piperazinyl benzimidazoles[6–8] including compound **1** (Table 1), which has demonstrated oral pharmacological activity by lowering plasma levels of LH in rats.[9] In order to further explore structural novelty, binding potency and pharmaceutical properties, we sought to compare several heterocyclic replacements for the benzimidazole core template of **1**. The substituted imidazole derivative **2** had better solubility[10] in aqueous buffer than **1** and showed comparable binding potency at both the human and rat GnRH receptors. Hence, the substituted imidazole side chain was kept constant in our comparison of replacements for the benzimidazole core to ensure reliable behavior in screening assays and consistency across templates in data analysis.

The heterocyclic replacements (**3–10**) for the benzimidazole core template are shown in Scheme 1. Each template adds, changes or repositions heteroatoms across the fused ring system. Templates **3** and **4** incorporate an additional nitrogen atom, giving imidazopyridines. Imidazopyridines **5** and **6** formally migrate one of the benzimidazole nitrogens of **1** into the six-membered ring. Templates **7** and **8** replace the benzimidazole system with regioisomeric benzoxazoles, while **9** and **10** are the corresponding benzothiazoles.

Schemes showing the preparation of **3–10** are available as Supplementary data. Additional details describing the preparation of **3** and **4**,[11] **5** and **6**,[12] and **7**–**10**[13] have been reported elsewhere. Compounds **9** and **10** are also available by a tandem benzothiazole ring closure/Buchwald coupling reaction.[14]

Benzimidazoles **1** and **2** were prepared as described previously by reductive amination of the appropriate aldehyde with the corresponding piperazine derivative.[8,15] As shown in Scheme 1, imidazole derivatives **3a** through **10a** were prepared using the analogous reaction with 2-ethyl-5-methyl-imidazole-4-carboxaldehyde and piperazinyl derivatives **3** through **10** (Scheme 1). Yields were generally high.

Compounds **3a** through **10a** were assayed for receptor binding potency against the human GnRH receptor as shown in Table 2. Binding data for **3a** and **4a** indicates the formal incorporation of

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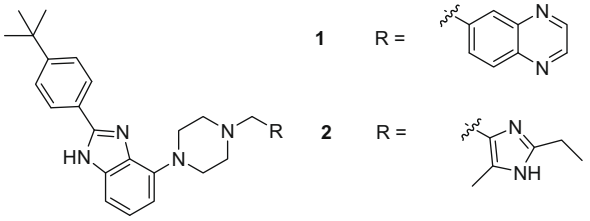
Table 1
Structures, GnRH receptor (GnRH-R) binding data and solubility for lead benzimidazole derivatives



Compound	IC_{50} (nm) hGnRH-R binding[a,b,c]	IC_{50} (nm) rGnRH-R binding[a,b,c]	Solubility[d] (μg/mL)
1	12	72	1
2	10	34	20

[a] Binding to overexpressed human or rat GnRH receptors in competition with ^{125}I-(D-Trp^6)-GnRH (see Ref. 8).
[b] Results are reported as an average of two independent experiments run in triplicate.
[c] Standard deviations for assay results were within ±50% of the average.
[d] Concentration is measured after dilution of a DMSO stock solution into pH 7.4 buffer followed by overnight equilibration. See Ref. 10 for details.

Table 2
GnRH receptor binding activity of 2-methyl-5-ethyl-imidazolyl derivatives of core templates **3**–**10** (Scheme 1)

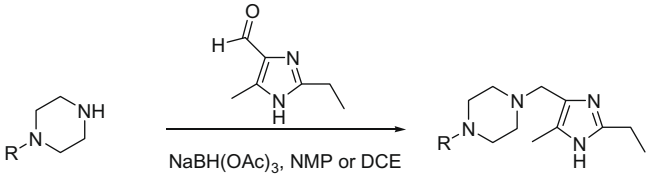
Compound	hGnRH receptor binding IC_{50}[a,b,c] (nM)	rGnRH receptor binding IC_{50}[a,b,c] (nM)
3a	(32%)[d]	
4a	58	290
5a	>10,000	
6a	9.8	37
7a	(61%)[d]	
8a	>10,000	
9a	(70%)[d]	
10a	>10,000	

[a] Binding to overexpressed human or rat GnRH receptors in competition with ^{125}I-(D-Trp^6)-GnRH (see Ref. 8 for details).
[b] Results are reported as an average of two independent experiments run in triplicate.
[c] Standard deviations for assay results were within ±50% of the average.
[d] Values in parentheses indicate percent inhibition at 500 nM.

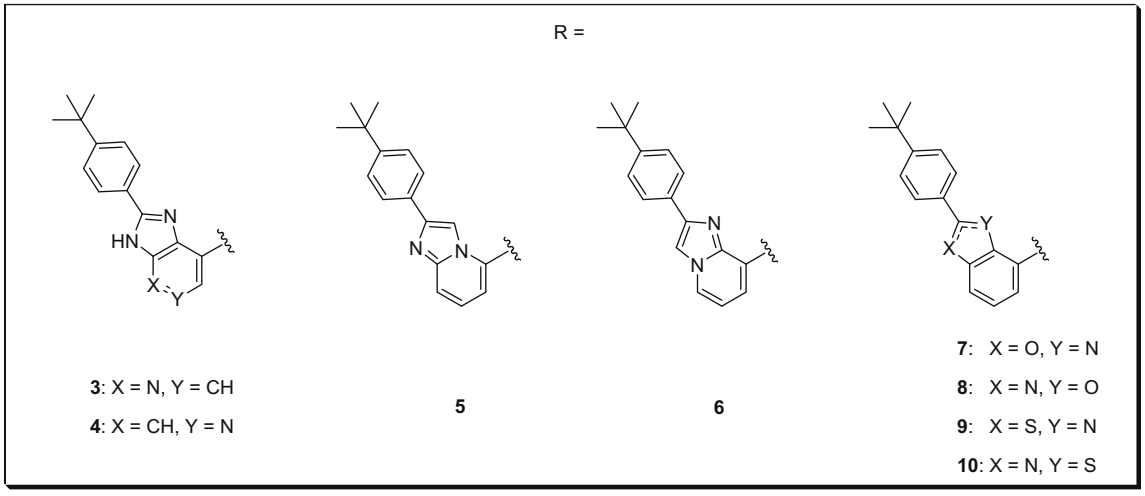
a pyridine-type nitrogen into the six-membered ring of the benzimidazole core is tolerated only in the 4-position (**4a**) while the 5-analogue is much less potent (**3a**).

The imidazopyridines **5a** and **6a** also show disparate binding potencies with **6a** displaying low nanomolar binding affinity at both the human and rat GnRH receptors. This level of binding potency is comparable to that of benzimidazole **2**. Compound **5a**, however, is significantly less potent, indicating that transposing the benzimidazole nitrogen distal to the piperazine ring has little effect on receptor binding. Benzoxazoles **7a** and **8a** are both significantly less active than **2**, as are benzothiazoles **9a** and **10a**. However, when the oxygen or sulfur of both analogue pairs is proximal to the piperazine ring (i.e., **8a** and **10a**) the analogues are significantly less active than their regioisomeric counterparts (**7a** and **9a**). Overall, these observations indicate that a nitrogen atom in the five-membered ring which is situated proximal to the piperazinyl substituent is an important determinant of GnRH receptor binding activity. Moreover the receptor binding data for this series suggested that the imidazopyridine template **6** would be a good surrogate for the benzimidazole core of compounds **1** and **2**.

We recently reported the beneficial effects on potency using the appended imidazoles, uracils and quinoxalines shown in Figure 1.[7–9] To investigate further the properties of **6** as a template for



3-10 **3a-10a**



Scheme 1. Reductive amination with templates **3**–**10** and 2-ethyl-4-methylimidazol-5-carboxaldehyde provided final products **3a** through **10a**. See Supplementary data for synthetic schemes leading to compounds **3**–**10**.

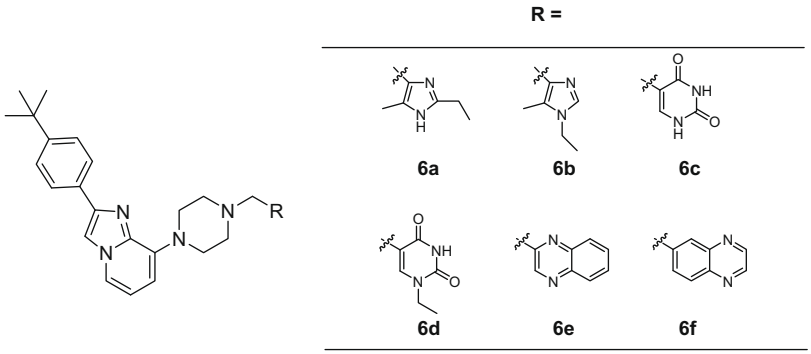


Figure 1. Derivatives of imidazopyridine template **6**.

GnRH antagonists, several additional analogs were synthesized and assayed for both receptor binding and functional antagonist activity. Hence compounds **6b–6f** (Fig. 1) were prepared by reductive amination of the corresponding aldehydes using template **6**. Along with **6a**, these compounds were assayed for receptor binding to the human and rat GnRH receptors. Additionally, all six compounds were tested for functional antagonist activity by measuring the in vitro inhibition of inositol phosphate in recombinant cells overexpressing the human GnRH receptor. As an additional measure of functional activity, the vitro inhibition of LH release in primary cultures of rat anterior pituitary cells was assessed.

As shown in Table 3 **6a–6f** demonstrated nanomolar binding potency at both the rat and human GnRH receptors, indicating that template **6** is a general mimic of the benzimidazole core in terms of receptor binding potency. Moreover, **6a–6f** also showed good levels of in vitro functional antagonist activity against the GnRH

Table 3
Receptor binding and functional antagonist potency for analogs derived from template **6**

Compound	hGHRH binding IC_{50}[a,b,c] (nM)	rGHRH binding IC_{50}[a,b,c] (nM)	Human IP inhibition IC_{50}[d] (nM)	Rat LH release IC_{50}[e] (μM)
6a	9.8	37	60	0.92
6b	6.7	12	41	0.42
6c	4.3	95	20	4.4
6d	2.3	5.7	5.0	0.26
6e	44	29	39	1.5
6f	47	81	90	3.4
1b (Fig. 2)	5.8	24	15	0.20
1	12	72	28	0.36
2	10	34	18	0.16

[a] Binding to overexpressed human or rat GnRH receptors in competition with ^{125}I-(D-Trp^6)-GnRH (see Ref. 8 for details).
[b] Results are reported as an average of two independent experiments run in triplicate.
[c] Standard deviations for assay results were within ±50% of the average.
[d] Compound driven IP reduction in whole cells following stimulation with (D-Trp^6)-GnRH (see Ref. 8 for details).
[e] Compound driven reduction in LH release from primary rat pituitary cells stimulated with (D-Trp^6)-GnRH (see Ref. 8 for details).

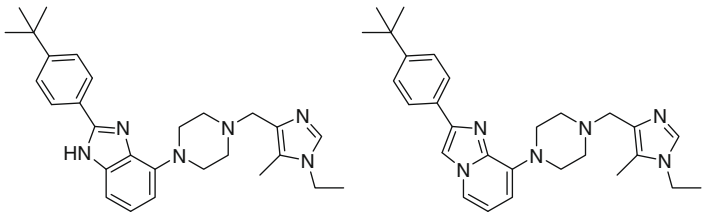


Figure 2. Benzimidazole and imidazopyridine derivatives chosen for pharmacokinetic evaluation.

Table 4
Pharmaceutical properties of selected imidazopyridine analogs[a]

Compound	PAMPA (Pe × 10^{-6} cm/s)	Solubility (μg/mL)	Rat liver microsome $t_{1/2}$ (min)	Cyp450 2C9% inhib.	Cyp450 2D6% inhib.	Cyp450 3A4% inhib.
6a	0.2	3	21	6	8	78
6b	1.1	9	11	10	9	54
6d	1.2	6	6	0	0	54
1b	2.7	3	21	27	14	73

[a] See Ref. 10 for experimental details.

Table 5
Pharmacokinetic parameters for **6b** and **1b**

Parameter	Compound (route of administration)[a,b,c]			
	6b (iv)	**6b** (po)	**1b** (iv)	**1b** (po)
Dose (mg/kg)	3	30	1	30
T_{max} (h)	—	0.8 ± 0.4	—	3.7 ± 2.5
C_{max} or C_0 (ng/mL)	1764 ± 353	788 ± 146	458 ± 75	533 ± 78
$T_{1/2}$ (h)	1.4 ± 0.1	1.1 ± 0.2	0.4 ± 0.0	2.2 ± 0.9
AUC_{0-inf} (ng * h/mL)	685 ± 149.3	1946 ± 394	124 ± 5.9	3444 ± 933
Cl (mL/min/kg)	75.1 ± 14.8	—	134 ± 6.5	—
V_{ss} (L/kg)	3.5 ± 0.7	—	2.7 ± 0.4	—
F (%)	—	28	—	93

[a] Single-dose pharmacokinetic studies using orchidectomized Sprague-Dawley rats ($n = 3$).
[b] Dosing vehicle: iv = DMSO/PEG200, po = PEG400.
[c] Values represent the mean ± standard deviation.

receptor as measured by inhibition of inositol phosphate and LH release. Compound **6b** and its direct comparator from the benzimidazole series (**1b**, Fig. 2) have very comparable levels of receptor binding and in vitro functional activity. Three analogs (**6a**, **6b** and **6d**) showed sub-micromolar potency in the in vitro inhibition of rat LH release, comparable to the level of activity of lead benzimidazoles **1** and **2**. These results demonstrate that the surrogacy of the imidazopyridine template **6** extends beyond binding affinity to in vitro functional antagonism, heightening our interest in pursuing compounds of this type.

In order to assess further the differences between the imidazopyridine and benzimidazole derivatives, we sought to compare the pharamocokinetics of the two templates with the side chain moiety being held constant. In selecting a compound for further evaluation, we examined the pharmaceutical properties of the most active members of the imidazopyridine series, as shown in Table 4. While **6d** showed the highest level of potency (Table 3) in all four in vitro assays, it suffered from a short half-life in rat liver microsomes. Compound **6a**, which had the longest microsomal half-life, showed reduced cell permeability and a relatively high level of Cyp450 3A4 isozyme inhibition. While all compounds shown in Table 3 exhibited relatively low solubility in pH 7.4 buffer, **6b** was modestly more soluble than the others. Consistent with our previous efforts to balance structure–property relationships with structure–activity relationships to obtain an orally active molecule,[9] **6b** appeared to have the best overall combination of in vitro activity and pharmaceutical properties.

Imidazopyridine **6b** and its benzimidazole comparator **1b** were dosed in orchidectomized Sprague-Dawley rats at 1 or 3 mg/kg iv and 30 mg/kg orally. Pharmacokinetic parameters are shown in Table 5. Following oral dosing at 30 mg/kg, imidazopyridine **6b** exhibited both a lower exposure and a shorter half-life than **1b**. The oral bioavailability of **6b** and **1b** were determined to be 28% and 93%, respectively. Greater oral bioavailability and exposure observed for **1b** can be attributed to several factors. Compound **1b** has better permeability properties than **6b**, making it a better candidate for intestinal absorption following oral administration. Also, based on liver microsome stability data, **1b** is most likely more stable to first pass metabolism, ensuring higher blood levels. This latter property is supported by the short oral plasma half-life of **6b** (1.1 h, Table 5). The significantly lower plasma levels of **6b,** compared to **1b**, do not support in vivo efficacy studies. As these results suggest, the program continues to focus on benzimidazoles.

In conclusion, eight alternative heterocyclic templates were prepared and derivatized to yield a set of analogs (**3a–10a**) which were evaluated for GnRH receptor binding potency. Imidazopyridine analog **6a** showed binding potency comparable to that of the benzimidazole leads **1** and **2**. Several additional imidazopyridine derivatives (**6b–6f**) demonstrated both binding and functional GnRH antagonist activity in vitro. A comparative pharmacokinetic study of an imidazopyridine analog and the corresponding benzimidazole showed the latter having better oral pharmacokinetics as measured by oral exposure and bioavailability. The benzimidazole series continues to show superior in vitro and in vivo properties when compared to derivatives of templates **3–10**.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.02.099.

References and notes

1. Matsuo, H.; Baba, Y.; Nair, R. M. G.; Arimura, A.; Schally, A. V. *Biochem. Biophys. Res. Commun.* **1971**, *43*, 1334.
2. Millar, R. P.; Lu, Z.-L.; Pawson, A. J.; Flanagan, C. A.; Morgan, K.; Maudsley, S. R. *Endocr. Rev.* **2004**, *25*, 235.
3. Schally, A. V.; Arimura, A.; Kastin, A. J.; Matsuo, H.; Baba, Y.; Redding, T. W.; Nair, R. M. G.; Debeljuk, L.; White, W. F. *Science (Washington, DC, United States)* **1971**, *173*, 1036.
4. Chengalvala, M. V.; Pelletier, J. C.; Kopf, G. S. *Curr. Med. Chem.: Anti-Cancer Agents* **2003**, *3*, 399.
5. Moreau, J.-P.; Delavault, P.; Blumberg, J. *Clin. Therap.* **2006**, *28*, 1485.
6. Green, D. M.; Goljer, I.; Andraka, D. S.; Chengalvala, M.; Shanno, L.; Hurlburt, W.; Pelletier, J. C. *J. Combin. Chem.* **2009**, *11*, 117.
7. Hauze, D. B.; Chengalvala, M. V.; Cottom, J. E.; Feingold, I. B.; Garrick, L.; Green, D. M.; Huselton, C.; Kao, W.; Kees, K.; Lundquist, J. T.; Mann, C. W.; Mehlmann, J. F.; Rogers, J. F.; Shanno, L.; Wrobel, J.; Pelletier, J. C. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1986.
8. 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.; Mann, C.; Mehlmann, J.; Rogers, J.; Shanno, L.; Wrobel, J. *Bioorg. Med. Chem.* **2008**, *16*, 6617.
9. Pelletier, J. C.; Chengalvala, M. V.; Cottom, J. E.; Feingold, I. B.; Green, D. M.; Hauze, D. B.; Huselton, C. A.; Jetter, J. W.; Kopf, G. S.; Lundquist, J. T. I.; Magolda, R. L.; Mann, C. W.; Mehlmann, J. F.; Rogers, J. F.; Shanno, L. K.; Adams, W. R.; Tio, C. O.; Wrobel, J. E. *J. Med. Chem.* **2009**, *52*, 2148.
10. Di, L.; Kerns, E. H. *Curr. Opin. Drug Disc. Dev.* **2005**, *8*, 495.
11. Pelletier, J. C.; Rogers, J. F. U.S. Patent Application 2006189617, 2006.
12. Lundquist, J. T.; Pelletier, J. C.; Vera, M. D. U.S. Patent Application: US2006270848, 2006.
13. Green, D. M.; Hauze, D. B.; Mann, C. W.; Pelletier, J. C.; Vera, M. D. US Patent: US 7531542 B2, 2009.
14. Vera, M. D.; Pelletier, J. C. *J. Combin. Chem.* **2007**, *9*, 569.
15. Garrick, L. M.; Hauze, D. B.; Kees, K. L.; Lundquist Iv, J. T.; Mann, C. W.; Mehlmann, J. F.; Pelletier, J. C.; Rogers, J. F., Jr.; Wrobel, J. E. Application: WO 2006009734, 2006.