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Synthesis and structure–activity relationships of thieno[2,3-*b*]pyrroles as antagonists of the GnRH receptor

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Abstract—A new class of small-molecule GnRH antagonists, the thieno[2,3-*b*]pyrroles, was designed. Herein, the synthesis and structure–activity relationships are described. Substitution at the C4 position was investigated; during this study, it was observed that introducing piperazines and piperidines improved the physical properties of the compounds while retaining good in vitro potency. This exploration led to the discovery of amidopiperidines with improved pharmacokinetic properties.

Gonadotropin-releasing hormone (GnRH), also known as LHRH, is a decapeptide hormone secreted and produced by the hypothalamus. It binds to GnRH receptors on the pituitary and stimulates the release of follicle stimulating hormone (FSH) and luteinising hormone (LH) which regulate gonadal steroid hormone production regulating in turn progesterone, oestrogens and androgens.[1,2] Several hormone dependent diseases such as endometriosis, breast and prostate cancer, caused by over-stimulation of the gonadal steroids, can be treated by GnRH peptide-based agonists. Antagonism of the GnRH receptor constitutes a valid alternative to agonists. They compete with endogenous GnRH at the receptor level and provoke a rapid suppression of the formation of LH without the concomitant 'flare' effect which is induced by peptide agonists in reaction to the initial over-stimulation of the receptors.[3a] Several peptide antagonists have been evaluated in the clinic for the treatment of hormone-sensitive diseases.[3] Orally administered small-molecule GnRH antagonists would present an advantage over the peptide antagonists and efforts towards orally active GnRH receptor antagonists have been reported in the literature (Fig. 1).[4] T-98475 (**1**) and analogues were the first orally active small molecules to be disclosed.[5] Indole derivatives (**2**) were reported to be potent and orally active GnRH antagonists.[6] Uracil derivatives (**3**) with potent antagonist activity have also been disclosed.[7]

In this paper, we describe our efforts towards potent orally bioavailable small-molecule GnRH antagonists and disclose the SAR study of thieno[2,3-*b*]pyrroles **4** (Fig. 2).[8]

The thienopyrrole core **5** with the functional amino group on the C4 side-chain was constructed by a *Fischer* indole approach according to Scheme 1.[9]

Commercially available ethyl thiophen-2-ylacetic acid ester was dimethylated with an excess of CH_3I in the presence of NaH. The resulting thiophene **7** was selectively nitrated with NO_2BF_4 to form **8** which was reduced by catalytic hydrogenation over palladium on charcoal to give the desired 2-aminothiophene. Boc protection of the amine gave carbamate **9** which was deprotonated with NaH and aminated with *O*-(diphenylphosphinyl)hydroxylamine, to afford hydrazine **10**.[10] Ketone **11** was condensed with hydrazine **10** under tailored *Fischer* indole conditions to give protected thieno[2,3-*b*]pyrrole **12**.[8] Deprotection and Boc reprotection provided intermediate **13**. Introduction of

Keywords: GnRH; Receptor; Antagonist; Thieno[2,3-*b*]pyrrole; LHRH.
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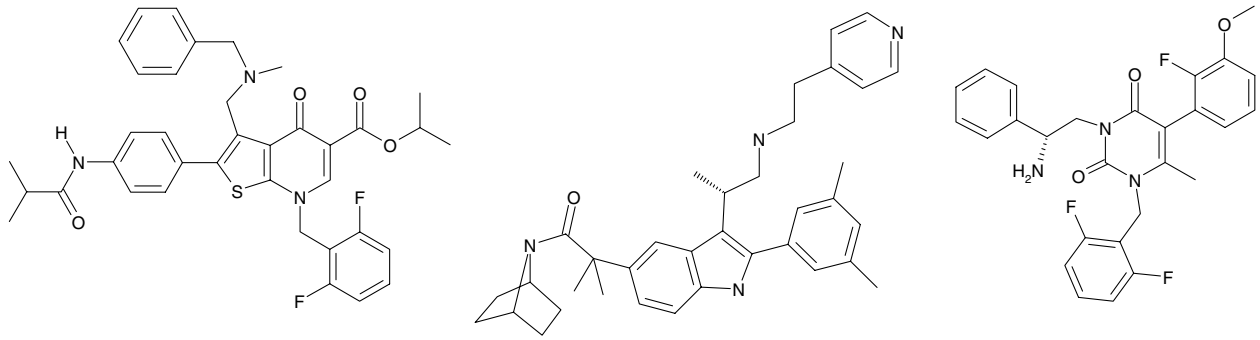


Figure 1. Small-molecule GnRH receptor antagonist.

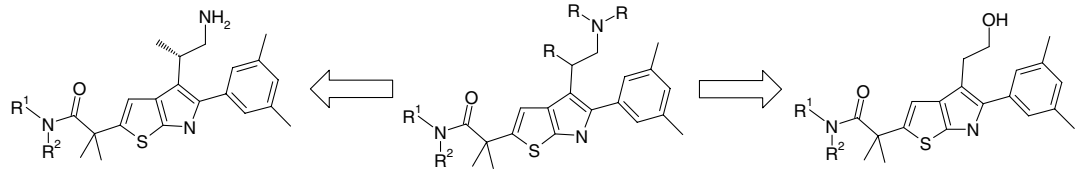
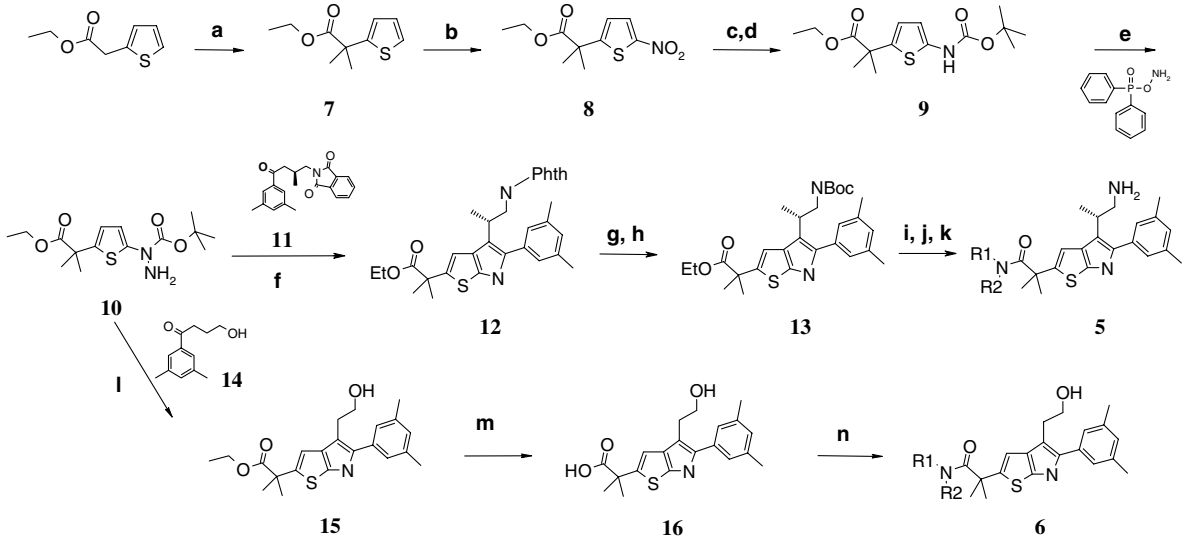


Figure 2. General synthesis of thieno[2,3-*b*]pyrroles.

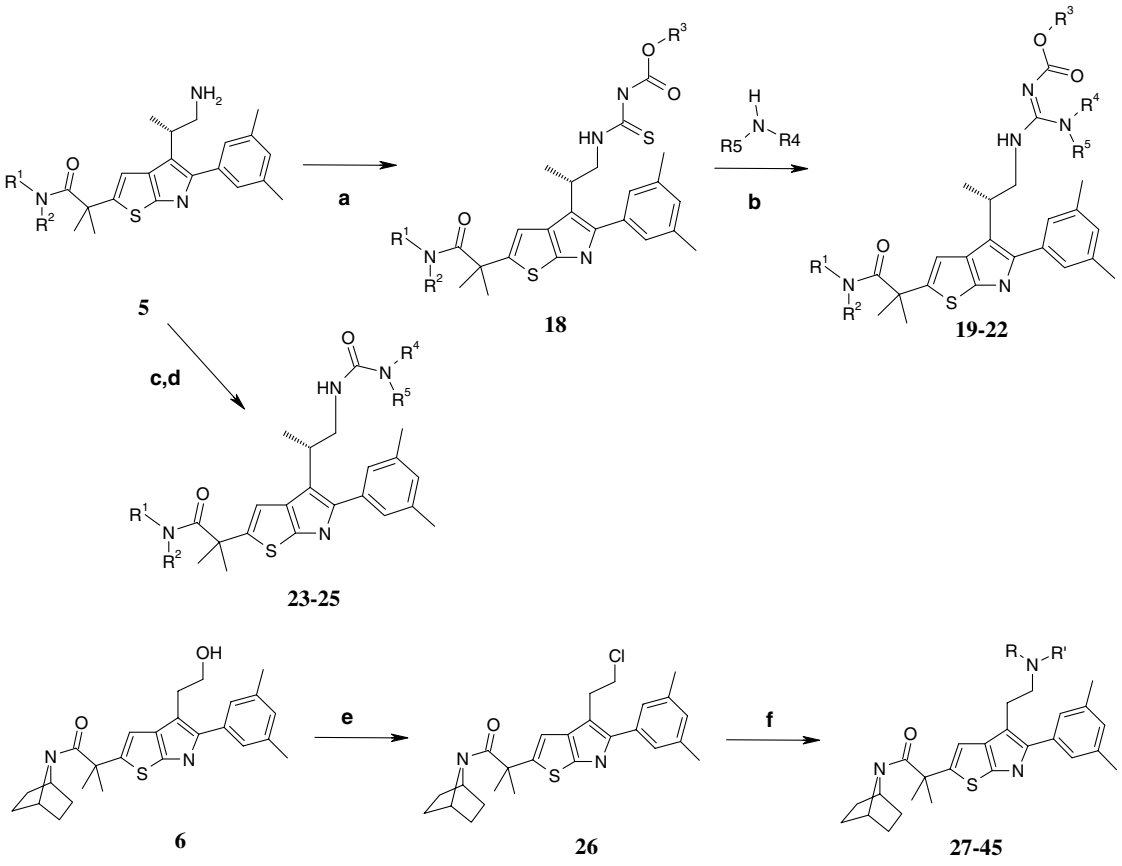


Scheme 1. Reagents and conditions: (a) NaH 60%, THF, 0 °C; CH_3I, THF, 18-crown-6, 5 h; 90%; (b) NO_2BF_4, DME, −55 °C 5 h then −5 °C overnight, 88%; (c) 10% Pd/C, EtOH/EtOAC, H_2, 9 h, 100%; (d) Boc-*O*-Boc, THF, reflux, 16 h, 68%; (e) NaH 60%, DMF, 5 °C, 2 h, overnight, 95%; (f) **11**, 2-butanol, $ZnCl_2$ 120 °C, 6 h; (g) EtOH, NH_2–NH_2, H_2O, 75%, 4 h; (h) MeOH, TFA, Boc-*O*-Boc, 60 °C, 4 h, quant; (i) NaOH 1 N, EtOH, 60 °C, 4 h, quant; (j) 7-azabicyclo[2.2.1]heptane, HCl, DIPEA, HATU, CH_2Cl_2, 0.5 h; quant; (k) CH_2Cl_2, TFA, 15 min, 57%; (l) **14**, 2-butanol, $ZnCl_2$, 110 °C, 5 h, 60%; (m) EtOH, NaOH 1 N, 60 °C, 4 h, 95%; (n) 7-azabicyclo[2.2.1]heptane, HCl, DIPEA, HATU, CH_2Cl_2, 0.5 h, 70%.

the amide group at the C2 position was achieved by saponification and a subsequent amide coupling under basic conditions using HATU.[11] Deprotection with TFA afforded the key intermediate **5**. A similar approach gave the hydroxy thienopyrrole core **6**.

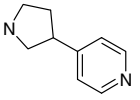
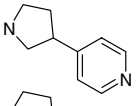
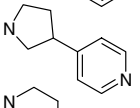
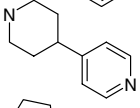
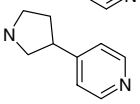
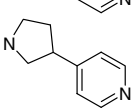
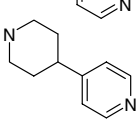
A series of thienopyrroles incorporating non-basic *N*-acylguanidino and urea groups was prepared according to Scheme 2. Synthesis of acylguanidines **19**–**22** was achieved by the addition of *N*-alkoxycarbonylisothiocyanates to core **5** and subsequent reaction of the intermediate **18** with secondary amines. Ureas **23**–**25** were prepared by reaction with *p*-nitrophenylchloroformate and further reaction with amines.

The synthesized compounds[12] were evaluated for their ability to compete for [^{125}I]-D-Trp^6 GnRH radioligand binding to rat and human receptors.[13] Their functional antagonism activity was established by inhibition of GnRH stimulated LH release.[14]



Scheme 2. Reagents and conditions: (a) alkoxycarbonylisothiocyanate, CH_2Cl_2, TFA, 2 h, quant; (b) CH_2Cl_2, EDCI, DIEA, NR_4R_5, overnight, 63%; (c) 4-nitrophenylchloroformate, Et_3N, CH_2Cl_2, 0 °C, 10 min, 0.5 h, quant; (d) R_4R_5NH, 2 h 30, 50%; (e) $SOCl_2$, DMF (cat), CH_2Cl_2, 0 °C, 10 min; 25 °C, 2 h, 85%; (f) K_2CO_3, NR_1R_2, CH_3CN, DMF, 80 °C.

Table 1. Binding and functional affinities of thienopyrroles **19**–**25** towards the rat and human GnRH receptors

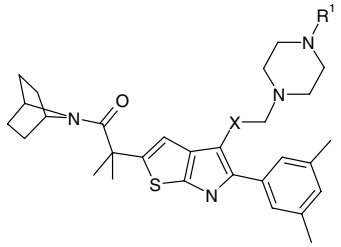
Compound	R^1R^2N	R^4R^5N	R^3	r IC_{50}, nM[a]	h IC_{50}, nM[a]	r cell IC_{50}, nM[a]	Sol. μM[b]
19	Me_2N		*i*-Pr	100	244	452	—
20			*i*-Pr	15	55	3	0.8
21			Me	8	35	3	4.6
22			*i*-Pr	27	79	12	1.4
23			—	64	96	445	1.5
24			—	<1	2	13	—
25			—	16	56	29	0.6

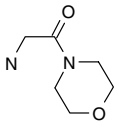
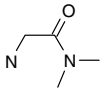
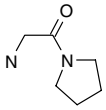
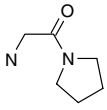
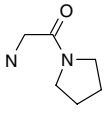
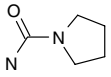
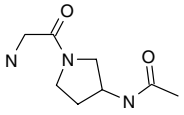
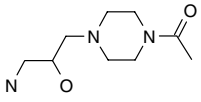
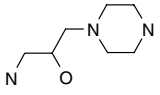
r, rat; h, human; sol., solubility.

[a] Values are geometric means of several experiments.

[b] Phosphate buffer 0.1 M, pH 7.4, 25 °C.

Table 2. Binding and functional affinities of piperazine thienopyrroles **27**–**35** towards the rat and human GnRH receptors



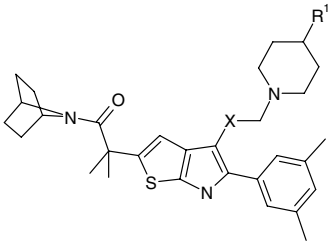
Compound	X	NR^1	r IC_{50}, nM[a]	h IC_{50}, nM[a]	r cell IC_{50}, nM[a]	Sol. μM[b]
27	CH_2		110	162	184	87
28	CH_2		172	177	209	104
29	CH_2		74	195	89	39
30	—		116	495	161	2
31	C=O		101	170	197	71
32	CH_2		529	696	—	—
33	CH_2		104	345	947	107
34	CH_2		155	331	280	236
35	CH_2		55	74	171	425

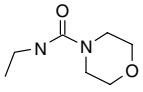
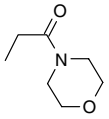
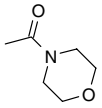
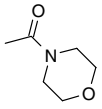
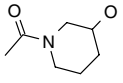
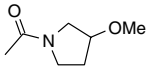
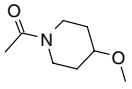
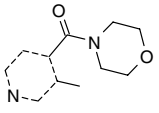
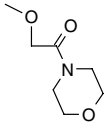
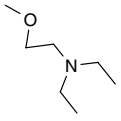
r, rat; h, human; sol., solubility.
[a] Values are geometric means of several experiments.
[b] Phosphate buffer 0.1 M, pH 7.4, 25 °C.

Table 1 lists the binding affinity data, functional activity and solubility for compounds **19**–**25**. Replacement of the diethylamide or pyrrolidine amide group at the C2 position by more lipophilic bicyclic amides led to a dramatic improvement in the affinity for GnRH receptors (rat and human) which translated into the functional assay (**20** vs **19**; **24** vs **23**). Solubility and free drug (not shown) were improved by replacement of the isopropoxy group by methoxy group (**21** vs **20**). Replacement of the pyrrolidinopyridine group by piperidinopyridine led to less potent compounds (**22** vs **20**; **25** vs **24**).

Overall, the introduction of acylguanidine or urea side-chains at the C4 position of the thieno[2,3-*b*]pyrrole gave very potent compounds. This suggests that there is no requirement for the side-chain to be basic.[6] Nevertheless, the physical properties were less than desired,

Table 3. Binding and functional affinities of piperidine thienopyrroles **36**–**46** towards the rat and human LHRH receptors



Compound	X	R^1	r IC_{50}, nM[a]	h IC_{50}, nM[a]	r cell IC_{50}, nM[a]	AUC μM.h
36	CH_2		51	178	191	0.006[b]
37	CH_2		94	275	743	0.018[b]
38	CH_2		51	132	111	0.041[b]
39	C═O		108	371	238	0.360[b]
40	CH_2		64	233	381	27.1[d]
41	CH_2		30	169	214	39.3[d]
42	CH_2		62	173	210	190[d]
43	CH_2		76	184	216	358[d]
44[c]	CH_2		43	88	220	0.254[d]
45	CH_2		116	338	117	0.526[d]
46	CH_2		30	33	36	0.002[b]

[a] Values are geometric means of several experiments.
[b] AUC dosed in rat at 1 mg/kg po (vehicle: propylene glycol).
[c] Drawing represents piperidine-R^1.
[d] AUC dosed in rat at 10 mg/kg po (vehicle: propylene glycol).

resulting in poor bioavailabilities, therefore further improvement was needed.

Introduction of piperazine, pyrrolidine and piperidine groups was then considered in an attempt to improve the physical properties. These compounds were prepared by reaction of the appropriate amines with the chloro intermediate **26** derived from core **6**, in the presence of a base (Scheme 2).

Right-hand side-chains with substituted piperazines contributed to improved physical properties, more specifically the solubility (Table 2). This was achieved to the detriment of binding and functional activities that were generally reduced. A rigid one-carbon linker between the thienopyrrole nucleus and the piperazine led to poor solubility (**30** vs **29**). On the contrary, introduction of a carbonyl function on the linker generally improved solubility (**31** vs **29**).

Variation of R_1 showed that amidomethyl substitution on the piperazine was giving good potency associated with overall good physical properties. Potency decreased dramatically with amide substitution (**32**). We observed during our investigation that functional activity was correlated with lipophilicity (**33** vs **29**). We also discovered during this study that some compounds were inhibitors of the cytochrome P450 3A4 enzyme. In fact, susceptibility to Cyp 3A4 is a general feature of this class of lipophilic antagonists.[6h,i] Nevertheless, we succeeded in diminishing the 3A4 inhibition with a variety of our piperazine compounds, as exemplified by **34** and **35** (3A4 IC_{50}: 6 μM and >10 μM, respectively, compared to 0.1 μM for **27**).[15]

The replacement of piperazines by piperidines appeared to be advantageous in giving better pharmacokinetics. Our exploration of piperidine side-chains was therefore driven towards combining good potency and improved pharmacokinetics (Table 3).

We rapidly observed that the 4-amidopiperidine series gave high blood levels (**40**–**43**). This was also true with a carbonyl function on the linker (**39**). Other series such as the amine (**36**), the methyl urea (**37**) or the amidomethyl (**38**) gave much poorer exposure. Good pharmacokinetic properties were restricted to close analogues of **40**. Indeed, minor modifications, as illustrated by **44**, **45** or by the replacement of the piperidine by a pyrrolidine (not shown), resulted in a dramatic loss of oral exposure. During our extensive exploration of the piperidine substitutions, we also discovered that binding affinities to rat and human receptors could be improved by 4-disubstitution (**46**). However, these compounds had poor blood levels.

Based on the attractive properties of these amidopiperidines, full pharmacokinetic profile in rat (po and iv) was obtained. These compounds were cleared from the plasma more slowly (**40**: 4.0; **41**: 1.3; **42**: 0.8; **43**: 0.1 ml/min/kg compared to **27**: 153 ml/min/kg) and had therefore longer terminal half-lives. This translated into better oral bioavailability as exemplified by **42** and **43** (38% and 35%, respectively, compared to 4% for **27**). Furthermore, those compounds showed an ability to inhibit the production of LH after oral administration in both acute and chronic in vivo rat models. For example, compound **40** administered orally at 75 mg/kg bid provided prolonged suppression of circulating LH levels in a castrated rat model.[16]

In conclusion, we have developed a route to a new class of small-molecule antagonists of the GnRH receptor. Introduction of substituted piperazines and piperidines at the C4 position substantially improved the physical properties. In these series, amidopiperidines showed improved pharmacokinetic parameters while retaining good potency.

Acknowledgments

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References and notes

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12. All the final products described in this paper have been fully characterised by LC–MS and ^{1}H NMR spectroscopy.
13. *Ligand displacement assay*. Crude membranes were prepared from human embryonic kidney (HEK) cells stably expressing human GnRH receptors. [^{125}I]-D-Trp^{6} GnRH having specific activity of 20 Ci/mmol was used as the radiolabelled ligand. Competitive binding was measured in a 80 mM Tris (4 mM MgCl) buffer containing 0.25% bovine serum albumin BSA, pH 7.4, and the compound of interest at concentrations between 0.3 and 3000 nM in a final concentration of 0.5% DMSO with a total incubation volume of 50 μl at 4 °C for approx 20 h. The membranes were then washed and harvested onto a GF/C filtermat and counted in a scintillation counter. The binding activity is reported as an IC_{50} value which is the antagonist concentration required to inhibit the specific binding of [^{125}I]-D-Trp^{6} GnRH to receptors by 50%.
14. *Functional assay*. Primary pituitary cells were isolated from >12-week-old female AP Han Wistar rats by enzymatic digestion with a mixture of collagenase and pancreatin. The suspended cells were then cultured for 4 days in 24-well tissue culture plates. On the day of the assay, the cells were washed and then treated with medium containing 10 nM GnRH and the compound of interest at concentrations between 3 and 3000 nM in a final concentration of 0.1% DMSO. After a 4-h incubation, the medium was removed and centrifuged. The supernatant was then analyzed for content of LH using a EIA kit specific for rat LH (Amersham). The results are reported as an IC_{50} value, which is the antagonist concentration, required to inhibit the GnRH stimulated LH release by 50%.
15. The microsomes were obtained from yeast expressing the human *CYP3A4* and the human cytochrome P450 reductase and were provided by AstraZeneca Biotech Laboratories (ABL) Sodertalje, Sweden. IC_{50} was determined using 7-benzyloxy-4-(trifluoromethyl)-coumarin (BFC) as the substrate.
16. *Chronic LH suppression.* 12-week-old male AP Han Wistar rats were castrated. At least 10 days post surgery compound (75 mg/kg) or vehicle (ICME Imwitor 988 49%, Cremaphor RH40 27%, Miglyol 12%, ethanol 13%) alone was administered by oral gavage bid at a dose volume of 5 ml/kg. Terminal blood samples were taken 12 h after either the first or second dose. Plasma LH levels were measured by rat LH specific radioimmunoassay (Amersham). Inhibition is reported as a percentage of control plasma LH.