Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 2597–2602

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Orally Bioavailable, Indole-Based Nonpeptide GnRH Receptor Antagonists with High Potency and Functional Activity

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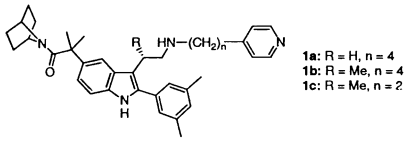
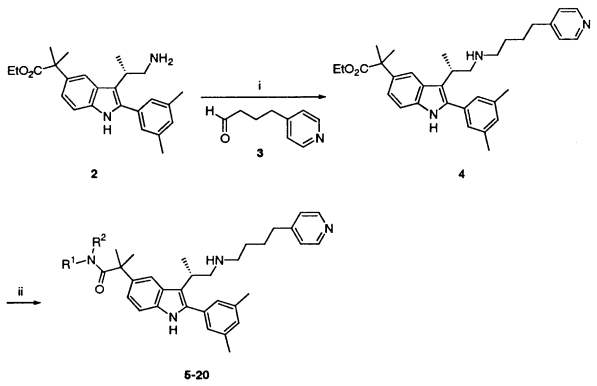
Received 29 May 2001; accepted 20 July 2001

Abstract—Stereospecific introduction of a methyl group to the indole-3-side chain enhanced activity in our tryptamine-derived series of GnRH receptor antagonists. Further improvements were achieved by variation of the bicyclic amino moiety of the tertiary amide and by adjustment of the tether length to a pyridine or pyridone terminus. These modifications culminated in analogue **24**, which had oral activity in a rat model and acceptable oral bioavailability and half-life in dogs and monkeys.

Orally active, nonpeptide receptor antagonists of gonadotropin-releasing hormone (GnRH; also known as luteinizing hormone-releasing hormone, or LHRH) represent a new class of experimental pharmaceuticals. They could possess advantages over existing therapies for chronic suppression of sex hormone levels, as in the treatment of certain hormone-dependent cancers, uterine fibroids, and endometriosis, or for short-term applications, as in assisted reproduction protocols.[1] Building upon previous work from these laboratories,[2] we have reported[3] that the tryptamine derivative **1a** is an effective GnRH antagonist in vitro. Introduction of a chiral methyl substituent, as in **1b**, has recently been achieved.[4] We now present a structure–activity study of amides related to **1b**[4] and its chain-shortened analogue **1c**.[4]



For the first series of compounds, containing a four-carbon tether between the side-chain amine and the pyridine ring, the (*S*)-β-methyltryptamine derivative **2**[4a] was reacted with 4-(4-pyridyl)butyraldehyde (**3**)[3] under reductive amination conditions[2e,3] to give **4** (Scheme 1). Saponification of the ester and coupling with amines in the presence of PyBOP reagent as previously described[2e,3]



Scheme 1. Conditions: (i) (a) $MgSO_4$, $CDCl_3$, −5 to −10 °C; (b) $NaBH_4$, MeOH, −5 °C; (ii) as described in refs 2e and 3.

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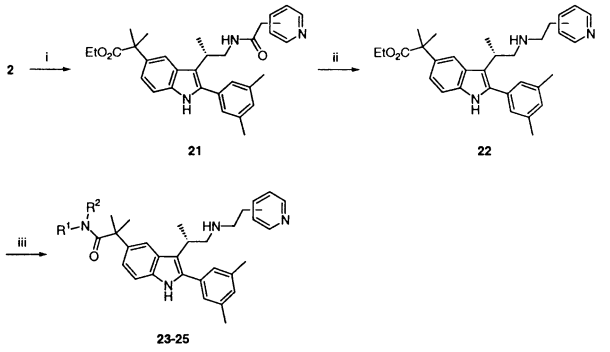
PII: S0960-894X(01)00512-1

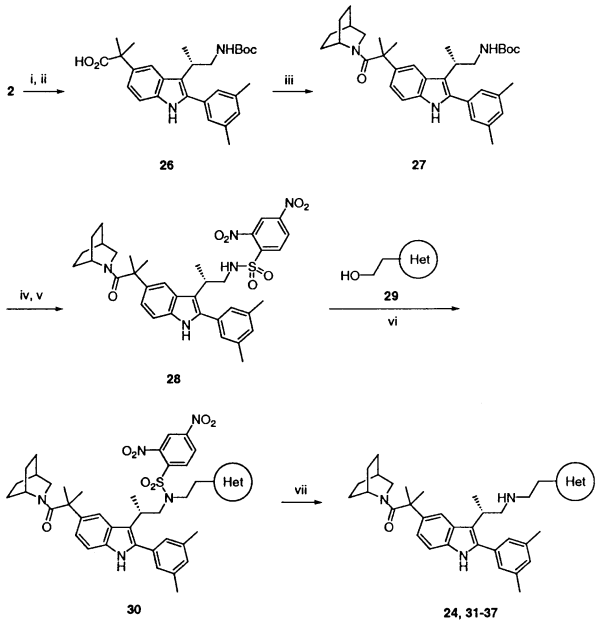
yielded the desired amides **5**–**20**. Noncommercial amines were prepared by literature methods.[5–15]

Analogues with a two-carbon, side-chain tether were obtained by acylation of **2** with pyridylacetic acids in the presence of PyBOP (Scheme 2). Reduction of the amides **21** with borane furnished the secondary amines **22**. Further transformation as described for Scheme 1 yielded the targets **23**–**25**.

Scheme 3 outlines the preparation of a series of analogues of **24** and **25** in which the unsubstituted pyridine ring is replaced by related heterocycles. Boc protection of the amino group of **2** followed by saponification of the ester yielded **26**. Treatment with isoquinuclidine hydrochloride[13] in the presence of PyBOP gave **27**. After deprotection with trifluoroacetic acid, the resulting amine was reacted with 2,4-dinitrobenzenesulfonyl chloride under modified Schotten–Baumann conditions to afford the activated sulfonamide **28**. Using the Fukuyama variation of the Mitsunobu reaction,[16] **28** was *N*-alkylated with heterocyclic ethanols **29**. Deprotection of the resulting **30** with neat propylamine[16] furnished the new derivatives **31**–**37** and also provided an alternative route to **24**.

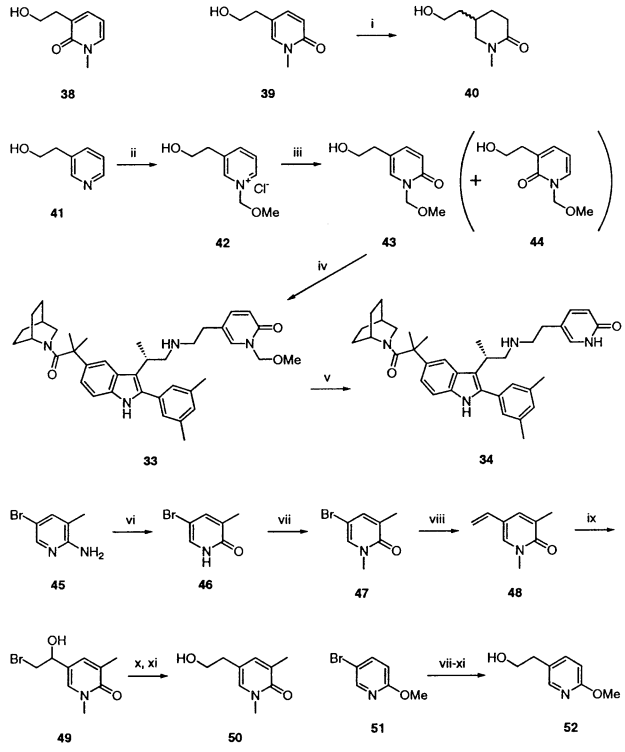


Scheme 2. Conditions: (i) 4- or 3-pyridylacetic acid hydrochloride, PyBOP, Et_3N, 1,2-dichloroethane; (ii) (a) BH_3, THF; (b) MeOH; (c) $Me_2N(CH_2)_2OH$, Δ; (iii) as described in refs 2e and 3.



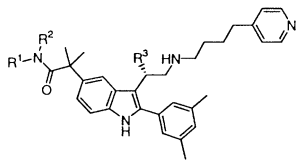
Scheme 3. Conditions: (i) Boc_2O, K_2CO_3, THF–H_2O; (ii) (a) KOH, MeOH–H_2O, Δ; (b) H^+; (iii) isoquinuclidine hydrochloride, PyBOP, Et_3N, CH_2Cl_2; (iv) (a) TFA, anisole, CH_2Cl_2; (b) aq Na_2CO_3; (v) 2,4-dinitrobenzenesulfonyl chloride, satd aq $NaHCO_3$, CH_2Cl_2; (vi) Ph_3P, DEAD, benzene; (vii) $PrNH_2$.

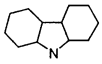
The synthesis of intermediate alcohols **29** is illustrated in Scheme 4. Isomeric pyridones **38** and **39** were prepared and separated by the method of Möhrle and Weber.[17] Hydrogenation of **39** over palladium on carbon furnished **40**. By a procedure analogous to that reported for **39**,[17] 2-(3-pyridyl)ethanol **41**[17] was quaternized by treatment with chloromethyl methyl ether to give **42**, which was then reacted with potassium ferricyanide and sodium hydroxide. The desired pyridone **43**, after separation from the isomeric product **44**, was converted to **33** by the methods of Scheme 3. Acid hydrolysis removed the methoxymethyl group to yield **34**. Commercial aminopyridine **45** was diazotized and hydrolyzed to **46**,[18] then *N*-methylated with methyl iodide in the presence of methanolic potassium hydroxide to give **47**.[19] Next, the vinyl group was introduced by a Stille coupling, yielding **48**. Reaction with *N*-bromosuccinimide in aqueous DMSO provided bromo alcohol **49**. Treatment with potassium carbonate resulted in formation of an epoxide (not isolated), which was reduced regiospecifically to alcohol **50** with ammonium formate and palladium catalyst. A similar reaction sequence converted 5-bromo-2-methoxypyridine (**51**) to the hydroxyethyl derivative **52**.



Scheme 4. Conditions: (i) H_2, Pd/C, EtOH; (ii) $MeOCH_2Cl$, CH_2Cl_2, 0 °C; (iii) $K_3Fe(CN)_6$, NaOH, H_2O, 0 °C; (iv) as in Scheme 3; (v) (a) concd HCl–EtOH (1:3), Δ; (b) aq Na_2CO_3; (vi) $NaNO_2$, aq H_2SO_4, 0–50 °C; (vii) MeI, KOH, MeOH, Δ; (viii) $Bu_3SnCH{=}CH_2$, $(Ph_3P)_2PdCl_2$, DMF; (ix) NBS, DMSO–H_2O; (x) K_2CO_3, DMF–H_2O; (xi) ammonium formate, Pd/C, EtOH.

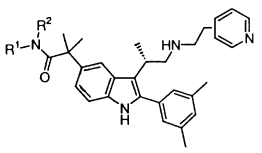
Table 1. Inhibition of GnRH receptor binding and PI turnover by tryptamine derivatives with four-carbon tether to pyridine ring



Compd	R^1R^2N	Amine source	R^3	hGnRH IC_{50} (nM)[a]	PI turnover IC_{50} (nM)[b]
1a[c]		Ref 5	H	1.4	18
1b[d]		Ref 5	Me	0.7	5.7
5		Ref 6	Me	0.7	21
6		Ref 7	Me	1.3	39
7		Ref 8	Me	2.7	93
8		Comm.[e]	Me	0.6	66
9		Comm.[e]	Me	6.1	220
10		Ref 9	Me	4.4	124
11		Comm.[e]	Me	5.6	14
12		Ref 10	Me	1.4	66
13		Comm.[e]	Me	21	31
14		Ref 11	Me	0.8	8.8
15		[f]	Me	0.4	8.7
16		[f]	Me	1.0	4.0
17		Ref 13	Me	1.0	2.8
18		Ref 14	Me	2.1	13
19		Ref 15	Me	4.8	6.8
20		Comm.[e]	Me	4.8	6.4

[a]Inhibition of [^{125}I]buserelin binding to human pituitary GnRH receptor.
[b]Inhibition of GnRH-stimulated [^{3}H]inositol phosphate hydrolysis.
[c]Ref 3.
[d]Ref 4a.
[e]Commercial.
[f]Made from commercial lactam precursor by method in ref 12.

Table 2. Inhibition of GnRH receptor binding and PI turnover by tryptamine derivatives with two-carbon tether to pyridine ring



Compd	R^1R^2N	Pyridine linkage	hGnRH IC_{50} (nM)[a]	PI turnover IC_{50} (nM)[b]
1c[c]		4	0.8	7.0
23		4	1.2	1.9
24		4	0.6	7.2
25		3	0.6	2.6

[a]Inhibition of [^{125}I]buserelin binding to human pituitary GnRH receptor.
[b]Inhibition of GnRH-stimulated [^{3}H]inositol phosphate hydrolysis.
[c]Ref 4b.

Compounds were evaluated for their ability to compete with the GnRH receptor agonist [^{125}I]buserelin for binding to the human GnRH receptor[20] in the presence of 0.1% BSA. In addition, functional antagonism in vitro was determined via inhibition of GnRH-stimulated phosphatidylinositol (PI) hydrolysis in cloned Chinese hamster ovary (CHO) cells stably expressing the human GnRH receptor.[20]

The beneficial effect of the (*S*)-β-methyl substituent on the tryptamine side chain was apparent (cf. **1b**[4] vs **1a**;[3] Table 1). Optimization of the tertiary amide substituent at the indole-5-position, which had been studied in the absence of the β-methyl substituent,[3] was reinvestigated here (Table 1). Compared to the 7-azanorbornane lead **1b**,[4] *gem*-dimethyl or spiro-fused azetidines or piperidines (**5**–**8**) generally maintained high affinity to the human GnRH receptor but clearly suffered a reduction in potency in the PI turnover functional assay. The piperazines **9** and **10** proved to be even more detrimental in the PI assay. In contrast, the diisobutyl amide **11**, although not especially potent in the binding assay, was relatively effective in the functional assay ($IC_{50} = 14$ nM). Whereas the fused pyrrolidines **12** and **13** did not produce favorable results, the products from several bicyclic amines **14**–**20** were effective GnRH antagonists, with all but **18** having functional IC_{50} values below 10 nM. Particularly noteworthy was the isoquinuclidine amide **17**, which had IC_{50} values of 1.0 and 2.8 nM in the binding and functional assays, respectively. Nevertheless, the oral bioavailability of **17** was less than desired (14% in rats, 7% in dogs), so further improvement was needed.

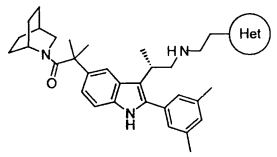
It was anticipated that shortening the side-chain tether between the pyridine ring and the secondary amine might improve oral bioavailability. In an earlier series lacking an indole 5-substituent and the β-methyl group, such a modification was significantly detrimental to receptor binding affinity.[2d] Remarkably, compound **1c**,[4b] the two-carbon tether analogue of **1b**, not only maintained excellent potency in the in vitro assays (Table 2) but also had oral bioavailability of 23% in rats. However, its bioavailability in dogs (14%) and rhesus monkeys (6.4%) was still deficient. Incorporation of some of the best bicyclic amino groups from Table 1 into this series gave **23**–**25**, which all displayed high potency in the binding and functional assays (Table 2). Although oral bioavailability in dogs was still unsatisfactory for **23** (8.5%) and **25** (12%), compound **24**, the isoquinuclidine amide bearing a 4-pyridyl terminus, had promising pharmacokinetic properties. Thus, the oral bioavailability of **24**, while only 8% in rats, was 25% in dogs and 21% in rhesus monkeys. The terminal half-life of **24** was determined to be 1 h in rats, 3.9 h in dogs, and 3.3 h in rhesus monkeys.

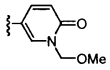
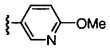
Replacement of the pyridine by a pyridone (or related heterocycle) was also investigated (Table 3). Of the isomeric 1-methylpyridin-2(1*H*)-ones **31** and **32**, linkage at the 5-position (**32**) resulted in a dramatic potency advantage over linkage at the 3-position (**31**). All of the analogues with the preferred substitution pattern (**32**–**34** and **36**) maintained very high potency. This suggests that there is no requirement for the side-chain terminus to be basic, provided that it is a suitable hydrogen-bond acceptor. The methoxypyridine **35**, an isomer of **32**, was somewhat less active. Finally, the reduced analogue **37**, tested as a mixture of diastereomers, was only moderately less potent than its unsaturated parent **32**. Although some of the compounds in this pyridone series had satisfactory oral bioavailability in dogs (e.g., 35% for **36**), none of those tested had acceptable bioavailability in rhesus monkeys (e.g., 4% for **36**).

The effectiveness of orally administered **24** in blocking the release of luteinizing hormone (LH) was investigated in castrated male rats,[21] which have relatively stable and elevated circulating LH levels. It should be noted that **24** had an IC_{50} value of 1.7 nM against the rat GnRH receptor in a whole cell binding assay. Compound **24** dose-dependently inhibited LH release for periods ranging from 5–7 h at 5 mg/kg po to >15 h at 20 mg/kg po. In a representative experiment, a single dose of **24** at 10 mg/kg po completely suppressed plasma LH levels for 13 h (Fig. 1).

In conclusion, enhancements to our previously reported series of indole-based GnRH antagonists[3] were undertaken with the goal of improving pharmacodynamic and pharmacokinetic properties. With the addition of the (*S*)-β-methyl substituent to the tryptamine core, the amino moiety of the tertiary amide was re-investigated. Amides derived from compact, hydrophobic bicyclic amines were preferred, with the isoquinuclidine amide apparently optimum. Subsequently, it was found that, with these features in place, the side-chain tether linking

Table 3. Inhibition of GnRH receptor binding and PI turnover by tryptamine derivatives with two-carbon tether to a heterocycle



Compd	Het	hGnRH IC_{50} (nM)[a]	PI turnover IC_{50} (nM)[b]
31		5.3	82
32		0.2	1.4
33		0.4	3.9
34		0.3	1.0
35		1.0	11
36		0.6	4.0
37		0.4	8.2

[a]Inhibition of [^{125}I]buserelin binding to human pituitary GnRH receptor.
[b]Inhibition of GnRH-stimulated [^{3}H]inositol phosphate hydrolysis.

the NH to the pyridine ring could be contracted from four methylene units to two. This modification maintained high potency and improved pharmacokinetic properties, with the result that **24** had an acceptable half-life and oral bioavailability of >20% in both dogs and rhesus monkeys. Furthermore, a single oral dose of **24** at 10 mg/kg provided complete and prolonged suppression of circulating LH levels in a castrated rat model.

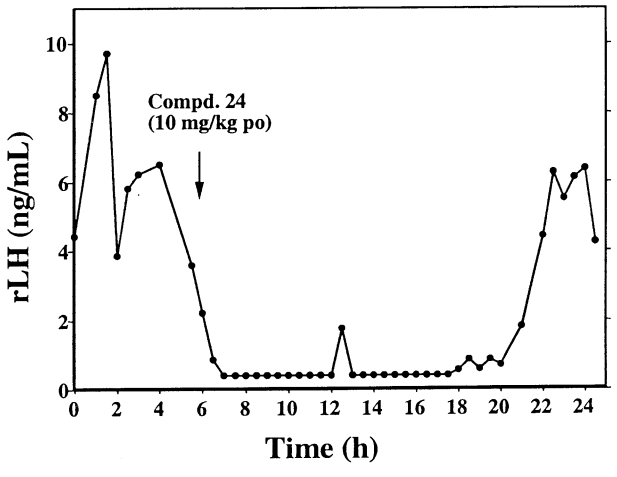


Figure 1. Effect of oral **24** (administration at time = 6 h indicated by arrow) on LH release in a castrated male rat, as described in ref 21.

Acknowledgements

We are grateful to Amy Bernick and Dr. Lawrence Colwell for providing mass spectral data, to Robert Frankshun, Amanda Makarewicz, Joseph Simeone, Mamta Parikh, and Dr. Peter Lin for preparation of certain intermediates, to Dr. Jonathan Young for supplying conditions for the Fukuyama–Mitsunobu amine alkylation methodology, and to Joseph Simeone and Robert Bugianesi for permission to use their unpublished data. We are indebted to Jinchang Chen and Mellissa Creighton in Drug Metabolism for pharmacokinetics support. For assistance in the animal experiments, we also thank Klaus Schleim in Animal Pharmacology, as well as Dr. Judy Fenyk-Melody, Dr. William Feeney, Dr. Susan Iliff, Donald Hora, Paul Cunningham, Bonnie Friscino, Alison Kulick, and Allison Parlapiano in Comparative Medicine.

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21. Castrated Wistar male rats (350–400 g) were surgically fitted with a chronic indwelling catheter inserted into the femoral artery for blood sampling. After the surgery, the rats were housed individually in metabolic cages to recover for at least 3 days. On the day of the experiment, the catheter was connected to an automatic blood micro-sampler via a swivel, which allowed the animal to move freely inside the cage. In this experiment, the animals were dosed orally with **24** at 10 mg/kg, and a 140 μL blood sample was withdrawn every 30 min for 24 h (6 h for pre-dosing baseline and 18 h post-dosing). After each blood sampling, the same amount of saline was returned to the animals to maintain the volume of blood constant. The LH content in serum was determined by a double antibody radioimmunoassay procedure.