Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3329–3332

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Modification of the Pyridine Moiety of Non-peptidyl Indole GnRH Receptor Antagonists

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Received 10 July 2002; accepted 12 August 2002

Abstract—The synthesis of a number of indole GnRH antagonists is described. Oxidation of the pyridine ring nitrogen, combined with alkylation at the two position, led to a compound with an excellent in vitro activity profile as well as oral bioavailability in both rats and dogs.

With the recent discovery of non-peptidyl Gonadotropin Releasing Hormone (GnRH) receptor antagonists the possibility arises for new and better treatment for hormone dependent diseases such as endometriosis, breast cancer, and prostate cancer. Many of the current therapeutic agents act as agonists to the receptor.[1] However, one of the major drawbacks of these agonists are that they cause an initial rise in hormone levels before the desensitization of the receptor occurs. This troublesome phenomenon, known as the 'flare effect', actually makes the symptoms of the disease worse for a short time. Due to the inherent nature of antagonist therapy, this problem does not arise. In addition, non-peptidyl receptor antagonists offer the possibility for oral administration.

Previously reported efforts in these laboratories have led to the discovery of two novel classes of non-peptide GnRH antagonists.[2,3] Recent modifications of the 2-aryl indole class (Fig. 1), such as incorporation of a chiral β-methyl substituent[4] and the truncation of the distal side chain to a two carbon tether[5] have produced improvements in these compounds in vitro potency as well as their pharmacokinetic profile. Recently, our efforts have concentrated on modification of the pyridine portion of the molecule. Results of this study are described in this letter.

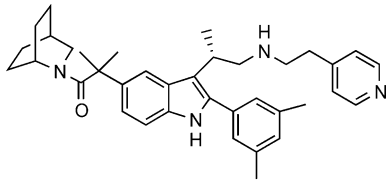
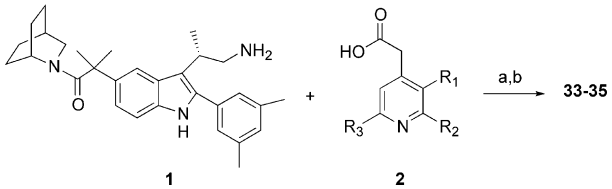


Figure 1. A Merck GnRH receptor antagonist.

Assembly of the compounds of interest was achieved via either coupling of tryptamine **1**[6] with pyridine acetic acid derivative **2** (Scheme 1) or dinitrobenzene sulfonamide



Scheme 1. (a) Et_3N, pyBOP, CH_2Cl_2, rt, 16 h; (b) 2 M $(CH_3)_2SBH_3$, rt, 16 h.

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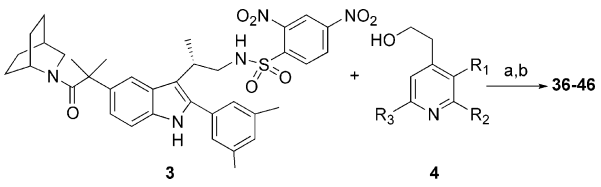
PII: S0960-894X(02)00751-5

tryptamine **3**[6] with pyridine ethanol derivatives **4** (Scheme 2). For the acids, standard peptide coupling conditions resulted in a diamide, of which, the secondary amide was selectively reduced using borane dimethylsulfide. In the case of the alcohols, coupling was achieved under Fukuyama Mitsunobu conditions[7] followed by deprotection with *n*-propylamine.

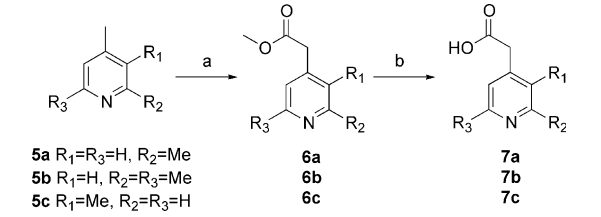


Scheme 2. (a) PPh_3, DEAD, CH_2Cl_2, rt, 3 h; (b) *n*-$PrNH_2$, CH_2Cl_2, rt, 20 min.

The acid and alcohol derivatives themselves were prepared through one of four different routes outlined below.

Methoxycarbonylation

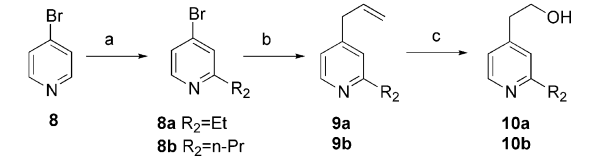
Methyl substituents were introduced around the ring via methoxycarbonylation of the appropriate lutidine or collidine with dimethyl carbonate (Scheme 3). After hydrolysis of the resulting methyl ester, pyridine acetic acids **7a**–**c** were obtained.



Scheme 3. (a) Diisopropylamine, *n*-BuLi, dimethyl carbonate, 0 °C, 2 h; (b) concd HCl, reflux, 1 h or NaOH, H_2O, THF, 4 h.

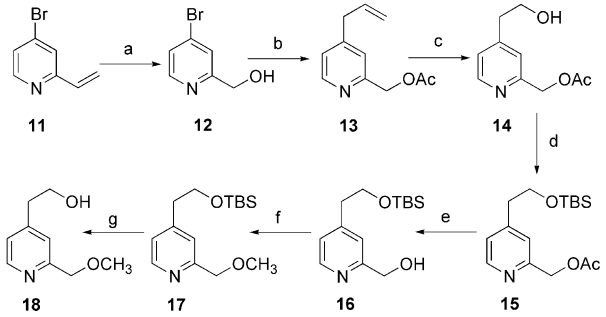
Comins Alkylation

Alternatively, the reaction conditions first reported by Comins[8] were used to prepare 2-ethyl and propyl derivatives (Scheme 4). The alkylation proceeds via activation of the pyridine ring with phenyl chloroformate. This is followed by addition of a Grignard reagent and treatment with *o*-chloranil. Stille coupling[9] of the alkylated bromides followed by ozonolysis and sodium borohydride reduction resulted in pyridine ethanols **10a** and **10b.**



Scheme 4. (a) (1) RMgX, phenylchloroformate −78 °C, 30 min; (2) *o*-chloranil, acetic acid, toluene, rt, 16 h; (b) allyl tributyltin, $Pd(PPh_3)_2Cl_2$, 90 °C, 90 min; (c) (1) ozone, 2 N HCl, H_2O, rt 1 h; (2) $NaBH_4$, CH_3OH, NaOH, 0 °C to rt 20 min.

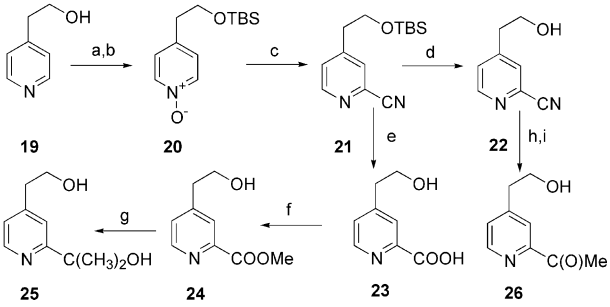
Compound **11** (Scheme 5) was prepared via the Comins method and converted to **12** by standard ozonolysis conditions followed by in situ sodium borohydride reduction. The resulting alcohol was protected as the acetate and allylated via Stille coupling in one pot to yield **13**. A second ozonolysis and reduction gave the acetoxy derivative **14** which was coupled to tryptamine **3**. Further elaboration of **14** was achieved via protection of the alcohol as the TBS ether **15** and saponification of the acetate. This was followed by alkylation of the alcohol **16** to the methyl ether **17** with iodomethane and removal of the TBS protecting group to give **18**.



Scheme 5. (a) (1) Ozone, 2 N HCl, H_2O, rt, 90 min; (2) $NaBH_4$, CH_3OH, NaOH, 0 °C to rt 2 h; (b) (1) acetic anhydride, triethylamine, rt, 3 h; (2) Allyl tributyltin, $Pd(PPh_3)_2Cl_2$, 90 °C, 90 min; (c) (1) ozone, 2N HCl, H_2O, rt 1 h; (2) $NaBH_4$, CH_3OH, NaOH, 0 °C to rt 20 min; (d) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 10 min; (e) 0.5 N KOH/CH_3OH, H_2O, rt, 10 min; (f) NaH, CH_3I, DMF, 0 °C, 1 h; (g) TBAF, THF, rt, 48 h.

Reissert Reaction

The third synthetic route (Scheme 6) involved commercially available 4-(2-hydroxyethyl)pyridine **19**. This was protected as the TBS ether and oxidized with hydrogen peroxide under methyl trioxorhenium catalysis[10] to give the *N*-oxide **20**. Treatment with trimethylsilylcyanide and dimethyl carbamoyl chloride under Reissert reaction conditions[11] resulted in the nitrile **21** which was deprotected with TBAF to give **22**. Compound **21** was further converted to the carboxymethyl analogue **24** via hydrolysis of the nitrile and esterification of the car-



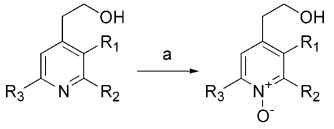
Scheme 6. (a) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 10 min; (b) $MeReO_3$, H_2O_2, CH_2Cl_2, rt, 1 h; (c) TMSCN, dimethylcarbamoyl chloride, CH_2Cl_2, rt, 23 h; (d) TBAF, THF, rt, 48 h; (e) 6 N HCl, reflux, 24 h; (f) methanol, cat. H_2SO_4, reflux, 16 h; (g) CH_3MgBr, THF, −20 °C, 30 min; (h) acetic anhydride, pyridine, rt, 16 h; (i) CH_3MgBr, THF, 0 °C to rt.

boxylic acid **23**. In addition, ester **24** was treated with two equivalents of methyl Grignard thereby yielding the tertiary alcohol **25**.

Conversion of the nitrile **22** to the ketone **26** was achieved in two steps, via treatment with acetic anhydride and pyridine followed by methyl Grignard addition.

Sharpless Oxidation

The *N*-oxide derivatives (Scheme 7) were prepared starting from the appropriate pyridine ethanol and using hydrogen peroxide under methyl trioxorhenium catalysis to oxidize the pyridine nitrogen.



27a R_1=R_2=R_3=H **28a**
27b R_1=R_3=H, R_2=Me **28b**
27c R_1=H, R_2=R_3=Me **28c**
27d R_1=Me, R_2=R_3=Me **28d**

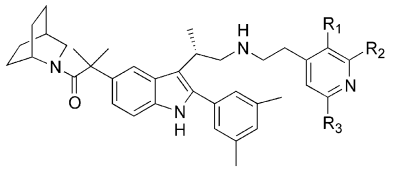
Scheme 7. (a) $MeReO_3$, H_2O_2, CH_2Cl_2, rt, 1–48 h.

Tables 1 and 2 list the activities of compounds[12] prepared using the protocols described above. Data from the hGnRH assay are expressed in terms of IC_{50}s for the inhibition of [I^{125}] labeled buserelin binding to the human GnRH receptor.[13] For the PI Turnover assay, activities are expressed as IC_{50} values representing the inhibition of phosphatidyl inositol hydrolysis in Chinese hamster ovary cells stably expressing human GnRH receptors.[13] All in vitro data represents an average of two experiments ($n=2$); except in cases where the standard error is given ($n\geq4$). Pharmacokinetic studies were conducted via IV and oral dosing of at least two subject animals with blood collected at various time points for analysis .

We began by assessing the effect of methyl group substitution at different locations on the ring. As previously disclosed, having the pyridine in a para linked configuration was important for maintaining oral activity.[5] As seen in Table 1, entries **29**–**31** are all potent binders of the human GnRH receptor. However, analogue **29**, which has a methyl group *ortho* to the pyridine nitrogen, was a much more effective antagonist in the PI turnover assay. In addition, **29** had excellent oral bioavailability in dogs (36%). Increasing the size of the alkyl chain by one or two carbons had a deleterious effect on activity in both assays, as seen from entries **32** and **33**.

The introduction of heteroatom functionality at the position *ortho* to the pyridine nitrogen, produced a number of subnanomolar binders to the human receptor, with the exception of the carboxylic acid **34**. In the PI turnover assay, those analogues containing an ether or ester functionality, such as **35**–**37**, performed poorly, as did the tertiary alcohol **38**. Substituents with less steric bulk around the heteroatom produced the most effective inhibitors, as can be seen from entries **39**–**42**.

Table 1. Substituent effects along various positions of the pyridine ring



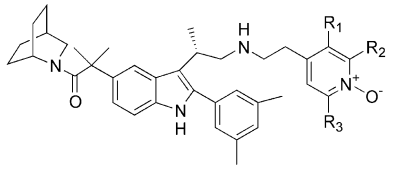
Compd	R_1	R_2	R_3	hGnRH IC_{50} (nM)	PI turnover IC_{50} (nM)
29	H	Me	H	0.9	8.7±4.6
30	H	Me	Me	0.4	24
31	Me	H	H	0.8	48
32	H	Et	H	3.3	19
33	H	*R*-Pr	H	4.6	62
34[a]	H	COOH	H	8.2	371
35	H	CH_2OAc	H	0.4	22
36	H	CH_2OMe	H	0.7	55
37	H	COOMe	H	0.9	25
38	H	$C(CH_3)_2OH$	H	0.7	106
39	H	CN	H	0.7	11
40	H	C(O)Me	H	0.6	12
41[b]	H	C(OH)Me	H	0.6	20
42[c]	H	CH_2OH	H	0.4	7.5±1.1

[a]Obtained from **37** under the following conditions: 0.5 N KOH in CH_3OH, H_2O, rt, 1 h.
[b]Obtained from **40** under the following conditions: $NaBH_4$, CH_3OH, rt, 20 min.
[c]Obtained from **35** under the following conditions: 0.5 N KOH/ CH_3OH, H_2O, rt, 10 min.

Of particular interest was the hydroxymethyl pyridine analogue **42**, which exhibited an excellent in vitro profile. However, its pharmacokinetic properties proved to be less than desirable, exhibiting only moderate bioavailability in dogs (15%) with a short terminal plasma half-life (2 h). Further exploration into substituents containing nitrogen and sulfur proved to be fruitless,[14] thus we turned our attention to modification of the pyridine nitrogen.

Oxidation of the pyridine nitrogen was thought to be a metabolic event upon oral dosing in certain members of this class of compounds. Therefore, we set out to prepare *N*-oxide derivatives with the hope of retaining GnRH antagonist activity, while at the same time improving the pharmacokinetic profile. We were pleased to find that in almost every case, the presence of the

Table 2. The effect of *N*-oxidation on GnRH antagonist activity



Compd	R_1	R_2	R_3	hGnRH IC_{50} (nM)	PI turnover IC_{50} (nM)
43	H	H	H	0.3	6.4
44	H	Me	H	0.3	5.9±0.4
45	H	Me	Me	0.5	10±3.4
46	Me	H	H	0.8	26

N-oxide boosted the activity in both assays (with the exception of **43**[5]). Table 2 shows the results of this study. While compounds **45** and **46** showed improvement over their reduced counterparts, they were weaker antagonists than both the unsubstituted pyridine *N*-oxide **43** and the ortho methyl pyridine *N*-oxide **44**. However, compound **45** did exhibit favorable pharmacokinetics in a single species (bioavailability 29%, half-life 7 h in dogs). In contrast, the bioavailability of **43** was rather low (14%). However, further evaluation of **44** resulted in calculated oral bioavailabilities of 25% in dogs (half-life 5 h) and 23% in rats (half-life 2 h).

In conclusion, modifications to the pyridine portion of the 2-aryl indole class of Gonadotropin Releasing Hormone receptor antagonists resulted in several potent new inhibitors. It was quickly determined that the position *ortho* to the nitrogen atom in the ring tolerated methyl substitution as well as a variety of oxygen containing functional groups. In addition, the *ortho* methyl analogue **29** exhibited excellent oral bioavailability in dogs. Oxidation of the pyridine nitrogen coupled with methyl substitution at various positions on the ring resulted in three compounds with excellent in vitro profiles. Among these, compound **45** had significant oral levels in dogs, while the *ortho* methyl pyridine *N*-oxide **44**, showed greater than 20% bioavalibility in two species.

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