Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1727–1731

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Potent Nonpeptide GnRH Receptor Antagonists Derived from Substituted Indole-5-carboxamides and -acetamides Bearing a Pyridine Side-Chain Terminus

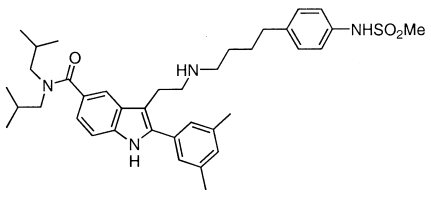
Wallace T. Ashton,[a,*] Rosemary M. Sisco,[a] Yi Tien Yang,[b] Jane-Ling Lo,[b] Joel B. Yudkovitz,[b] Patrice H. Gibbons,[b] George R. Mount,[b] Rena Ning Ren,[b] Bridget S. Butler,[b] Kang Cheng[b] and Mark T. Goulet[a]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065-0900, USA*
[b]*Department of Biochemistry and Physiology, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065-0900, USA*

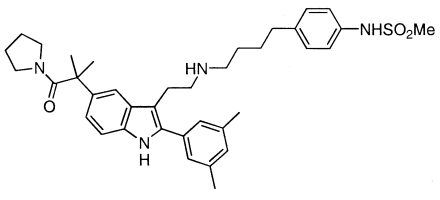
Received 16 March 2001; accepted 19 April 2001

Abstract—A pyridine side-chain terminus has been incorporated into the indole-5-carboxamide and indole-5-acetamide series of GnRH antagonists. Potent activity was observed in binding and functional assays. Certain branched or cyclic tertiary amides were identified as preferred in each series. Alkylation of the side-chain secondary amine had generally unfavorable effects. Variations of the *gem*-dialkyl substituents in the indole-5-acetamide series were also investigated.

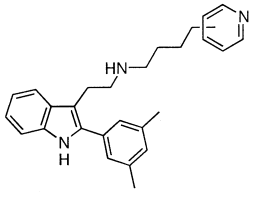
As described in our previous paper,[1] we have sought orally active, nonpeptide receptor antagonists of gonadotropin-releasing hormone (GnRH; also known as luteinizing hormone-releasing hormone, or LHRH). Such compounds could be desirable drugs for therapeutic applications requiring suppression of sex hormone levels.[2] Potential uses include treatment of certain hormone-dependent cancers, uterine fibroids, and endometriosis, as well as assisted reproduction protocols.[2,3] Following previous work from these laboratories,[4] we reported potent GnRH antagonists derived from indole-5-carboxamides and -acetamides, exemplified by structures **1** and **2**.[1] Our original compounds contained phenol or aryl methanesulfonamide groups terminating the side chain at the 3-position of the indole. Because of unfavorable pharmacokinetics, alternative end groups had been investigated. It was reported[5] that 3-pyridyl and 4-pyridyl termini, as in **3** and **4**, were compatible with good potency at the GnRH receptor. We now describe the incorporation of the pyridyl terminus into the indole-5-carboxamide and -acetamide series of GnRH antagonists. A series of indole-5-carboxamides incorporating pyridyl end groups was prepared according to Scheme 1. The 3- and 4-pyridylbutanols **5**[6] were oxidized under Swern conditions[5] to the aldehydes **6**.[7] Reductive amination with the



1



2



3 (pyridin-3-yl)
4 (pyridin-4-yl)

*Corresponding author. Fax: +1-732-594-5350; e-mail: wally_ashton@merck.com

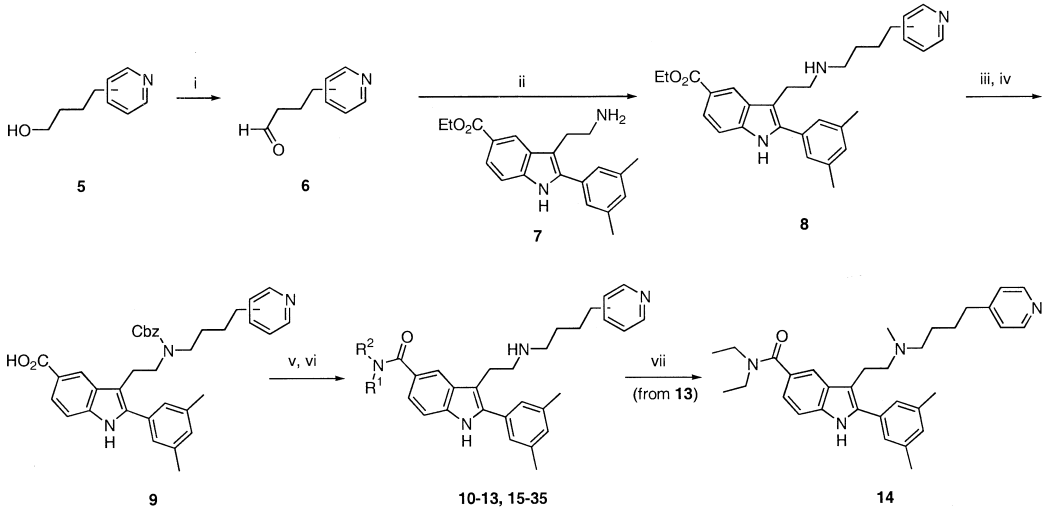
0960-894X/01/$ - see front matter
PII: S0960-894X(01)00275-X

tryptamine derivative **7**[1] furnished **8**. After saponification of the ester, the secondary amine was protected to give Cbz derivative **9**. Next, carboxylic acid **9** was coupled with amines in the presence of PyBOP reagent. Finally, deprotection by catalytic hydrogenolysis afforded the amide products **10**–**13** and **15**–**35**. The *N*-methylated analogue **14** was obtained from **13** by reductive amination using paraformaldehyde and sodium cyanoborohydride.

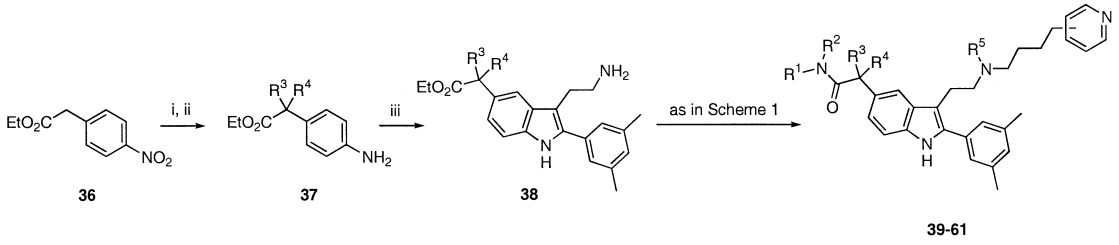
A series of analogues containing *gem*-dialkylacetamide substituents, as in **2**, was prepared as shown in Scheme 2. By known methods,[8] ethyl (4-nitrophenyl)acetate (**36**) was dialkylated and then hydrogenated to give **37**. These intermediates were converted to the tryptamines **38** by our previously described procedures.[1] Further elaboration to target compounds **39**–**61** was carried out as described for Scheme 1.

In the binding assay, compounds were initially tested for the ability to compete with the GnRH receptor agonist [^{125}I]-buserelin for binding to the rat GnRH receptor[9] in the presence of 0.1% BSA. During the course of this study, the rat receptor binding assay was replaced by a similar human GnRH receptor binding assay,[9] which was more sensitive to structure–activity relationships and was considered more relevant to the drug design process. Two functional assays were used to further characterize many of the compounds: inhibition of GnRH-stimulated LH release from rat primary pituitary cells[9] and inhibition of GnRH-stimulated phosphatidylinositol (PI) hydrolysis in cloned Chinese hamster ovary (CHO) cells stably expressing the human GnRH receptor.[9]

It is apparent from the data in Table 1 that addition of a carboxamide to the 5-position of the indole dramatically increases binding affinity to the rat GnRH receptor compared to the parent analogues **3** and **4**. Although potency at the rat receptor did not vary greatly among the amides, all of which had subnanomolar IC_{50} values, greater differences were seen for the human receptor. Several bulky, hydrophobic, branched or cyclic tertiary amides (**24**–**28** and **31**) were most favorable for binding to the human GnRH receptor. Some of these same compounds, notably **26**–**28**, were also among the most effective in the LH release and PI turnover assays. Nevertheless, the results from the functional assays did not always track with those of the binding studies. Thus, the diethyl amide **13** proved to be considerably more active ($IC_{50} = 12$ nM) in the PI inhibition experiment than would have been predicted from its performance in the other tests. In all of the assays, the attachment point of the terminal heterocycle (i.e., 3-pyridyl or 4-pyridyl) had little effect.



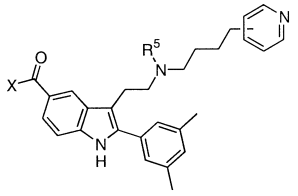
Scheme 1. Conditions: (i) (a) oxalyl chloride, DMSO, CH_2Cl_2, −60 °C; (b) Et_3N, −65 °C; (ii) (a) $MgSO_4$, $CDCl_3$, −5 to −10 °C; (b) $NaBH_4$, MeOH, −5 °C; (iii) Cbz-Cl, *i*-Pr_2NEt, CH_2Cl_2–THF, −78 °C; (iv) (a) KOH, MeOH–H_2O, Δ; (b) H^+; (v) R^1R^2NH, PyBOP, Et_3N, CH_2Cl_2; (vi) H_2, $Pd(OH)_2/C$, 2-methoxyethanol; (vii) paraformaldehyde, $NaBH_3CN$ (4 equiv), AcOH (10 equiv), powdered 4 Å molecular sieves, MeOH–THF.



Scheme 2. Conditions: (i) (a) NaH, DMF; (b) R^3I, R^4I; (ii) H_2, Pd/C, EtOH; (iii) procedures described in ref 1.

Except for the primary amide **39**, most of the indole-5-acetamides (Table 2) were potent antagonists at the human GnRH receptor, several having IC_{50} values of 1–3 nM. Low nanomolar IC_{50} values at this receptor were achieved with smaller amide *N*-substituents than in the indole-5-carboxamide series. Again, 3- and 4-pyridyl analogues were similar in potency. Among the most effective compounds in the binding and functional

Table 1. Inhibition of GnRH receptor binding, LH release, and PI turnover by indole-5-carboxamides with pyridine end groups

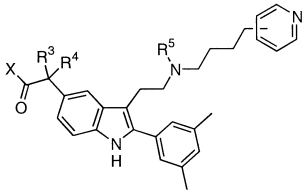


Compd	X	Pyridine link	R^5	GnRH binding IC_{50} (nM)[a]		LH release IC_{50} (nM)[b]	PI turnover IC_{50} (nM)[c]
				Rat	Human		
3[d]	[H][e]	3	H	40			
4[d]	[H][e]	4	H	16			
10	Me_2N	3	H	0.7		2600	
11	Me_2N	4	H	0.8		1460	
12	Et_2N	3	H	0.6	23	400	190
13	Et_2N	4	H	0.5	18	470	12
14	Et_2N	4	Me		36	430	58
15	$HOCH_2CH_2N(Et)$	3	H	0.6		1200	
16	$HOCH_2CH_2N(Et)$	4	H		36	1000	260
17	$(MeOCH_2CH_2)_2N$	3	H		45	1300	610
18	$(CF_3CH_2)_2N$	4	H	0.6	36	3600	91
19	*i*-PrNH	3	H		55	>1300	1200
20	*i*-PrN(Et)	3	H		11	830	110
21	*i*-Pr_2N	4	H	0.3		400	
22	*n*-Bu_2N	3	H	0.4		1000	
23	*n*-Bu_2N	4	H	0.4		240	56
24	*i*-Bu_2N	3	H	0.7	5.0	150	120
25	*i*-Bu_2N	4	H	0.6	7.1	380	80
26	Cyclohexyl-N(Et)	3	H	0.2	2.3	140	16
27	Cyclohexyl-N(Et)	4	H	0.4	2.8	130	22
28	Cyclooctyl-N(Et)	4	H		1.9	81[f]	15
29		3	H	0.6		5100	
30		4	H	0.5		>1300	
31		3	H		3.6	360	62
32		4	H	0.3		340	
33		3	H	0.4		1600	
34		4	H	0.3		1400	
35		4	H		32		260

[a]Inhibition of binding of [^{125}I]buserelin to rat or human pituitary GnRH receptor.
[b]Inhibition of GnRH-stimulated LH release from rat pituitary cells.
[c]Inhibition of GnRH-stimulated [^{3}H]inositol phosphate hydrolysis.
[d]Ref 5.
[e]Substituent in place of XCO- at indole 5-position.
[f]Modified assay conditions using 0.2 nM instead of 2 nM GnRH.

assays were the 7-azanorbornane amides **53** and **54** despite the relatively poor performance of the corresponding analogue **35** in the indole-5-carboxamide series. Other particularly active analogues in the PI turnover assay included the diisobutyl amide **45** and the 2,5-dimethylpyrrolidine amide **50**. *N*-Methylation of the secondary amine (**55**; cf. **54**) had no significant effect on potency in the receptor binding and LH release assays, as was also the case for **14** in Table 1. However, a 6-fold loss in activity was observed for **55** versus the parent **54** in the PI turnover assay. Increasing the size of the *N*-alkyl group (**56**–**58**) tended to reduce activity in the assays. In our earlier series,[1] *gem*-dimethylation of the acetamide side chain was found to enhance activity, and this substitution pattern may also be favorable for reducing metabolism. Here, replacing one or both of the

Table 2. Inhibition of GnRH receptor binding, LH release, and PI turnover by indole-5-acetamides with pyridine end groups



Compd	R^3	R^4	X	Pyridine link	R^5	hGnRH IC_{50} (nM)[a]	LH release IC_{50} (nM)[b]	PI turning IC_{50} (nM)[c]
39	Me	Me	H_2N	3	H	32		350
40	Me	Me	Me_2N	3	H	[0.5][d]	680	
41	Me	Me	Me_2N	4	H	[0.3][d]	930	
42	Me	Me	Et_2N	3	H	3.3	240	94
43	Me	Me	Et_2N	4	H	3.2	770	58
44	Me	Me	*i*-Bu_2N	3	H	1.7	400	22
45	Me	Me	*i*-Bu_2N	4	H	2.8	140	8.3
46	Me	Me	*c*-HxN(Et)	4	H	2.3	960 350[e]	54
47	Me	Me		3	H	1.0	190	62
48	Me	Me		3	H	5.0	290	61
49	Me	Me		4	H	4.8	340	
50	Me	Me		4	H	2.8	160	15
51	Me	Me		4	H	1.8	1200	38
52	Me	Me		4	H	11	540	59
53	Me	Me		3	H	1.4	73[e]	14
54	Me	Me		4	H	1.4	71[e]	18
55	Me	Me		4	Me	1.5	71[e]	110
56	Me	Me		4	Et	2.2	730[e]	90
57	Me	Me		4	*i*-Pr	3.1		200
58	Me	Me		4	*i*-Bu	8.2		300

(*continued on next page*)

Table 2 (*continued*)

Compd	R^3	R^4	X	Pyridine link	R^5	hGnRH IC_{50} (nM)[a]	LH release IC_{50} (nM)[b]	PI turning IC_{50} (nM)[c]
59	Me	Et		4	H	1.0	240[e]	38
60	Et	Et		4	H	1.7	260[e]	40
61	-$(CH_2)_3$—			4	H	1.2		170

[a]Inhibition of binding of [^{125}I]-buserelin to human pituitary GnRH receptor.
[b]Inhibition of GnRH-stimulated LH release from rat pituitary cells.
[c]Inhibition of GnRH-stimulated [^{3}H]inositol phosphate hydrolysis.
[d]Data for inhibition of rat GnRH receptor.
[e]Modified assay conditions using 0.2 nM instead of 2 nM GnRH.

gem-dimethyl substituents by ethyl (**59** and **60**) or connecting them in a four-membered ring (**61**) was reasonably well tolerated but offered no advantages.

In summary, the combination of a 3- or 4-pyridyl terminus on the indole-3-side chain and carboxamide or acetamide substituents at the indole 5-position afforded potent antagonists at rat and human GnRH receptors. These compounds were also effective in blocking GnRH-stimulated release of LH from rat pituitary cells and GnRH-stimulated PI turnover in CHO cells expressing cloned human GnRH receptors. Nonpolar tertiary amines were most potent in each series. Among the indole-5-carboxamides, a preferred analogue was the bulky *N*-cyclooctyl-*N*-ethyl amide **28**, with IC_{50} values of 1.9, 81, and 15 nM in the receptor binding, LH release, and PI turnover assays, respectively. A somewhat different structure–activity relationship was observed for amides in the indole-5-acetamide series. Here the most favorable amides were those derived from diisobutylamine (**45**), 2,5-dimethylpyrrolidine (**50**), and 7-azanorbornane (**53** and **54**). For example, **53** had IC_{50} values of 1.4, 73, and 14 nM in the binding, LH release, and PI turnover assays, respectively. In both series, other structural modifications, involving variation of the *gem*-dialkyl substituents or alkylation on the secondary amine of the side chain, had neutral or negative effects. Further structural variations, leading to GnRH antagonists with improved pharmacokinetics and in vivo activity, will be described in a subsequent report.

Acknowledgements

The authors are grateful to Amy Bernick and Dr. Lawrence Colwell for providing mass spectral data and to Mark Levorse, Robert Frankshun, Glenn Reynolds, Judith Pisano, Joseph Leone, Dr. Ranjit Desai, Joseph Simeone, Mamta Parikh, and Dr. Peter Lin for preparation of certain intermediates.

References and Notes

1. Ashton, W. T.; Sisco, R. M.; Yang, Y. T.; Lo, J.-L.; Yudkovitz, J. B.; Cheng, K.; Goulet, M. T. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1723.
2. (a) Cho, N.; Harada, M.; Imaeda, T.; Imada, T.; Matsumoto, H.; Hayase, Y.; Sasaki, S.; Furuya, S.; Suzuki, N.; Okubo, S.; Ogi, K.; Endo, S.; Onda, H.; Fujino, M. *J. Med. Chem.* **1998**, *41*, 4190. (b) Millar, R. P.; Zhu, Y.-F.; Chen, C.; Struthers, R. S. *Brit. Med. Bull.* **2000**, *56*, 761.
3. (a) Schally, A. V.; Comaru-Shally, A. M. *Adv. Drug Deliv. Rev.* **1997**, *28*, 157. (b) Kutscher, B.; Bernd, M.; Beckers, T.; Polymeropoulos, E. E.; Engel, J. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2148. (c) Felberbaum, R. E.; Ludwig, M.; Diedrich, K. *Mol. Cell. Endocrinol.* **2000**, *166*, 9.
4. (a) Chu, L.; Hutchins, J. E.; Weber, A. E.; Lo, J.-L.; Yang, Y. T.; Cheng, K.; Smith, R. G.; Fisher, M. H.; Wyvratt, M. J.; Goulet, M. T. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 509. (b) Chu, L.; Lo, J.-L.; Yang, Y. T.; Cheng, K.; Smith, R. G.; Fisher, M. H.; Wyvratt, M. J.; Goulet, M. T. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 515. (c) Lin, P.; Marino, D.; Lo, J.-L.; Yang, Y.-T.; Cheng, K.; Smith, R. G.; Fisher, M. H.; Wyvratt, M. J.; Goulet, M. T. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1073.
5. Lin, P.; Parikh, M.; Lo, J.-L.; Yang, Y.-T.; Cheng, K.; Smith, R. G.; Fisher, M. H.; Wyvratt, M. J.; Goulet, M. T. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1077.
6. Mayer, J. M.; Testa, B. *Helv. Chim. Acta* **1982**, *65*, 1868.
7. For 3-pyridyl: Wanner, M. J.; Koomen, G.-J. *Eur. J. Org. Chem.* **1998**, 889.
8. (a) Munakata, H.; Kobayashi, M.; Wagatsuma, K.; Sato, S.; Tsurufuji, M.; Matsumura, S.; Enomoto, H. European Patent Application 52,296, 1982; *Chem. Abstr.* **1982**, *97*, 182862. (b) Carney, R. W. J.; de Stevens, G. U.S. Patent 3,641,040, 1972; *Chem. Abstr.* **1972**, *77*, 5532.
9. Walsh, T. F.; Toupence, R. B.; Young, J. R.; Huang, S. X.; Ujjainwalla, F.; DeVita, R. J.; Goulet, M. T.; Wyvratt, M. J., Jr.; Fisher, M. H.; Lo, J.-L.; Ren, N.; Yudkovitz, J. B.; Yang, Y. T.; Cheng, K.; Smith, R. G. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 443.