Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2801–2804

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

THE SYNTHESIS OF A NEW CLASS OF OXYTOCIN ANTAGONISTS

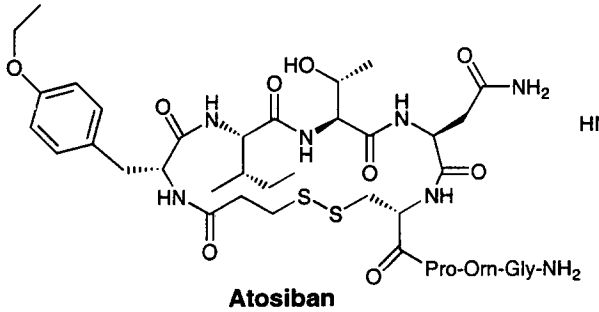
Kazimierz Wiśniewski,[a,*] Jerzy Trojnar,[a] Pierre Riviere,[a] Robert Haigh,[b] Chris Yea,[b] Doreen Ashworth,[b] Per Melin,[c] and Anders Nilsson[c].

[a]*Ferring Research Institute, 3550 General Atomic Ct, San Diego, CA 92121, USA*
[b]*Ferring Research Institute, Chilworth Research Centre, Southampton S016 7NP UK*
[c]*Ferring AB, Soldattorpsvägen 5, SE-200 61 Malmö, Sweden*

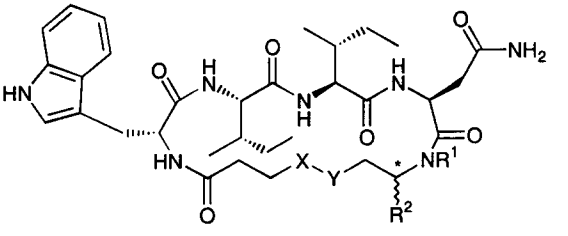
Received 14 April 1999; accepted 23 August 1999

Abstract: The synthesis of a new class of oxytocin antagonists, with significantly modified C-terminal part, is described. The chemistry of the Mitsunobu reaction was applied to obtain the key derivatives. In spite of the extensive modifications of previously described compound F792, the peptides retain biological activity as oxytocin antagonists.

Since the mid-seventies Ferring has been engaged in structure–activity studies on oxytocin antagonists. Modification of the parent hormone at positions 1, 2, 4, and 8 led to a selective antagonist of oxytocin, [D-Tyr(Et)^2,Thr^4,Orn^8]dOT, the drug candidate atosiban.[1,2] The atosiban molecule was further modified by increasing its ring hydrophobicity and shortening its C-terminus. One new lead compound F792, **1a**, was selected.[3,4] The peptide showed a significant improvement in potency, duration of action, and bioavailability as compared to atosiban.



Atosiban



1 (see Table)

The molecule has a hydrophobic oxytocin-like 20 membered ring, and the C-terminal tripeptide is replaced by N-α-MeOrn-NH_2. In order to explore the possibility of further decreasing the size of the C-terminus, we have

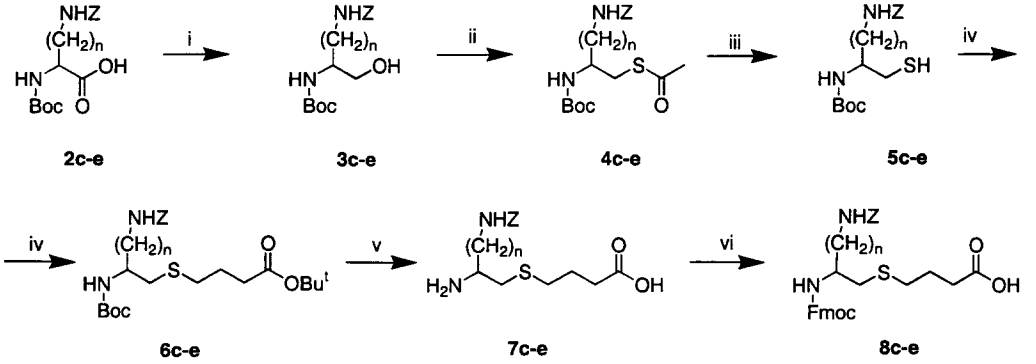
0960-894X/99/$ - see front matter
PII: S0960-894X(99)00478-3

designed and synthesized several analogs (**1c–1f**) of F792 of the structure **1**. We have employed the chemistry of the Mitsunobu reaction[5] to obtain the key derivatives for peptide synthesis.

Chemistry and Biology

The key part of the analog syntheses was the preparation of derivatives Fmoc-NR^1-CH$(CH_2)_n$NHZ-CH_2-S-$(CH_2)_3$-COOH, **8c–e**, (Scheme). The derivative **8f** was prepared analogously from Boc-Pro-OH as a substrate. Boc-amino acid mixed anhydrides were reduced with sodium borohydride to give the corresponding protected β-aminoalcohols, **3**.[6] In order to obtain the required thioether derivatives **6** we employed a strategy based on the Mitsunobu reaction. Extensive precedent for the utility of the Mitsunobu reaction in peptide chemistry[7] suggested that a pathway through an S-acetyl thiol derivative should provide the desired intermediates. Accordingly, the β-aminoalcohols **3** were subjected to standard Mitsunobu conditions (TPP and DEAD in THF)[8] to obtain the corresponding S-acetyl thiols, **4**. Alkaline hydrolysis (MeOH/2 M NaOH) of the thioesters **4**, resulted in the thiols **5**, which were alkylated in situ with 4-bromobutyric acid *t*-butyl ester to give the fully protected derivatives: Boc-NR^1-CHR^2-CH_2-S-$(CH_2)_3$-COO-*t*-Bu, **6**. The Boc and *t*-Bu protecting groups were removed with TFA and the amino function was reprotected with the Fmoc group.[9] The resulting Fmoc-ω-amino acids, **8c–f**, were attached to trityl resin,[10] and the peptides were assembled by manual SPPS using DIC/HOBt couplings. The side-chain protected peptides were cleaved from the resins with 50%TFA/DCM. Cyclizations of the crude peptides were accomplished with TBTU in DMF in high (60–80%) yields. Where necessary, the side-chain protection was removed with either low HF or 1 M TMSBr/thioanisol/TFA.[11] The crude peptides were purified by preparative HPLC. The purity and identity of peptides **1a–f** were assessed by analytical HPLC and MS.

Scheme.



i: 1/NMM, ClCOOBu^i, 2/$NaBH_4$; ii: CH_3COSH, DEAD, TPP; iii: NaOH; iv: $Br(CH_2)_3COOBu^t$; v: TFA/DCM; vi: FmocONSu, TEA.

To obtain compounds **8** from **3** we employed the following three-step procedure: **Step 1.** 13.65 g (52 mmol) of TPP and 8.19 mL (1equiv) of DEAD were dissolved in 130 mL of dry THF. The solution was stirred for 0.5

h at 0 °C resulting in yellow precipitate. To the above suspension a solution of 3.73 mL (1 equiv) of thiolacetic acid and 9.2 g (0.5 equiv) of Boc-Orn(Z)-ol, **3d**, in dry THF was added portionwise over a 1-hour period. After the substrate spot (TLC) disappeared the solvent was evaporated and the residue was dissolved in 100 mL of Et_2O. The resulting white precipitate was filtered off and the solvent was evaporated. The residue was subjected to chromatography on silica eluting with cyclohexane/EtOAc solvent system. 7.4 g (17.9 mmol, 69%) of compound **4d** was obtained. **Step 2.** 5.34 g (13 mmol) of Boc-NH-CH($(CH_2)_3$NHZ)-CH_2-S-CO-CH_3, **4d**, was dissolved in 50 mL MeOH. To the vigorously stirred solution 13 mL (26 mmol) of 2 M NaOH was added under nitrogen. After 2 min 3.05 g (1.05 equiv) of *t*-butyl 4-bromobutyrate was added. Stirring was continued until no intermediate thiol was detected (HPLC monitoring, ca. 6 hr). MeOH was then evaporated, the residual mixture was diluted with water and extracted with AcOEt. After drying over $MgSO_4$ the solvent was evaporated and the oily residue was treated with petroleum ether. 5 g (9.8 mmol, 75%) of a white powder **6d**, was obtained. **Step 3.** 4.53 g (8.9 mmol) of Boc-NH-CH($(CH_2)_3$NHZ)-CH_2-S-$(CH_2)_3$COOBu^t, **6d**, was treated with 40 mL TFA/DCM (1:1) for 0.5 h at room temperature. The solvents were evaporated and the residue was dissolved in 20 mL MeCN/H_2O (1:1) and pH was adjusted to 9 with triethylamine. To the vigorously stirred reaction mixture 2.86 g (0.95 equiv) of solid Fmoc-ONSu was added portionwise over the period of 15 min. The pH of the mixture was maintained at 9 with the addition of triethylamine. The product **8d** was isolated from the reaction mixture by a standard aqueous workup. Yield 4.3 g (7.4 mmol, 83%).

Human Oxytocin Receptor Assay. The cloned human oxytocin receptor[12] was overexpressed in a stable HEK293 cell line. Membranes were prepared by cell lysis (in Tris/HCl 15 mM, EDTA 3 mM, $MgCl_2$ 2 mM, pH7.5, 4 °C, 5 min.), homogenization (20 strokes in a glass/glass homogenizer) and centrifugation (40,000 x *g* for 60 min, 4 °C) collecting the membrane pellet.

Binding assays were performed in 96-well polypropylene microtitre plates in a volume of 100 µL where each well contained homogenized crude membranes (typically 4–8 µg membrane protein/data point) and [^{125}I]OVT (iodinated 8-ornithine vasotocin) diluted in binding buffer (Tris/HCl 100 mM, $MgCl_2$ 10 mM pH7.0, room temperature containing 0.2% (v/v) BSA). Nonspecific binding was determined with 1 µM OVT and 100% binding determined in the absence of any competing ligand but in the presence of vehicle. The plates were incubated at room temperature for 90 min when the assay was stopped by rapid filtration through a glass filter pre-treated with 1% BSA. The filters were washed with 2 x 1 mL/well ice-cold wash buffer followed by drying at 37 °C for 1 h. Scintillant was added to the filter and radioactivity was counted. K_i values were calculated using the method of Cheng and Prusoff[13] using a commercially available curve-fitting program (Graph-Pad Prism).

Results

The activity as oxytocin antagonists of compounds **1a–f** has been investigated in a human receptor assay as well as an in vivo rat model.[14] ID_{50} is defined as a dose of antagonist inhibiting the response of a dose of oxytocin to half of the response. The results are presented in the Table.

Table.

Structure and biological activity of compounds synthesized.

	Chemical structure					Biological activity	
entry	R^1	R^2	X	Y	Conf[a]	K_i, [nM][b]	ID_{50} [nmol/kg][c]
1a	H	-CO-MeOrn-NH_2	S	CH_2	S	-	2.9(9)[d]
1b	H	-CO-NH_2	S	CH_2	S	-	48(4)
1c	H	-$(CH_2)_4NH_2$	CH_2	S	R	2.8	17(4)
1d	H	-$(CH_2)_3NH_2$	CH_2	S	S	26	32(4)
1e	H	-$(CH_2)_3NH_2$	CH_2	S	R	4	5.4(3)
1f	-$(CH_2)_3$-		CH_2	S	S	1000	No activity(5)

[a] Absolute configuration of the marked carbon (see structure 1); [b] Binding constant, human oxytocin receptor assay; [c] Uterus inhibition, in vivo rat, intravenous ID_{50} = dose of an antagonist inhibiting the response of a dose of oxytocin to half of the response; [d] Number of animals in brackets.

The same trends for the activity are observed in both test models. As was shown previously,[3] peptide **1a** (F792) is a very potent oxytocin antagonist. Peptide **1b** (R^2 = -CO-NH_2) appears to be a weak oxytocin antagonist. Both **1a** and **1b** were used as reference compounds since carba-6 (X = S, Y = CH_2) and carba-1 peptides (X = CH_2, Y = S) exhibit a similar profile of biological activity.[15] Analogs in which the C-terminus was replaced by the side chain of ornithine (**1d,e**) and lysine (**1c**) retain antagonistic activity. Analogs **1c,e** with the same absolute configuration (R) at the marked carbon atom (formula **1**) as in oxytocin show higher potency than analog **1d**. The presence of the amino function seems to be crucial for high biological activity. This can be illustrated by the inactivity of analog **1f** in which the pyrrolidine ring in the C-terminal part of the molecule was introduced.

The results obtained indicate that the oxytocin molecule tolerates significant modifications in its C-terminal tripeptide. However, there are some limitations that have been exemplified by the inactivity of the analog **1f**. Further investigations are being carried out and will be reported in due course.

References

1. Melin, P.; Trojnar, J.; Johansson, B.; Vilhardt, H.; Akerlund, M. *J. Endocrinol.* **1986**, *111*, 125.
2. Melin, P.; Trojnar, J.; Vilhardt, H.; Akerlund, M. In *Peptides: Structure and Function* (Proceedings of the 8th American Peptide Symposium); Hruby V. J., Rich, D. H., Eds. Pierce Chemical Company, Rockford 1983; pp 361–364.
3. Aurell, C-J.; Melin, P.; Nilsson, A.; Trojnar, J. Patent WO 95/02609; *Chem. Abstr.* **1995**, *123*, 1047.
4. Nilsson, A.; Aurell, C-J.; Ekholm, K.; Johansson, E.; Melin, P.; Trojnar, J.; Walhagen, K.; Wisniewski, K. In *Peptides 1996* (Proceedings of the 24th European Peptide Symposium); Ramage, R., Epton, R., Eds. Mayflower, England 1997; pp 683–684.
5. Mitsunobu, O. *Synthesis* **1981**, 1.
6. Rodriguez, M.; Llinares, M.; Doulut, S.; Heitz, A.; Martinez, J. *Tetrahedron Lett.* **1991**, *32*, 923.
7. Wisniewski, K.; Kołodziejczyk. A. S.; Falkiewicz, B. *J. Peptide Sci.* **1998**, *4*, 1.
8. Volante, R. P. *Tetrahedron Lett.* **1981**, *22*, 3119.
9. Bolin, D. R.; Sytwu, I-I.; Humiec, F.; Meienhofer. J. *Int. J. Peptide Protein Res.* **1989**, *33*, 353.
10. Barlos, K.; Chatzi, O.; Gatos, D.; Stavropoulos, G. *Int. J. Peptide Protein Res.* **1991**, *37*, 513.
11. Fujii, N.; Otaka, A.; Sugiyama, N.; Hatano, M.; Yajima, H. *Chem. Pharm. Bull.* **1987**, *35*, 3880.
12. Kimura, T.; Tanizawa, O.; Mori, K.; Brownstein, M. J.; Okayama, H. *Nature* **1992**, *356*, 526.
13. Cheng, Y-C.; Prusoff, W. H.; *Biochem. Pharmacol.* **1973**, *22*, 3099.
14. Melin, P.; Vilhardt, H.; Larsson, L-E.; Akerlund, M. *J. Endocrinol.* **1981**, *88*, 173.
15. Jost, K. In *Handbook of Neurohypophyseal Hormone Analogs*; Jost, K., Lebl, M., Brtnik, F., Eds. CRC Press, Boca Raton, 1987; Vol 1, Part 2, pp 144-156.