Communications to the Editor

1-{1-[4-[(*N*-Acetyl-4-piperidinyl)oxy]-2-methoxybenzoyl]piperidin-4-yl}-4*H*-3,1-benzoxazin-2(1*H*)-one (L-371,257): A New, Orally Bioavailable, Non-Peptide Oxytocin Antagonist

Peter D. Williams,*,† Bradley V. Clineschmidt,‡ Jill M. Erb,† Roger M. Freidinger,† Maribeth T. Guidotti,‡ Edward V. Lis,‡ Joseph M. Pawluczyk,† Douglas J. Pettibone,‡ Duane R. Reiss,‡ Daniel F. Veber,† and Carla J. Woyden‡

Departments of Medicinal Chemistry and New Lead Pharmacology, Merck Research Laboratories, West Point, Pennsylvania, 19486

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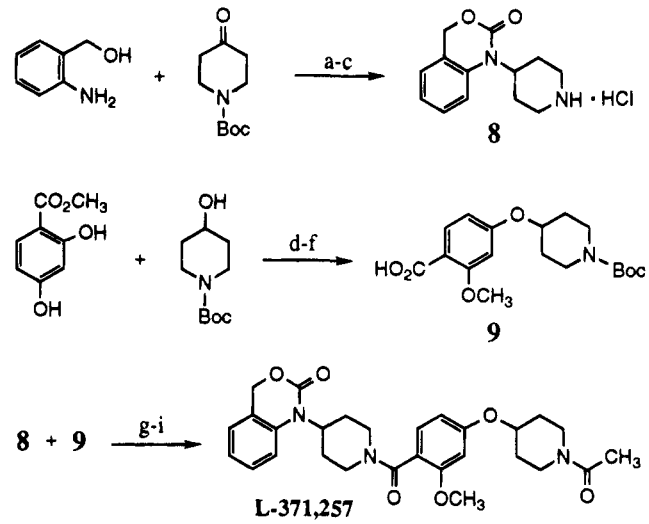
The identification of selective ligands for receptors of the structurally related nonapeptide hormones, oxytocin (OT) and arginine vasopressin (AVP), has been an area of active investigation for the last several decades.[1–3] Several important peptidic agonists and antagonists have resulted from structural modifications to the parent hormones,[4–7] and recently, a number of orally-active, non-peptide OT/AVP antagonists have been reported.[8–14] As part of our continuing interest in OT antagonists as potential new therapeutic agents for treating preterm labor,[15,16] we now report 1-{1-[4-[(*N*-acetyl-4-piperidinyl)oxy]-2-methoxybenzoyl]piperidin-4-yl}-4*H*-3,1-benzoxazin-2(1*H*)-one (L-371,257) as a representative member of a new class of potent, orally bioavailable OT antagonists with excellent selectivity for binding to human OT vs AVP receptors.

Several years ago, the first non-peptide AVP antagonists were reported by researchers at Otsuka. Significant rat AVP-V_{1a}/V_2 receptor selectivity (≥100-fold) and oral activity for inhibiting AVP-mediated responses in rats were demonstrated with OPC 21268[8] and OPC 31260.[10] Subsequent characterization of the AVP-V_{1a} receptor antagonist OPC 21268 revealed a marked species difference in which significantly lower affinity was found for binding to human vs rat AVP-V_{1a} receptors.[14,17,18] We now report that OPC 21268 exhibits moderate affinity for both rat and human uterine OT receptors. Using human tissues, OPC 21268 is a selective OT receptor ligand with >60-fold higher affinity for binding to uterine OT receptors vs AVP-V_{1a} receptors in platelets, uterus and liver (**1**, Table 1).[19]

Several types of structural modifications to OPC 21268 were examined with the goal of increasing human OT receptor affinity (Table 1).[20] Reasonably subtle changes in the lactam portion of the quinolinone ring had variable effects on OT receptor affinity: the quinazolinone **2** had poor affinity, whereas the benzoxazinone analog **3** left affinity essentially unchanged. Constraining the flexible acetamidopropyloxy terminus of **3** proved fruitful. Of the analogs in which the acetamido nitrogen is connected to one of the three carbon atoms in the propyloxy chain to form a six-membered ring (**4**–**6**), the achiral 4-piperidinyloxy variation (**6**) proved to be the most advantageous. Further improvement in receptor affinity was obtained by incorporation of a methoxy group at the 2-position of the central benzoyl ring (**7**, L-371,257). Thus, the structural changes present in **7** have provided >35-fold improvement in human OT receptor affinity vs OPC 21268. Compound **7** also exhibits excellent human OT/AVP receptor selectivity (>600-fold and >7000-fold selectivity for binding to the human uterine OT receptor vs the human platelet AVP-V_{1a} receptor and human kidney AVP-V_2 receptor, respectively). The human OT receptor affinity/selectivity profile of **7** compares favorably with the best of the camphor-based non-peptide OT antagonists.[16,21,22] Using rat tissues, **7** retains the original AVP-V_{1a} receptor selectivity of OPC 21268, but with 10-fold higher affinity at both OT and AVP-V_{1a} sites.

Compound **7** proved to be a potent OT antagonist in functional assays.[23,24] In an *in vitro* assay using isolated rat uterine tissue, **7** was found to be a potent (pA_2 = 8.44 ± 0.26) and competitive (Schild slope: 0.92 ± 0.24) antagonist of OT-induced contractions. Compound **7** also antagonized OT-induced uterine contractions *in vivo* in anesthetized rats in a dose-dependent manner after either intravenous (AD_{50} = 0.55 mg/kg) or intraduodenal (AD_{50} = 2.5 mg/kg) administration with a moderate duration of action (Figure 1). The oral bioavailability of **7** was determined in rats dosed orally at 10 mg/kg and intravenously at 3 mg/kg, using a radioreceptor assay to measure plasma levels of bioactivity. The oral/intravenous ratio of area under the plasma concentration vs time curves (0–6 h), adjusted for dose,

Scheme 1[a]



[a] Reagents: (a) toluene, CH_3CO_2H, reflux ($-H_2O$); $NaBH_3CN$, THF (78%); (b) $CO(OCCl_3)_2$, iPr_2NEt, THF (75%); (c) HCl(gas), EtOAc (99%); (d) diethyl azodicarboxylate, Ph_3P, THF (79%); (e) NaH, CH_3I, DMF (89%); (f) NaOH, H_2O, CH_3OH (95%); (g) 1-[3-(dimethylamino)propyl]-3-ethylcarbodimide hydrochloride, 1-hydroxybenzotriazole, iPr_2NEt, DMF (90%); (h) HCl(gas), EtOAc (99%); (i) $(CH_3CO)_2O$, iPr_2NEt, CH_2Cl_2 (97%).

† Department of Medicinal Chemistry.
‡ Department of New Lead Pharmacology.

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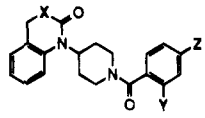
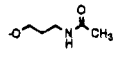
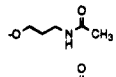
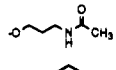
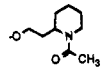
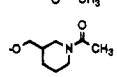
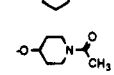
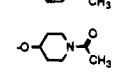
Table 1. Rat and Human OT/AVP Receptor Affinities



cmpd	X	Y	Z	K_i (nM)[a] OT: rat uterus / human uterus	AVP-V_{1a}: rat liver / human platelet	AVP-V_2: rat kidney / human kidney
1 (OPC 21268)	CH_2	H		230 ± 35 170 ± 49	32 ± 5.2 52,000 (14,000; 11,000)[b]	≥30,000 >81,000
2	NH	H		2,400 2,100	>1,000 62,000	>3,000 [c]
3	O	H		200 ± 12 130	31 ± 5.4 36,000	35,000 [c]
4[d]	O	H		130 150	15 22,000	11,000 [c]
5[d]	O	H		340 200	24 38,000	11,000 [c]
6	O	H		69 ± 22 24	5.6 12,000	35,000 [c]
7 (L-371,257)	O	OCH_3		19 ± 2.1 4.6 ± 0.25	3.7 ± 0.79 3,200 ± 82	≥30,000 37,000 ± 5,500

[a] Radioligand binding assays were performed as described in refs 15 and 17. K_i values are reported as group means ±SEM for compounds for which there were at least three separate determinations. OT column: displacement of [^{3}H]OT from specific binding sites in uterine tissue obtained from diethylstilbestrol propionate-pretreated rats or pregnant non-labor women undergoing cesarean section at 38–39 weeks gestation. AVP-V_{1a} column: displacement of [^{3}H]AVP from specific binding sites in rat liver or human platelets. AVP-V_2 column: displacement of [^{3}H]AVP from specific binding sites in kidney medulla obtained from rats or early postmortem human donors. [b] K_i values for inhibition of [^{3}H]AVP binding to AVP-V_{1a} sites in human liver and human uterus, respectively (ref 17). [c] Not determined. [d] Compound is racemic.

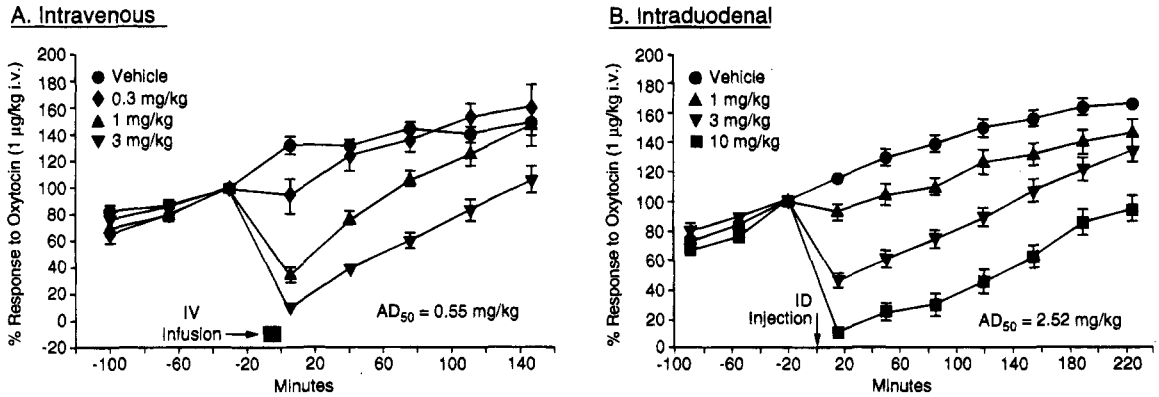


Figure 1. OT antagonist activity of L-371,257 in anesthetized rats. Uterine responses to intravenous bolus injections of OT were recorded before and after treatment with either vehicle or L-371,257 administered intravenously as a solution in saline containing 15% dimethyl sulfoxide and 15% emulphor (A) or intraduodenally as a suspension in saline containing 1% methocel (B). Data represent mean values ±SEM for four or five animals per dose group. Experimental details for this assay are reported in ref 15.

was 0.39 (data not shown). Thus, the *in vivo* functional assay and oral bioavailability study indicate that in rats **7** is well absorbed from the gut.

Compound **7** was synthesized in excellent overall yield using the sequence given in Scheme 1. *N*-(*tert*-Butyloxycarbonyl)-4-piperidinone was reductively aminated with 2-(hydroxymethyl)aniline. Cyclization of the resulting amino alcohol with triphosgene followed by acid-mediated removal of the *tert*-butyloxycarbonyl group gave the hydrochloride salt of piperidinylbenzoxazinone **8**. Methyl 2,4-dihydroxybenzoate was selectively alkylated on the 4-position hydroxyl group in a Mitsunobu coupling with *N*-(*tert*-butyloxycarbonyl)-4-piperidinol. Methylation of the remaining 2-hydroxyl group and saponification gave the benzoic acid derivative **9**. Amine **8** and carboxylic acid **9** were coupled using 1-[3-(dimethylamino)propyl]-3-ethylcarbodimide and hydroxybenzotriazole, and the resulting amide was converted to **7** by acid-mediated removal of the *tert*-butyloxycarbonyl group and acetylation with acetic anhydride.

In summary, the non-peptide AVP-V_{1a} receptor antagonist OPC 21268 was shown to have significant selectivity for binding to OT receptors in human uterus vs AVP receptors in human uterus, liver, platelets, and kidney. Structural modifications were identified that improved human OT receptor affinity to the low nanomolar range while maintaining excellent human OT/AVP receptor selectivity, as exemplified with **7**. Compound **7** was shown to be a potent antagonist of OT-induced contractions of the rat uterus *in vitro* and *in vivo* and was found to have significant oral bioavailability in rats. These findings highlight the promise for

optimization within this structural class to obtain an OT antagonist with suitable properties for clinical testing.

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(23) *In vitro* and *in vivo* functional assays and determination of oral bioavailability in rats were conducted using previously reported protocols: see refs 15 and 17 for details.

(24) L-371,257 was not tested in functional assays to assess AVP receptor agonist/antagonist activities, but several other closely related analogs exhibiting rat OT/AVP receptor binding profiles very similar to that of L-371,257, i.e., high affinity and selectivity for binding to rat AVP-V_{1a} receptors, were found to be potent AVP-V_{1a} antagonists *in vitro* (inhibition of AVP-induced contraction of isolated rat caudal artey rings) and *in vivo* (inhibition of the AVP-induced pressor response in pithed rats). These results will be reported in a full paper.

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