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NONPEPTIDE OXYTOCIN ANTAGONISTS: POTENT, ORALLY BIOAVAILABLE ANALOGS OF L-371,257 CONTAINING A 1-*R*-(PYRIDYL)ETHYL ETHER TERMINUS

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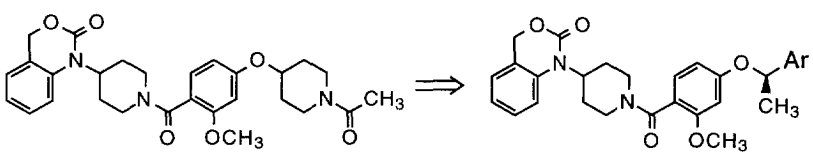
Abstract: Structure-activity studies on the oxytocin antagonist **1** (L-371,257) have identified a new series of high affinity, receptor-selective OT antagonists in which the N-acetyl-4-piperidinyl ether terminus in **1** has been replaced with a 1-(aryl)ethoxy group.

Preterm labor, defined as labor occurring prior to the completion of 37 weeks of gestation, often results in preterm delivery and affects approximately one in ten births in the United States and 13 million births world wide each year.[1] Although there are probably a number of different etiologies that result in preterm labor, it has been estimated that 10-30% of women suffering from this condition are eligible for tocolytic therapy.[2] The well-recognized limitations of existing treatments[3] have fueled the search for alternative methods with improved safety and efficacy. In this regard, the longstanding hypothesis of oxytocin (OT) receptor blockade as representing a potentially useful new method of tocolysis has received support from clinical studies with the peptidic OT antagonist, atosiban.[4] Our interest has been in developing an oral OT antagonist for use in preterm labor,[5] and toward this end, we recently reported the orally bioavailable nonpeptide **1** (L-371,257, Figure 1) as a promising new lead.[6] In this Letter, we describe structure-activity studies leading to a related series of potent, orally bioavailable OT antagonists in which the N-acetylpiperidinyl ether terminus in **1** has been replaced with a 1-*R*-(aryl)ethyl ether substituent.

Figure 1



1 (L-371,257)

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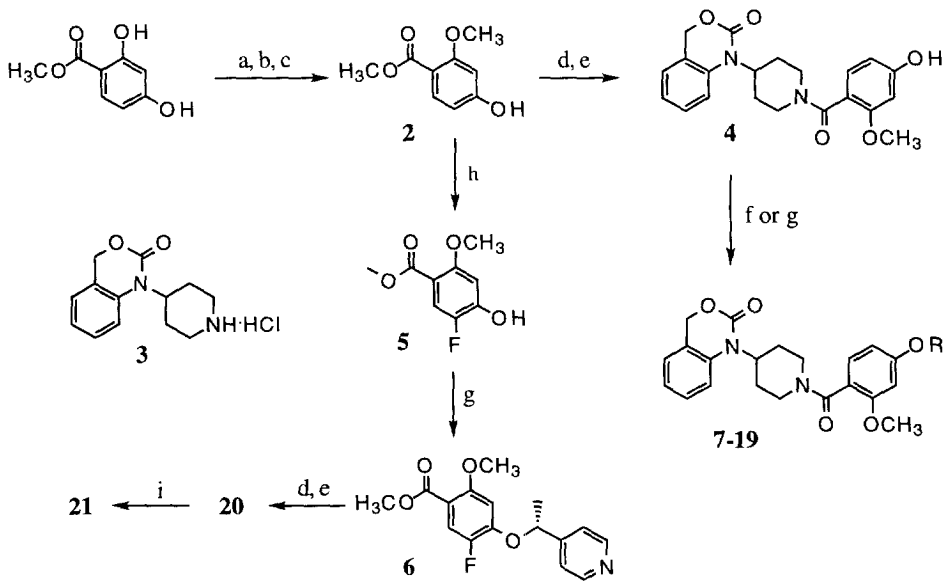
Chemical Methods

Preparation of the compounds listed in Table 1 began by functionalization of methyl 2,4-dihydroxybenzoate (see Scheme 1). Regioselective benzylation of the *para* hydroxyl group, followed by methylation of the *ortho* hydroxyl group and hydrogenolytic deprotection of the benzylic ether afforded methyl 4-hydroxy-2-methoxybenzoate **2**. Basic hydrolysis of the methyl ester and active ester coupling of the resulting acid to 1-(piperidin-4-yl)-4*H*-3,1-benzoxazin-2(1*H*)-one hydrochloride[6] (**3**) gave the common intermediate **4**. Phenol **4** was then alkylated with either an alkyl halide in the presence of cesium carbonate (**7-13**) or with an alcohol under Mitsunobu conditions (**14-19**) to produce the desired ether. Enantiomerically pure 1-*R*- and 1-*S*-(4-pyridyl)ethanol[7] were used in Mitsunobu reactions, which proceed with inversion of configuration,[8] to obtain the 1-*S*- and 1-*R*-(4-pyridyl)ethyl ethers, **18** and **19**, respectively. Compounds **20** and **21** utilized fluoro phenol **5** as an intermediate. The latter was obtained in moderate yield by fluorination of **2** using 3,5-dichloro-N-fluoropyridinium triflate.[9] Reaction of **5** with 1-*S*-(4-pyridyl)ethanol under Mitsunobu conditions gave ether **6**. Hydrolysis of **6** and coupling to **3** gave **20**, which was converted to the pyridine N-oxide derivative **21** by treatment with *meta*-chloroperoxybenzoic acid.

Scheme 1



(a) $PhCH_2Br$, K_2CO_3, acetone, 58%; (b) CH_3I, NaH, DMF, 96%; (c) H_2, Pd-C, CH_3OH, HOAc, 86%; (d) NaOH, CH_3OH, H_2O, 90-95%; (e) **3**, EDC, HOBT, $^iPr_2NEt$, DMF, 90-95%; (f) R-I or R-Cl, Cs_2CO_3, DMF, 50-65%; (g) R-OH, Ph_3P, DEAD, THF, 20-40%; (h) 3,5-dichloro-N-fluoropyridinium triflate, CH_2Cl_2, 38%; (i) MCPBA, $CHCl_3$, 70%

Biological Methods

Previously published procedures were utilized for OT receptor binding experiments, which measure the ability of test compounds to compete with tritiated OT for high affinity binding sites in uteri taken from rats

pretreated with diethyl stilbestrol (DES) or in human embryonic kidney cells expressing the cloned human OT receptor.[10-12] Results are expressed as inhibition constants (K_i values) and are given in Table 1. Compounds for which three or more independent K_i determinations were made include standard error of the mean calculations. The typical range of Ki values between experiments varied less than twofold. Arginine vasopressin (AVP) receptor binding experiments were also conducted using previously reported procedures[10,13] to determine the ability of test compounds to compete with tritiated AVP for high affinity binding sites in rat liver and human platelets (V_{1a} receptor), and in rat and human kidneys (V_2 receptor).

Several compounds were selected for evaluation in an in vivo functional assay to determine their ability to block OT-stimulated contractions of the in situ rat uterus. A detailed procedure has been previously reported.[11] Briefly, in anesthetized, DES-pretreated rats surgically prepared for recording of isomeric uterine contractions, an approximate ED_{50} intravenous (iv) dose of OT (1 μg/kg) was administered every 35 min for a total of eight injections. Fifteen minutes before the fourth injection, vehicle or the test compound was infused intravenously over a ten-minute period. Doses of 0.3, 1.0, and 3.0 mg/kg of test compound were used, with 4-5 animals per dose group. The uterine contractile response in the vehicle-treated group to the fourth injection of OT was set as 100%, and the AD_{50} value (i.e., the dose that antagonized the OT-induced contractile response in the vehicle-treated group by 50%) was determined by regression analysis.

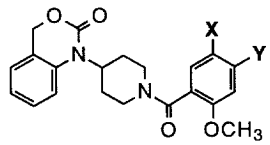
Procedures for the pharmacokinetic analyses have been described previously in detail.[11] Briefly, the test compound was dosed intravenously in rats at 3 mg/kg in saline-2% DMSO solution or orally at 10 mg/kg as a 1% methocel-saline suspension. Three animals per dose group were used. Blood samples were collected at eight time points over a period of six h. The plasma fraction was isolated by centrifugation and the plasma proteins were precipitated by treatment with methanol. The supernatant was collected and analyzed for OT receptor binding activity using the cloned human OT receptor binding assay. The concentration of test compound in each sample, expressed as μM equivalents, was determined from a standard curve generated by spiking rat plasma with known concentrations of test compound and working these samples up identically to the experimental plasma isolates. Elimination half-life was estimated from the concentration vs. time curve obtained from the iv experiment, and oral bioavailability (F) was determined by dividing the area under the concentration vs. time curve (0-6 h) from the oral experiment by the area under the concentration vs. time curve (0-6 h) from the iv experiment, and multiplying the result by 10/3 to adjust for the difference in dose.

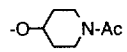
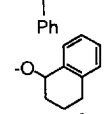
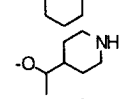
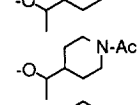
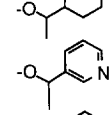
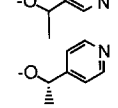
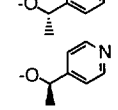
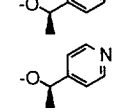
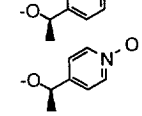
Results and Discussion

From our previous structure-activity studies that afforded lead compound **1**, it was found that modifications to the piperidinylbenzoxazinone portion of the structure were generally deleterious to OT receptor binding affinity.[14] On the other hand, modification of the piperidinyl ether terminus, and in particular modification of the N-substituent, was much better tolerated and provided a means to obtain potent OT antagonists with good pharmacokinetic properties.[15] The greater latitude for structural changes at the piperidinyl ether terminus motivated us to investigate analogs containing other more substantially modified ether end groups. The results from this line of investigation are described herein.

As a baseline compound, the simple methyl ether analog **7** was found to have a K_i of 87 nM at the cloned human OT receptor, which represents a ninefold loss of affinity compared to the piperidinyl ether lead compound **1** (see Table 1). Increasing the size of the ether substituent from methoxy to ethoxy (**8**) or isopropoxy (**9**) improved affinity to the 15 nM range, but larger ether substituents (e.g., benzyloxy **10**) offered little advantage vs. **7**. Interestingly, affinity of the benzyloxy analog improved significantly by introducing a methyl group at the benzylic carbon (**11**). Further changes to the benzylic substituent indicated that methyl was optimal: enlarging from methyl to ethyl (**12**) or phenyl (**13**) progressively reduced affinity, as did the introduction of a six-

Table 1 SAR of ether end group analogs



Compound	X	Y	K_i (nM)[b] chOTr	K_i (nM)[b] rOTr	in vivo rat: iv AD_{50} (mg/kg)	in vivo rat: pharmacokinetics 3 mg/kg iv 10 mg/kg orally
1	H		9.3 ± 1.4	19 ± 2.1	0.55	iv $T_{1/2}$ ~30 min. oral C_{max} = 3.3 μM eq[c] F = 39%
7	H		87 ± 7.5	120 ± 28		
8	H		15 ± 2.9			
9	H		17 ± 2.1			
10	H		73 ± 7.8			
11[a]	H		7.9 ± 0.58	44 ± 7.4		iv $T_{1/2}$ <30 min. oral C_{max} < 0.6 μM eq
12[a]	H		58 ± 8.3			
13	H		1800			
14[a]	H		260			
15[a]	H		73			
16[a]	H		54			
17[a]	H		5.4 ± 0.85	12 ± 0.45	1.2	iv $T_{1/2}$ ~30 min. oral C_{max} = 3.8 μM eq F = 30%
18	H		160			
19	H		3.9			
20	F		2.0 ± 0.61			iv $T_{1/2}$ ~30 min. oral C_{max} = 4.6 μM eq F = 97%
21	F		7.34	9.9 ± 1.8	0.78	

[a]compound is racemic; [b]chOTr = cloned human OT receptor; rOTr = rat uterine OT receptor; K_i values are reported as group means ± S.E.M. where ≥3 independent determinations were made; [c]dosed at 20 mg/kg orally

membered cyclic constraint (**14**). As the racemate, phenethyl ether **11** had the same affinity for binding to thecloned human OT receptor as lead compound **1**. Further examination of **11** for binding to receptors of the related peptide hormone, AVP, revealed a profile similar to that previously reported[6] for **1**, that is, **11** was found to possess excellent human OT vs. human AVP receptor selectivity (K_i = 3,200 nM, human platelet V_{1a} receptor; K_i = 8,100 nM, human kidney V_2 receptor) and exhibited a significant species difference with much higher affinity for rat vs. human AVP-V_{1a} receptors (K_i = 5.6 nM, rat liver V_{1a} receptor; K_i = 2,100 nM, rat kidney V_2 receptor).

A major goal for this structural series was to produce a compound that was orally bioavailable with an iv plasma half life of several hours. Compound **11**, however, was poorly bioavailable after a 10 mg/kg oral dose (plasma levels below the detection limit of 0.6 μM equiv at all time points), and the iv plasma half-life was short (<30 min). Speculating that the poor pharmacokinetics of **11** were due to its high lipophilicity and/or low aqueous solubility, analogs were synthesized in which the phenyl portion of the phenethyl moiety was replaced with more polar heterocycles. A tenfold loss of affinity resulted with the 4-piperidinyl analog **15**, and affinity of the latter was only marginally improved by acetylation of the basic nitrogen (**16**). The 3-pyridyl analog **17**, on the other hand, maintained good OT receptor affinity vs. **11** and also exhibited excellent selectivity vs. human AVP receptors (K_i = 2,100 nM, human platelet V_{1a} receptor; K_i = 7,500 nM, human kidney V_2 receptor). The phenyl to pyridyl change also improved oral bioavailability: **17** had a C_{max} of 3.8 μM equiv after a 10 mg/kg oral dose and an oral bioavailability of 30% in rats. Compound **17** was rapidly cleared, however, with an iv plasma half life of ~30 min. In an in vivo functional assay in rats, **17** behaved as a pure antagonist of OT-induced uterine contractions with an AD_{50} value of 1.2 mg/kg.

As a racemate, compound **17** thus exhibited a similar potency and pharmacokinetic profile to lead compound **1**. Having established these favorable attributes, attention was turned to preparing arylethyl ethers as single enantiomers to establish what, if any, preference might exist for binding to the OT receptor. The 1-*S*- and 1-*R*-(4-pyridyl)ethyl ethers (**18** and **19**) indeed showed a large difference in binding affinity, with the *R* enantiomer having fortyfold greater affinity than the *S* enantiomer. The more potent *R* isomer was additionally modified by incorporation of a fluorine at the 5-position on the central benzoyl ring, a modification which had been found to improve OT receptor affinity by two- to fivefold in the piperidinyl ether series.[15] A small affinity-enhancing effect was also obtained in this series (compare **19** and **20**). Compound **20** was well absorbed in rats after an oral dose of 10 mg/kg (C_{max} = 4.6 μM equiv), had good oral bioavailability (F = 97%), but possessed a short iv plasma half life (~30 min). The pyridine N-oxide **21** was also prepared as a potential metabolite of **20** based on the finding that a related series of OT antagonists containing a more distally placed pyridine ring underwent facile metabolic pyridine *N*-oxidation.[15] The OT receptor affinity of **21** was diminished by about threefold vs. **20**, and the former proved to be a reasonably potent OT antagonist in vivo (AD_{50} = 0.78 mg/kg). However, the duration of action of **21** in the functional assay (~50% recovery of the uterine response to OT at 1 h post dose) indicated that it, like the other compounds in this series, was rapidly cleared.

Summary and Conclusions

Structure-activity studies on lead compound **1** have identified a related series of potent OT antagonists in which the piperidinyl ether terminus in **1** is replaced with a 1-(aryl)ethylether substituent. Systematic structural variations revealed that affinity for human OT receptors is maximized by employing an α-branched ether substituent in which one of the two branching elements is a small alkyl group (i.e., methyl) and the other is an aromatic group. The large difference in OT receptor affinity observed for 1-*R*- and 1-*S*-(pyridyl)ethyl ether enantiomers suggests that the ether substituent makes a very specific contact with the receptor. That the receptor affinity was only minimally affected by the inclusion of polar heteroatoms in the aryl portion of the 1-(aryl)ethyl

ether substituent also suggests that the distal edge of the aryl ring is not in direct contact with the receptor. Several compounds described in this Letter possess noteworthy features, for example, they are pure antagonists of OT-induced uterine contractions in an in vivo functional assay, they possess human OT receptor affinity in the low nanomolar range, high human OT vs. human AVP receptor selectivity, and significant oral bioavailability. Improvements in in vivo potency and half life, however, would be desirable for a clinical candidate, and efforts to this end will be detailed in future communications.

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