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NONPEPTIDE OXYTOCIN ANTAGONISTS: ANALOGS OF L-371,257 WITH IMPROVED POTENCY

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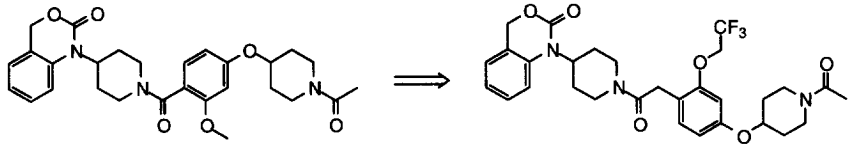
Abstract: Structure–activity studies on the oxytocin antagonist **1** (L-371,257; K_i = 9.3 nM) have led to the identification of a related series of compounds containing an ortho-trifluoroethoxyphenylacetyl core which are orally bioavailable and have significantly improved potency in vitro and in vivo, e.g., compound **8** (L-374,943; K_i = 1.4 nM).

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 four decades. Selective OT antagonists have been obtained by modifying the parent hormone structure,[1] and during the last decade, several structural classes of receptor selective, nonpeptide OT and AVP antagonists have been reported.[2] Our laboratory has been involved in an effort to identify an orally active OT antagonist for potential use in preterm labor[3] and in connection with this effort, we recently reported lead compound **1** (L-371,257; Figure 1) as a representative member of a promising new structural class.[4] In this Letter we disclose structure–activity studies based on **1** which have led to the identification of a series of orally bioavailable OT antagonists containing an ortho-trifluoroethoxyphenylacetyl core. Several analogs in this new series, e.g., **8** (L-374,943; Figure 1), offer significant improvements in potency vs **1**.

Figure 1



1 (L-371,257; K_i = 9.3 nM) **8** (L-374,943, K_i = 1.4 nM)

Chemical Methods

The compounds in Table 1 were prepared using methods given in Scheme 1. Starting materials **13**, **16**, **18**, and **21** were obtained from commercial sources. Preparation of piperidinylbenzoxazinone **15** has previously

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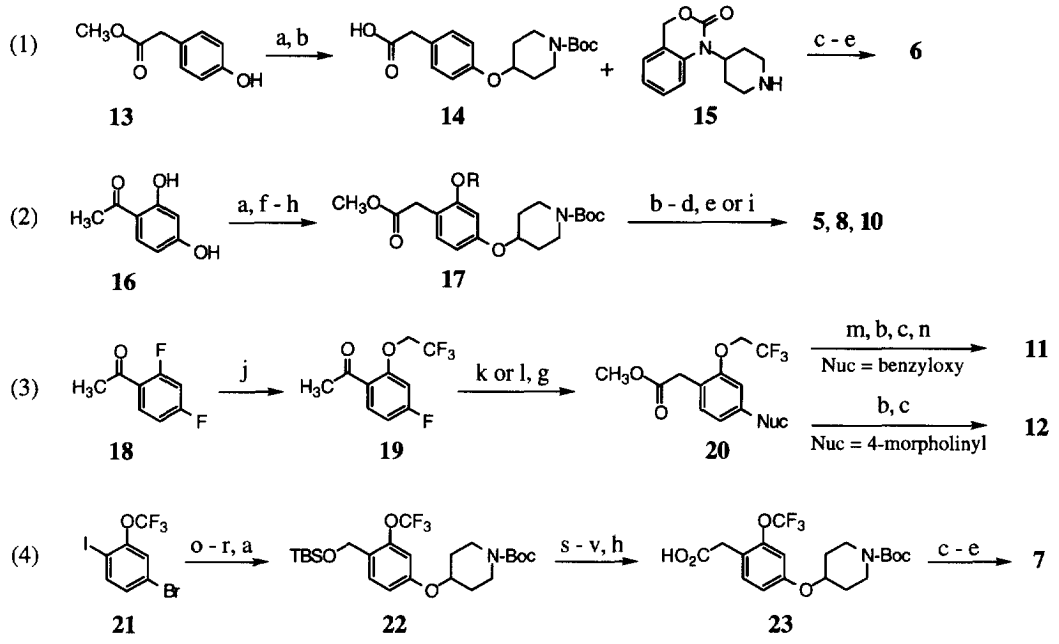
Scheme 1



(a) N-Boc-4-piperidinol, diethyl azodicarboxylate, Ph_3P, THF, 70-85%; (b) NaOH, MeOH, H_2O, 90-95%; (c) **15**, EDC, HOBT, $^iPr_2NEt$, DMF, 90-95%; (d) HCl(g), EtOAc, 95%; (e) Ac_2O, DIEA, CH_2Cl_2, 95%; (f) R-I or R-OTf, Cs_2CO_3, DMF, 80-90%; (g) $Tl(NO_3)_3$, $CH(OMe)_3$, MeOH, 70-80%; (h) Boc_2O, DIEA, DMF, 95%; (i) cyclopropane carboxaldehyde, $NaBH_3CN$, MeOH, 85%; (j) CF_3CH_2OH, KO^{t}Bu, THF, 65%; (k) morpholine, DMF, 75%; (l) $PhCH_2OH$, KO^{t}Bu, THF, 70%; (m) H_2, Pd black, MeOH, 90%; (n) N-(2-chloroethyl)-morpholine, Cs_2CO_3, DMF, 85%; (o) nBuLi, N-formylmorpholine, THF, 85%; (p) $NaBH_4$, MeOH, 95%; (q) ^{t}Bu(Me)$_2$SiCl, 4-(dimethylamino)pyridine, CH_2Cl_2, 95%; (r) nBuLi, THF; $B(OMe)_3$; H_2O_2, HOAc, 80%; (s) Bu_4NF, THF, 95%; (t) CBr_4, Ph_3P, ether, 85%; (u) NaCN, DMF, 90%; (v) conc. HCl, HOAc, 75%

been described.[5] Two methods were employed for preparation of the phenylacetic acid intermediates: the thallium(III) mediated acetophenone to phenylacetic ester rearrangement[6] (equations 2 and 3), and displacement of a benzyl halide with cyanide ion followed by acidic hydrolysis (equation 4). Mitsunobu reaction of **13** with N-Boc-4-piperidinol followed by basic hydrolysis gave **14**, which was converted in a straightforward fashion to compound **6**. Acetophenone **16** was derivatized with complete regioselectivity at the para hydroxyl group under Mitsunobu conditions and then converted in a straightforward manner to **17**. The acidity of the thallium(III) rearrangement in step g of equation 2 caused partial loss of the Boc group, so the crude product was treated with Boc anhydride to improve the yield of **17**. The latter was then used to obtain compounds **5**, **8**, and **10**. Treatment of difluoroacetophenone **18** with the potassium salt of trifluoroethanol in THF resulted in regioselective displacement of the ortho fluorine to give **19** as the major product (ortho:para ~3.5:1). This result contrasts the high para selectivity reported for reaction of potassium alkoxides with 2,4-difluorobenzonitrile.[7] The remaining fluorine in **19** could then be displaced with oxygen or nitrogen nucleophiles followed by thallium(III) rearrangement to give phenylacetic esters **20**, which were converted in a straightforward manner to compounds **11** and **12**. Selective halogen-metal exchange reactions with trifluoromethoxy dihaloarene **21** allowed efficient

production of **22** which was converted in a straightforward fashion to the phenylacetic acid **23** and thence to compound **7**.

Biological Methods

Previously published procedures were utilized for OT receptor binding experiments which measure the potency 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 (HEK 293-EBNA) cells stably expressing the cloned human OT receptor.[8,9] 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. Typical range of K_i values between experiments varied less than two-fold. Arginine vasopressin (AVP) receptor binding experiments measuring the potency of test compounds to compete with tritiated AVP for binding to high affinity sites in rat liver and human platelets (V_{1a} receptor), and in rat kidney (V_2 receptor) were conducted as previously reported.[8] Using methods similar to what has previously been reported for the cloned human OT receptor,[9] an assay was developed to measure potency of test compounds to compete with tritiated AVP (0.5 nM) for binding to cloned human AVP-V_2 receptors stably expressed in HEK 293-EBNA cells (B_{max} = 350 fMol/mg protein; AVP K_d = 0.80 nM).

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.[8] 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. For the iv experiment, vehicle or the test compound were infused iv over a 10 min period 15 min before the fourth injection of OT. For the intraduodenal (id) experiment, the test compound was administered intraduodenally 5 min before the fourth injection of OT. Three or four dosing levels of test compound were used, with four or five 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 (the dose which 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.[8] 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 6 h. The plasma fraction was isolated by centrifugation and the plasma proteins were precipitated by treatment with methanol. The supernatant was collected and analyzed by HPLC. The concentration of test compound in each sample 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. Plasma half-life was calculated 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 3/10 to adjust for the difference in dose given orally vs iv.

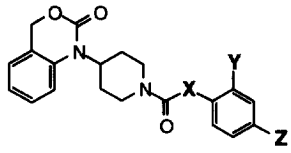
Results and Discussion

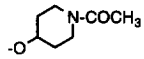
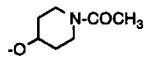
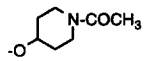
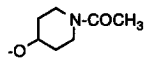
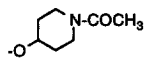
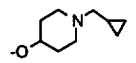
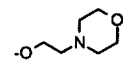
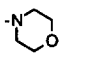
Compound **1** has served as an attractive starting point for our discovery program to obtain an orally active OT antagonist for potential use in preterm labor. **1** has high affinity for the cloned human OT receptor (K_i = 9.3 nM), good selectivity for binding to human OT receptors vs. human AVP receptors (K_i = 3,200 nM, human platelet V_{1a} receptor; K_i = 37,000 nM, human kidney V_2 receptor), is a competitive antagonist of OT-induced uterine contractions in functional assays, and is orally bioavailable in rats and rhesus monkeys.[4] In vivo potency and pharmacokinetic half-life are two areas where improvements were needed in order to obtain a suitable clinical candidate. Previous work had shown that modifications to the piperidinylbenzoxazinone portion of compound **1** were generally deleterious to OT receptor binding affinity,[4] whereas significantly more latitude was available for modifying the piperidinyl ether terminus.[5,10] In the present study, the initial line of inquiry was to determine the effect of changes in the central portion of the molecule, and in particular, the effect of inserting one or more methylene "spacers" to allow for more conformational mobility. Three prototype compounds **2–4** show that elongation from benzoyl to phenylacetyl improved OT receptor affinity, but further elongation to the dihydrocinammoyl derivative caused a loss of receptor affinity. The benzoyl to phenylacetyl modification also proved to be well tolerated with the piperidinyl ether in place (compare **1** and **5**). Aryl ring substituent effects revealed some interesting differences in the phenylacetyl vs. benzoyl series. For example, a much greater contribution of the ortho substituent to OT receptor binding affinity was seen in the phenylacetyl series compared to the benzoyl series. In the latter, a three- to five-fold improvement in affinity was typically obtained by introducing an ortho methoxy group, whereas the same modification in the phenylacetyl series produced an approximately forty-fold gain in affinity (compare **5** and **6**). Furthermore, several modifications of the ortho alkoxy substituent which were found to be detrimental to receptor affinity in the benzoyl series turned out to be beneficial in the phenylacetyl series. In particular, the trifluoromethoxy and trifluoroethoxy substituents contained in compounds **7** and **8** provided improvements of approximately four- and seven-fold to human OT receptor affinity, respectively, vs. the methoxy analog **5**. Administered iv to rats, both **7** and **8** proved to be potent antagonists of OT-induced uterine contractions, with the rank order of the in vivo potencies of compounds **1**, **7**, and **8** paralleling their affinities for the rat uterine OT receptor.

Compound **8** (L-374,943) was studied in more detail and was found to possess a number of favorable attributes. In addition to its high affinity for the human OT receptor, **8** exhibited good selectivity for binding to human OT vs. human AVP receptors (K_i = 740 ± 66 nM, human platelet V_{1a} receptor; K_i = 130 nM, cloned human V_2 receptor). Rat AVP receptor binding data indicated that **8**, like its progenitor compound **1**, exhibits a significant species difference with much higher affinity for rat vs. human V_{1a} receptors (K_i = 2.0 ± 0.27 nM, rat liver V_{1a} receptor; K_i = 150 ± 26 nM, rat kidney V_2 receptor). In a functional assay using isolated rat uterine tissue, **8** proved to be a potent and competitive antagonist of OT-induced contractions (pA_2 = 9.2; Schild slope = 1.0). Compound **8** also performed well as an OT antagonist in vivo in rats when administered intraduodenally (id AD_{50} = 0.64 mg/kg), indicating that it was well absorbed from the gut. Indeed, **8** is one of the most potent oxytocin antagonists discovered to date in this assay. A pharmacokinetic study in rats showed that **8** produced good plasma levels after an oral dose of 10 mg/kg (C_{max} = 570 nM) and had an oral bioavailability of 19%.

The phenylacetyl and benzoyl series also behaved differently in terms of para substitutent effects. For example, replacing the piperidinyl ether terminus in **1** with hydrogen reduced receptor affinity by greater than one

Table 1.



cmpd	X	Y	Z	K_i (nM)[a] chOTr	K_i (nM)[a] rOTr	in vivo rat AD50 (mg/kg) iv	AD50 (mg/kg) id	pharmacokinetics 3 mg/kg iv 10 mg/kg orally
1	bond	OCH_3		9.3 ± 1.4	19 ± 2.1	0.55	2.5	F = 39%; iv $t_{1/2}$ ~0.5 h[b]
2	bond	OCH_3	OCH_3	87 ± 7.5	120 ± 28			
3	CH_2	OCH_3	OCH_3	33 (1)	37 (2)			
4	CH_2CH_2	OCH_3	OCH_3	240 (1)	520 (1)			
5	CH_2	OCH_3		9.8 (1)	36 (1)			
6	CH_2	H		390 (1)	1600 (1)			
7	CH_2	OCF_3		2.4 ± 0.75	2.8 (1)	0.27		
8	CH_2	OCH_2CF_3		1.4 ± 0.75	0.71 ± 0.04	0.060	0.64	F = 19%; iv $t_{1/2}$ = 0.27 h
9	CH_2	OCH_2CF_3	H	4.5 ± 0.63	6.0 (2)	1.0	>10	
10	CH_2	OCH_2CF_3		1.0 (2)	1.2 (1)	0.69	2.2	F = 45%; iv $t_{1/2}$ = 1.7 h
11	CH_2	OCH_2CF_3		1.4 (1)	2.1 (1)	0.13		F = 31%; iv $t_{1/2}$ = 0.28 h
12	CH_2	OCH_2CF_3		0.86 (2)	0.60 (1)	0.077	0.97	

[a]chOTr = cloned human OT receptor, rOTr = rat uterine OT receptor; K_i values are reported as group means ± S.E.M. for compounds with three or more K_i determinations, otherwise group means are reported with the number of K_i determinations given in parentheses. [b]dosed at 20 mg/kg orally; results are given from determination of plasma levels by bioassay

hundred-fold, whereas a loss of only about three-fold was seen in the phenylacetyl series (compare **8** and **9**). Also, revealing a basic nitrogen by replacing the acetyl group in **1** with a cyclopropylmethyl group reduced OT receptor affinity by twenty-fold, whereas potency was maintained in the phenylacetyl series (compare **8** and **10**). These findings suggested that the para position substituent in the phenylacetyl series could be used to alter physicochemical properties and hence, in vivo properties, while maintaining high levels of OT receptor affinity. This supposition was borne out with numerous additional para position analogs of **8** that were prepared. Such analogs were also found to maintain the same OT vs AVP receptor binding selectivity as **8**. Clear-cut trends relating the influence of structural changes in the para position substituent and in vivo properties, however, were difficult to establish. In general, the best in vivo potency was obtained with polar, non-charged or weakly basic

para position substituents (e.g., compounds **8**, **11**, and **12**), but an acceptable pharmacokinetic half life and/or duration of action in the functional assay was not obtained with such compounds. Good pharmacokinetic properties were obtained with a subset of the compounds containing a basic group, however, in vivo potency was typically compromised with these analogs, for example, as seen with compound **10**.

In summary, structure–activity studies on the OT antagonist lead compound **1** (L-371,257; K_i = 9.3 nM) have led to the identification of a potency-enhancing modification which involves replacing the central ortho-methoxybenzoyl subunit in **1** with an ortho-trifluoroethoxyphenylacetyl moiety. The ortho-trifluoroethoxy group was found to be critical for obtaining high levels of OT receptor affinity. Para position substituents on the phenylacetyl ring had much less impact on receptor affinity but were useful for varying physicochemical and in vivo properties. This new design yielded a number of orally bioavailable, receptor-selective OT antagonists with improved potency vs. lead compound **1**, e.g., compound **8** (L-374,943; K_i = 1.4 nM). With continued optimization, particularly with respect to pharmacokinetic half-life, this new series of OT antagonists offers the potential of providing a compound suitable for clinical study as an oral agent in preterm labor.

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