Drugs of the Future 2006, 31(4): 345-353

CCC: 0377-8282/2006
DOI: 10.1358/dof.2006.031.05.980655

Review Article

Nonpeptide oxytocin agonists

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Abstract

Oxytocin is a neurohypophyseal hormone that acts at the oxytocin receptor localized both peripherally and centrally and is particularly highly expressed in the pregnant human myometrium. Synthetic oxytocin has long been used to induce labor and oxytocin antagonists have been introduced for the treatment of preterm labor. New compounds are described that are potent, selective and efficacious oxytocin receptor agonists with potential utility in promoting labor and for the treatment of conditions such as male and female sexual dysfunction. These new agents may also be useful as tools for further elucidating the roles of the oxytocin receptor system. Furthermore, as they are nonpeptidic in nature, they may offer advantages over peptide oxytocin analogues in terms of oral availability and central penetration. This article describes the various approaches that have been used to discover such compounds. Compounds such as **27**, the first nonpeptide oxytocin agonists reported, are currently being investigated as pharmacological tools in animal models of oxytocin activity.

Introduction

Oxytocin is a cyclic peptide consisting of 9 amino acids with a disulfide bridge between the cysteines at the 1 and 6 positions (Fig. 1). Oxytocin is a neurohypophyseal hormone secreted by the posterior pituitary gland that acts at the oxytocin receptor, a member of the G-protein-coupled receptor (GPCR) superfamily (1). The oxytocin receptor is found in both the periphery and in central tissues. Within the periphery, it is localized in the uterus and the mammary glands. The receptor is particularly highly expressed in the pregnant human myometrium, where a 100-200-fold increase in concentration occurs during gestation, and the hormone is involved in the onset and progress of labor (2). Thus, synthetic oxytocin has long been used as a drug to induce labor. It is also used just after birth to prevent postpartum hemorrhage and to induce milk let-down. Long-acting synthetic analogues such as carbetocin (DURATOCIN) have also been developed for the prevention of uterine atony and postpartum hemorrhage following cesarean section (3). Peptide antagonists of the oxytocin receptor are also in use, including atosiban (TRACTOCILE), which is utilized in the treatment of preterm labor (4). In addition, the highly potent and long-acting peptide oxytocin antagonist barusiban is currently in clinical development for the treatment of preterm labor (5). Centrally, oxytocin has been localized in tissues such as the paraventricular nucleus, where it is involved in the regulation of both male and female sexual responses (1, 6).

The importance of oxytocin as a pharmaceutical agent was recognized recently in the journal *Chemical and Engineering News* by the American Chemical Society, which placed it on their list of top drugs that have made a major impact on human health and society (7). However, the range of oxytocin's physiological roles has

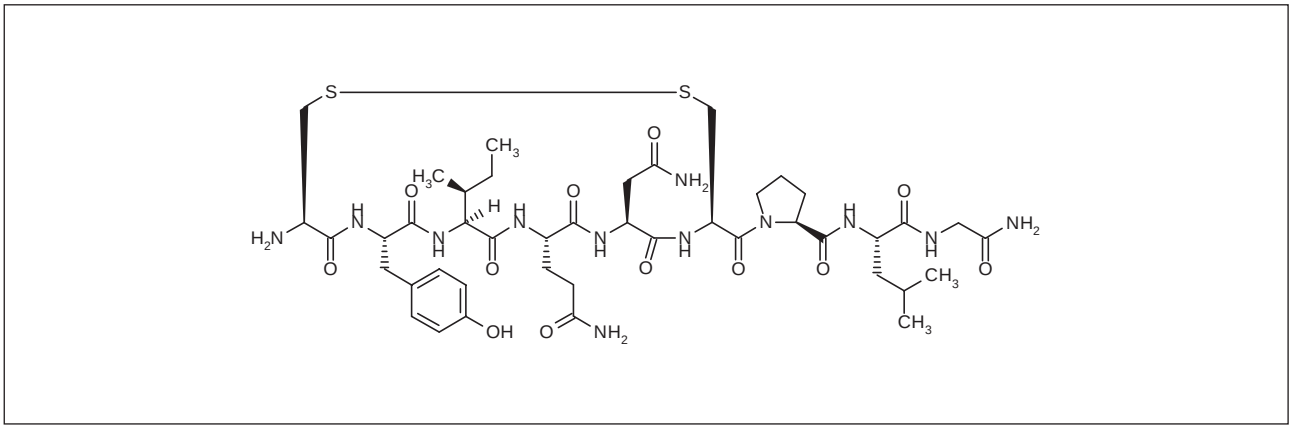


Fig. 1. Structure of oxytocin.

not yet been fully elaborated. This is particularly the case in the brain, where the role of oxytocin is largely unexplored. Thus, a clear need exists for new compounds that are potent, selective and efficacious agonists at the oxytocin receptor for use as tools to explore the pharmacology and physiological function of the oxytocin receptor system. Such compounds may also be potential drugs for indications in which oxytocin function is compromised. More specifically, they may have utility as alternatives to oxytocin in treating postpartum bleeding, promoting labor and increasing milk let-down. They may also offer new centrally driven treatments for sexual disorders such as male erectile dysfunction, female sexual dysfunction and psychiatric disorders such as anxiety or autism (1).

With regard to the male erectile response, there are multiple central pathways involved, and new treatments targeting some of these pathways are under development. For example, the melanocortin agonist PT-141 has been reported to have significant efficacy in phase II clinical trials (8). Also, apomorphine, a dopamine D2 agonist, is marketed for the treatment of erectile dysfunction (9). However, these treatments suffer from certain limitations. PT-141, for example, is an analogue of α-melanocyte-stimulating hormone (α-MSH) with limited oral availability, and apomorphine, when delivered orally, produces nausea that is sometimes severe. Oxytocin itself is known to induce erections when injected into the paraventricular nucleus in laboratory animals, but its peptidic nature, like carbetocin, is likely to limit the intact penetration of these compounds through the blood-brain barrier (6). Thus, their efficacy when delivered to the periphery is likely to be poor, precluding their use as therapeutics in humans.

Our aim was to develop nonpeptide oxytocin mimetics with an increased likelihood of penetration through the blood-brain barrier and possible oral bioavailability. Such mimetics would have potential utility in, for example, the treatment of erectile dysfunction and other male and female sexual dysfunctions. Furthermore, they would be more convenient for the patient than PT-141 and without the unpleasant side effects, such as nausea, associated with apomorphine.

Approaches for the discovery of novel oxytocin agonists

Given that no nonpeptide oxytocin mimetics existed before the beginning of this work, one approach that we took to discover such compounds was to examine the biological activity of mimetics of the closely related hormone arginine vasopressin (AVP) (10). Like oxytocin, AVP is a neurohypophyseal hormone secreted by the posterior pituitary gland. AVP acts at the vasopressin receptor, which has three subtypes: V_{1a}, V_{1b} and V_2. In addition, AVP also binds to the oxytocin receptor, although with a potency 10 times lower than that of oxytocin itself (11, 12). In spite of the perception that it is difficult to identify nonpeptide agonists of peptide hormone receptors, principally because of their much reduced molecular size compared to the parent hormone, a number of agonists at the vasopressin V_2 receptor have indeed been reported and are shown in Figure 2 (13, 14).

One of these V_2 receptor agonists is WAY-VNA-932 (**1**), an orally active compound under phase I clinical development at Wyeth (15). Another orally active V_2 receptor agonist, OPC-51803 or SOU-003 (**2**), is currently in phase II trials and is being developed by Sosei for the potential treatment of urinary incontinence (16). Ferring also has new, highly orally available V_2 agonists in phase II development for use as antidiuretics. One compound from this series is compound **3** (17). Given the close relationship between oxytocin and AVP, our hypothesis was that a library of structures designed around nonpeptide V_2 agonists would likely yield compounds with oxytocin-agonist activity as well.

A second approach was to design libraries of structures around known oxytocin antagonists (Fig. 3) (18, 19). Compound **4** is an example of a novel series of diketopiperazines recently published by GlaxoSmithKline (20). Serono has published the pyrrolidinone oxime **5**, which has undergone phase I clinical testing, and more recently a sulfonamide-based series exemplified by **6**, which was shown to be potent, selective and orally active (21, 22). Additionally, Merck Research Laboratories and GlaxoSmithKline have disclosed structure-activity rela-

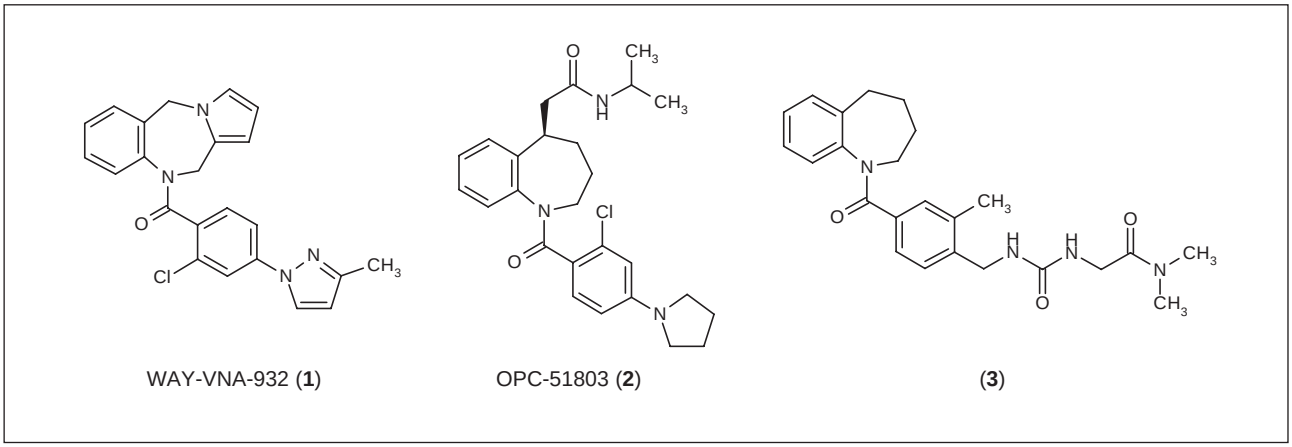


Fig. 2. Known nonpeptide vasopressin V2 agonists.

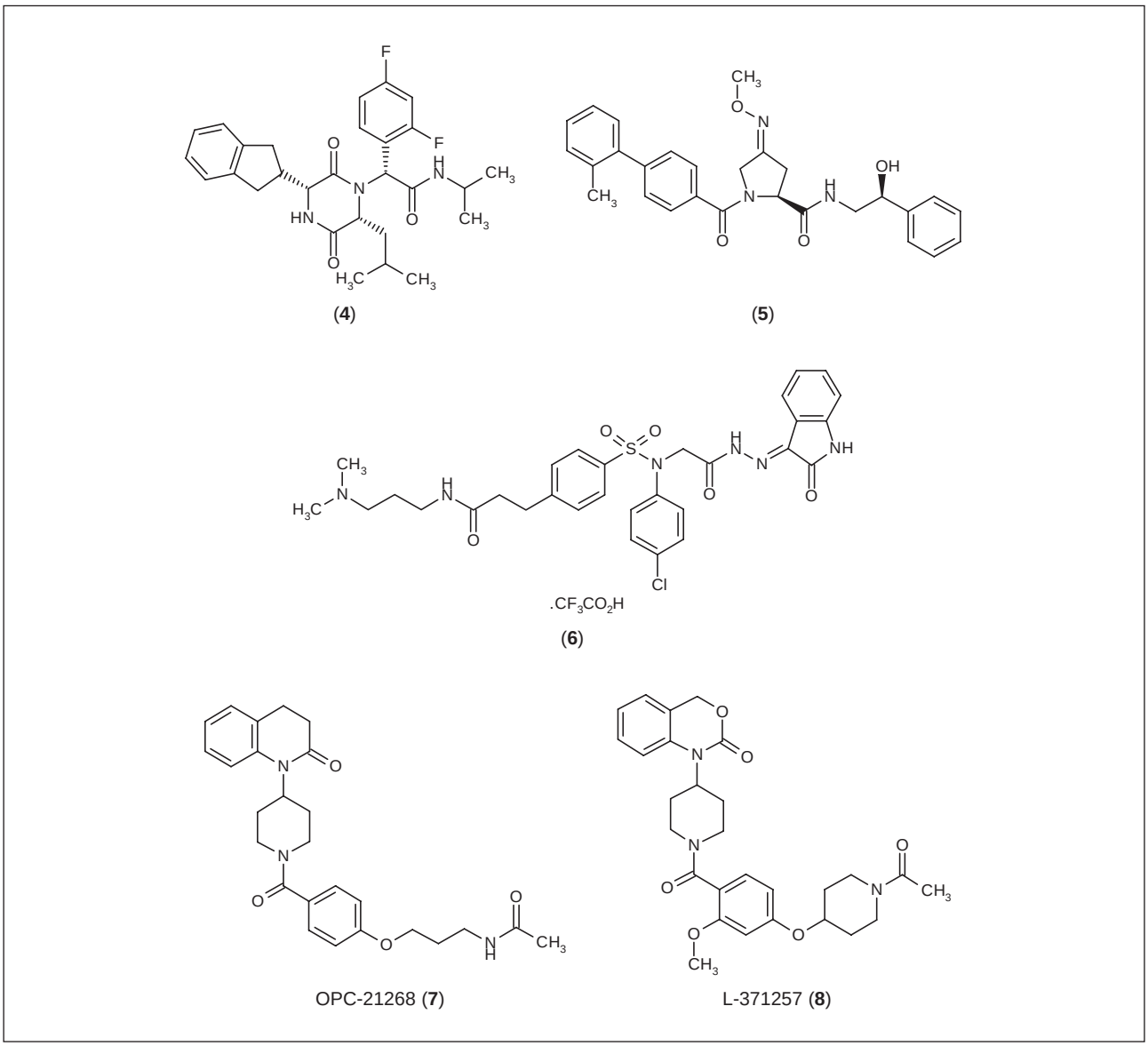


Fig. 3. Published nonpeptide oxytocin antagonists.

tionship (SAR) studies on the series related to OPC-21268 (**7**) and including the benzoxazinones exemplified by L-371257 (**8**) (23, 24). The utility of these compounds is generally cited as being related to the treatment of preterm labor.

As a result of information available in the literature, as well as data from our own studies on the oxytocin-agonist activities of structures related to compounds **1-8**, it was concluded that only compound **3** was able to elicit a response at the oxytocin receptor. In fact, in a functional reporter gene assay for activity at the human oxytocin receptor, compound **3** gave a response relative to oxytocin of 33% at a concentration of 3 μM (25). Based on this result, a library of 50,000 vasopressin-targeted structures related to **3** was screened for oxytocin-agonist activity.

Identification and optimization of selective oxytocin agonists

Screening of the library of compounds related to **3** revealed three further hits for the oxytocin receptor (Fig. 4). It was apparent that the hit structures shared some common features, such as a fused bicycle/tricycle, a 3-substituted benzamide linker and a urea. The compounds **9**, **10** and **11** were differentiated by the nature of the urea substituent, which was a substituted phenyl, a proline amide and an *N*-substituted piperazine, respectively. Initially, we chose to focus on the proline-based series. One advantage of selecting this series was that the proline amide functionality allowed for rapid analoging. Thus, over 100 new diverse amides were synthesized, but the majority of these lost all oxytocin-agonist activity. Where activity was retained, such as in the case of compound **11**, the structures tended to include a basic amine (Table I). For example, compounds **13-15** showed activity at the oxytocin receptor, but reduced potency compared to the hit compound **10.** Even greater potency was lost if, in contrast to compounds **13-15**, the tertiary amine was acyclic in nature, as in compound **16**. Within these new compounds, the greatest potency was observed with compound **12** (EC_{50} = 1500 nM), which contained the weakly basic pyridine and was almost equipotent with **10**. It was found that the only modification of the proline amide functionality that resulted in improved potency was conversion of the amide to a thioamide. The resulting thioamide analogues **17-20** all gave at least a 3-fold improvement over the corresponding amides. The most potent thioamide was **17**, which had an EC_{50} value of 190 nM and was based on the original dimethyl proline amide analogue.

Despite identifying improved potency with the thioamide, the amide substituents themselves failed to produce any improvement in potency at the oxytocin receptor. Therefore, attention was turned towards the piperazine-based series (compound **11**). In this series, we decided that we might make better progress by focusing on an alternative area of the structure. Observing that oxytocin activity had been seen with at least two different fused-ring systems in hits **9**, **10** and **11**, a number of new fused-ring analogues were prepared (Table II).

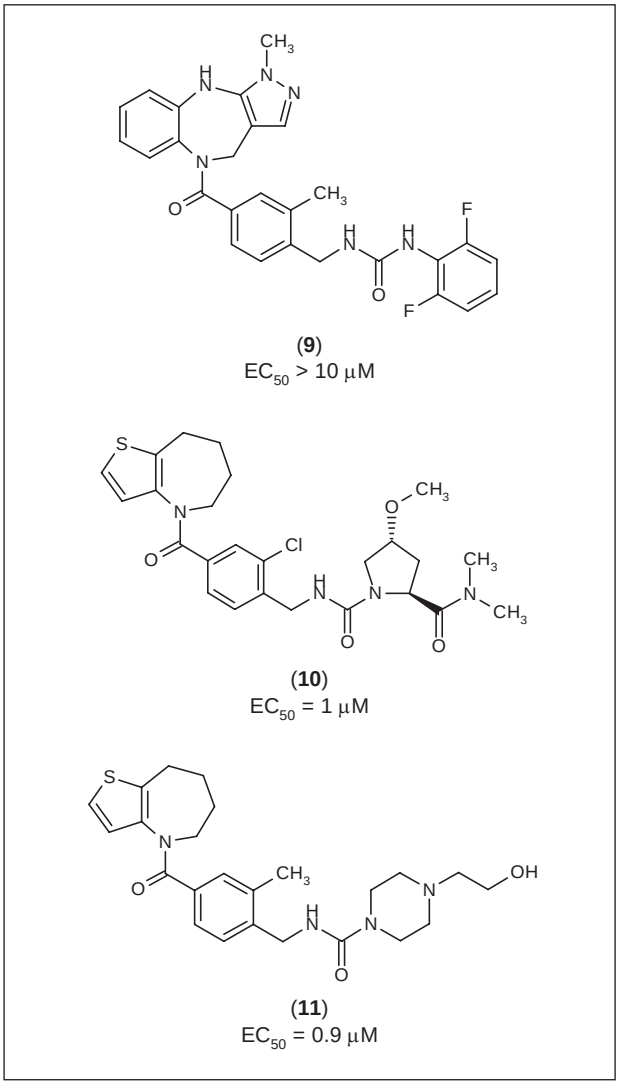
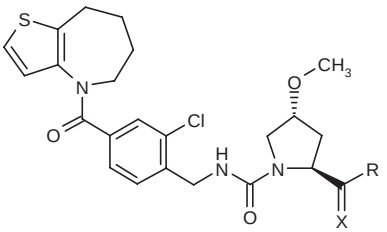


Fig. 4. Structures of the oxytocin agonist hits obtained by screening the vasopressin-targeted library.

Replacement of the 5,6,7,8-tetrahydro-4*H*-thieno[3,2-*b*]azepine group in compound **11** by the tricycle featured in compound **9** resulted in a 9-fold improvement in potency in compound **21** (EC_{50} = 100 nM). The 1,5-diazepine that this structure contained was also present in the potent compounds **22** and **25**. However, switching to tricycles containing a 1,4-diazepine gave a reduction in potency, as demonstrated by compounds **23** and **24**.

Thus, improvements in potency were achieved in both the proline- and the piperazine-based series to afford compounds **17** and **21**. However, since the hit compounds for these series had originated from a library based on the V_2-active compound **3**, it was not surprising to find that these compounds retained some V_2-agonist activity (Table III). In fact, the proline-based compound **17** showed > 30-fold selectivity for the V_2 receptor (EC_{50} = 5 nM). Interestingly, we observed that the V_2 activity in the series of proline thioamides **17-20** was somewhat intoler-

Table I: Structure-activity relationships (SAR) of proline amide/thioamide series.



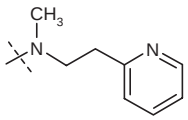
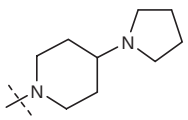
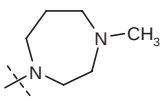
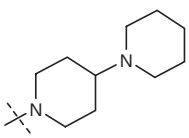
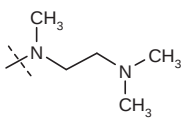
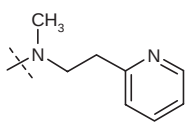
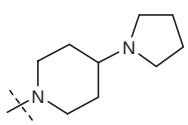
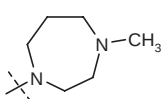
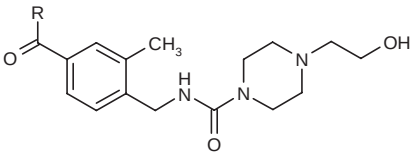
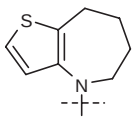
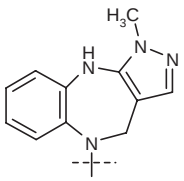
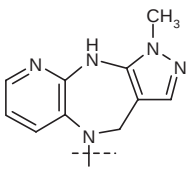
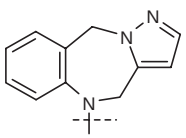
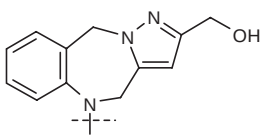
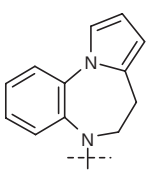
Compound	R	Oxytocin EC_{50} (nM)
X=O		
10		1000
12		1500
13		2300
14		2800
15		2200
16		> 10,000
X=S		
17		190
18		470
19		480
20		430

Table II: SAR of fused bicycle/tricycles.

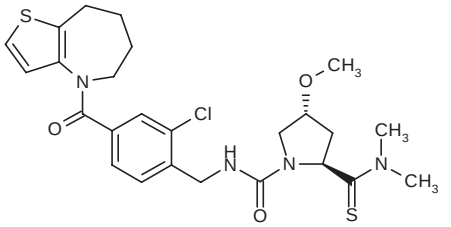
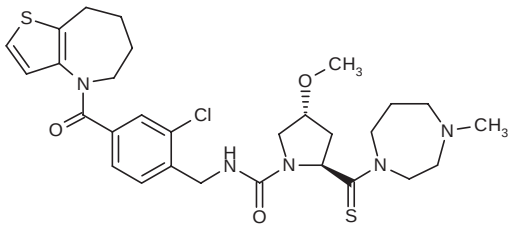
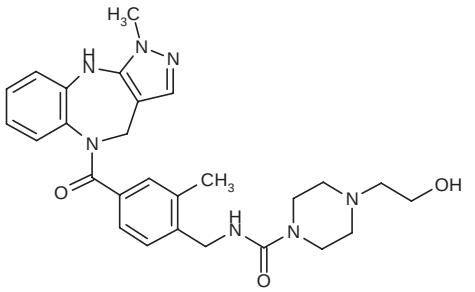
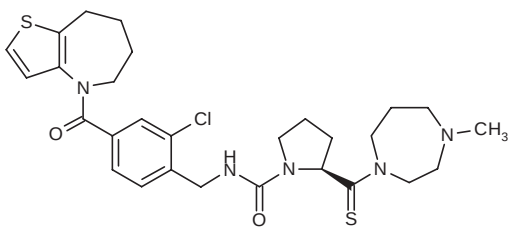
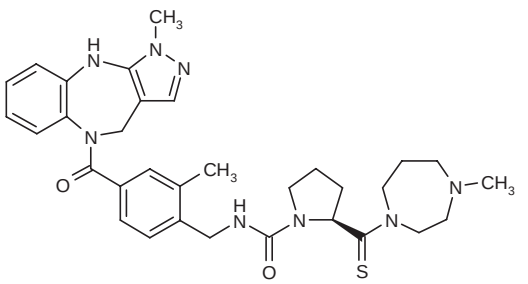


Compound	R	Oxytocin EC_{50} (nM)
11		900
21		100
22		520
23		5000
24		> 10,000
25		150

ant of the basic amine that had been introduced earlier. This had been shown to be a functionality that retained oxytocin activity (Table I). Thus, we had a means by which to increase selectivity in favor of oxytocin-agonist activity. This is shown in Table III by compound **20**, in which introduction of the basic methylhomopiperazine functionality improved oxytocin selectivity, although it remained only half as potent at oxytocin as at V_2 receptors. Removal of the stereogenic methoxy group resulted in the first oxytocin-selective compound, **26** (EC_{50} = 810 and 1800 nM at the oxytocin and V_2 receptor, respectively).

Turning our attention to the piperazine-based series, it was pleasing to discover that although **21** still possessed significant V_2 activity, its selectivity already favored oxytocin (EC_{50} = 100 and 380 nM at the oxytocin and V_2 receptor, respectively) (Table III). This structure

Table III: Oxytocin/V_2 selectivity of lead compounds.

Compound	Structure	Oxytocin EC_{50} (nM)	V_2 EC_{50} (nM)	Selectivity
17		190	5	0.03x
20		430	230	0.5x
26		810	1800	2x
21		100	380	4x
27		33	850	25x

contains a basic amine and this is consistent with the above observation that such a functionality is important for oxytocin over V_2 receptor selectivity. The SAR data shown in Table II showed that incorporation of the 1,5-diazepine-based tricycle first identified in the hit compound **9** may be important in improving potency. Therefore, it was hypothesized that a chimeric structure incorporating both a 1,5-diazepine-based tricycle and the homopiperazine proline thioamide would yield a potent and selective oxytocin agonist. Thus, compound **27** was synthesized and proved to be the most potent oxytocin agonist prepared, with an EC_{50} value of 33 nM and > 25-

fold selectivity for the oxytocin receptor over the V_2 receptor (Table III). This compound shows 100% relative efficacy at the oxytocin receptor in the reporter gene assay, and no agonist activity was observed at V_{1a} and V_{1b} receptors (25).

In vivo confirmation of oxytocin-agonist activity

Compounds were tested for their species selectivity at the rat oxytocin receptor by investigating their efficacy at stimulating contractions of rat uterine strips in an organ bath (data not shown). Compounds that were potent at the human receptor generally were also found to be potent in this assay. In order to confirm their oxytocin activity, compounds **17** and **27** were tested in an *in vivo* rat model of uterine contractility. In this assay, uterine contractile activity was monitored by recording pressure in the lumen of the right uterine horn in Sprague-Dawley rats anesthetized with thiobutabarbital sodium. The rats were pretreated with the synthetic estrogen diethylstilbestrol (DES) 18-24 h before the experiment to induce pharmacological estrus and increase uterine reactivity to oxytocin agonists through increased expression of the oxytocin receptor. Response (intrauterine pressure AUC) to a reference dose of oxytocin (1 μg/kg; ~ED_{75}) administered by i.v. bolus was stabilized with 4 repeated administrations at 30-min intervals. The test compound (or the oxytocin reference dose as a time control) was then administered via the same route, and response was expressed as a percentage of the preceding response to the oxytocin reference dose. Figure 5 shows the response to **17** and **27**, each at 0.3 mg/kg, and for the time-control oxytocin reference dose compared to spontaneous contractile activity (vehicle). All three compounds similarly augmented uterine contractile activity, thus demonstrating that the nonpeptides are as effective as oxytocin at stimulating the oxytocin receptor *in vivo*.

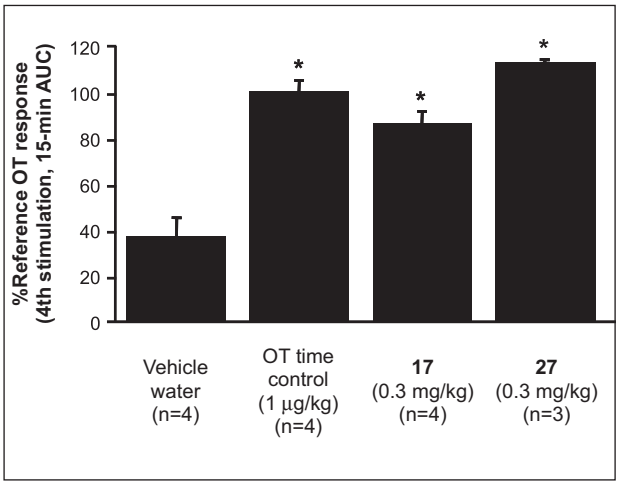


Fig. 5. Data from a rat model of uterine contraction showing AUC for oxytocin (OT), **17** and **27**. *$p < 0.001$ *vs*. vehicle, one-way ANOVA followed by Tukey test.

Conclusions

The compounds described herein are the first reported nonpeptide, small-molecule agonists of the hormone oxytocin. These agonists are potent and effective at both the human and rat receptors. Two approaches were undertaken to identify the initial hits. The first was to search for oxytocin activity in reported vasopressin V_2 agonists, and the second was to search for oxytocin-agonist activity in compounds known to have affinity for the oxytocin receptor. Both approaches utilized a functional reporter gene assay for initial screening. In accordance with the perception that it is difficult to identify nonpeptide agonists of peptide hormones, no hits were identified from the selection of oxytocin ligands screened. However, the successful identification of oxytocin-agonist activity in one of the V_2 agonists screened demonstrates the effectiveness of the first approach. The subsequent screening of a library of related compounds yielded three further structural types (phenylurea, prolylurea and piperazinylurea), demonstrating the effectiveness of the functional screening strategy. These three hits had in common a fused bicyclic or tricyclic group, and this functionality appears to be important for activity at both the V_2 and oxytocin receptors. We have suggested that this functionality might be termed a privileged structure, but it still remains to be seen whether it is privileged for nonvasopressin G-protein-coupled receptors (13, 25).

SAR studies led to improved potency at the oxytocin receptor, with compound **27**, for example, exhibiting an EC_{50} value of 33 nM. Crucially, using information from all three structural types, we were able to improve selectivity for the oxytocin receptor to 25-fold that expressed over the vasopressin V_2 receptor. The resulting compounds, such as **27**, have been shown to be active *in vivo* in a model of uterine response. These compounds are currently under further investigation as pharmacological tools in animal models of oxytocin activity, particularly in models of erectile function. Given their oxytocin-agonist activity, these small molecules have potential as drugs for diseases where oxytocin activity is compromised. They may be especially useful in the treatment of various male and female sexual disorders, including male erectile dysfunction.

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