Small Molecule Ligands for Oxytocin and Vasopressin Receptors

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I. INTRODUCTION

A. Endogenous Hormones and Receptors

The neurohypophyseal hormones oxytocin (OT) and arginine vasopressin (AVP) are structurally related cyclic nonapeptides with distinct biological functions. The peptide sequences differ only in the amino acids at the 3 and 8 positions, but for both hormones disulfide formation between cysteines at the 1 and 6 positions results in a 20-membered ring (Fig. 1). OT binds to OT receptors in the uterus and mammary glands to mediate important functions in parturition involving contraction of the uterine myometrium during labor and the mammary myoepithelium postpartum to elicit milk letdown.[1] In addition, OT receptors are located in a variety of other peripheral tissues as well as the brain where OT may have physiological effects on cardiovascular, renal, endocrinological, and behavioral functions.[2] The primary role of AVP involves regulation of renal and cardiovascular function where its antidiuretic actions on kidney AVP-V_2 receptors correct fluctuations in blood osmolality, and its pressor actions on vascular smooth muscle AVP-V_{1a} receptors help to maintain peripheral resistance under certain adverse conditions (e.g., hemorrhage).[3] Binding of AVP to this latter receptor subtype also stimulates glycogenolysis in the liver[4] and mediates platelet aggregation.[5] In addition, hypothalamic AVP stimulates adrenocorticotropic hormone release from the pituitary via more recently discovered V_{1b} (or V_3) receptors.[6] All of the currently known OT/AVP receptor subtypes are members of the G protein-coupled superfamily, and rat and human oxytocin,[7] human and rat V_{1a},[8] human and rat V_2,[9] and rat[10] and human V_{1b}[11] receptors have been cloned and characterized.

The diverse functions of OT and AVP have led to a number of therapeutic possibilities via modulation of their receptor activity. Two examples of current therapeutics are OT it-

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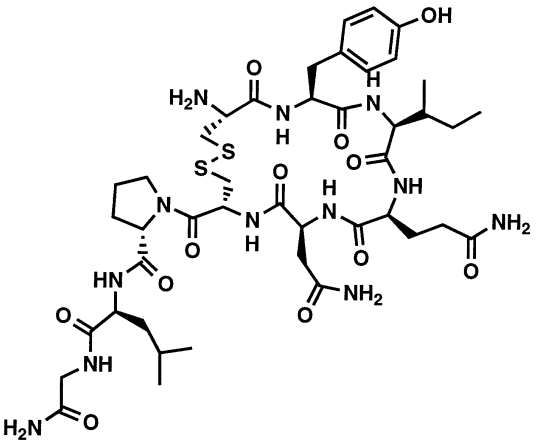

H-Cys-Tyr-**Ile**
Cys-Asn-Gln
Pro
Leu
Gly-NH_2

Oxytocin

H-Cys-Tyr-**Phe**
Cys-Asn-Gln
Pro
Arg
Gly-NH_2

Arginine Vasopressin

Figure 1. Structures of oxytocin and arginine vasopressin.

self, for the promotion of labor and delivery,[12] and desmopressin, a selective AVP-V_2 agonist for the treatment of diabetes insipidus in which there is a deficiency of circulating AVP.[13] Alternatively, based on clinical studies now ongoing with the OT antagonist atosiban (Fig. 2),[14] blockade of uterine OT receptors may provide a unique approach to the treatment of preterm labor.[15] Blockade of the vascular-type AVP-V_{1a} receptors may have utility in hypertension[16] and dysmenorrhea,[17] whereas an aquaretic response (water diuresis) caused by antagonism of renal AVP-V_2 receptors could be useful in counteracting

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Roger M. Freidinger *received a B.S. in chemistry from the University of Illinois in 1969 and a Ph.D. in organic chemistry from the Massachusetts Institute of Technology in 1975. Since 1975, he has been at the Merck Research Laboratories in West Point, Pennsylvania, where he is currently Executive Director in Medicinal Chemistry. His research has encompassed several therapeutic areas involving chemistry of peptides, peptidomimetics, and small molecule ligands for peptide receptors. Specific examples are orally active nonpeptide cholecystokinin and oxytocin antagonists.*

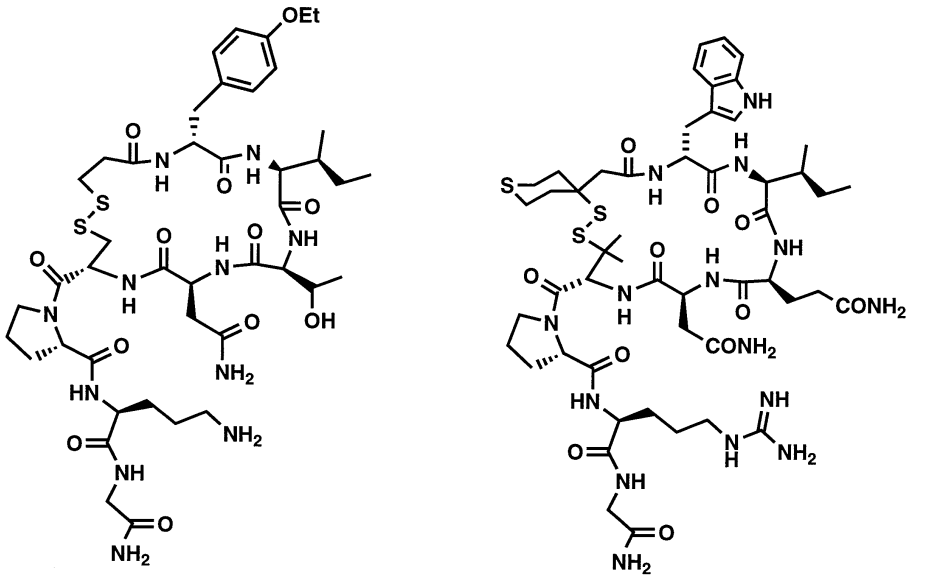


Figure 2. Representative peptide oxytocin antagonist analogs.

fluid retention and hypoosmolality resulting from a variety of conditions.[18] An AVP-V_2 antagonist may also be useful in the treatment of congestive heart failure, particularly in combination with an AVP-V_{1a} antagonist.[19]

B. Peptide Structure-Activity Studies

OT and AVP were the first peptide hormones prepared by total synthesis.[20] Since that time, due to the important physiological and pathophysiological roles of these peptides, numerous analogs covering modifications in all regions of the native structures have been prepared. Peptide agonists and antagonists, many with receptor subtype selectivity, have originated from modification of the native hormone structures. Several of these agents are important pharmacological tools with potential in therapy.[21] X-ray crystal structure determination and NMR solution conformational studies have enhanced the understanding of key features of OT and AVP.[22] Conformationally restricted analogs have been designed using this information.[23] A potent and long acting analog termed Antag III (Fig. 2) has recently been reported.[24] Recently, it has also been shown that the 20-membered ring is not required for activity, and structure-activity relationships for these less constrained linear analogs have been developed.[21] None of the peptide OT and AVP receptor ligands have significant oral activity.

II. SMALL MOLECULE VASOPRESSIN RECEPTOR SELECTIVE ANTAGONISTS

A. AVP V_{1a} Selective Antagonists

The first orally active antagonist of AVP was reported by a group from Otsuka Pharmaceuticals,[25] and it has entered clinical trials for hypertension and congestive heart fail-

ure.[26] This nonpeptidyl dihydroquinolinone structure, termed OPC-21268 (Fig. 3), was developed by optimization of a lead molecule found through random screening of several thousand compounds. The original lead compound was a 2-thiophenecarbonyl derivative of the parent piperidinylquinolinone, and it has an IC_{50} = 2.5 μM for binding to rat liver V_{1a} receptors.[27] Extensive analog work focusing on variously substituted aromatic groups in place of the thiophene resulted in the more potent OPC-21268 (IC_{50} = 0.44 μM). Additional structure-activity studies identified the importance of a hydrogen bonding element at the 2-position of the benzoyl ring.[28] The highest affinity analog is the 2-methoxy, 4-ethoxy derivative (Fig. 3) with IC_{50} = 62 nM. Very high selectivity versus V_2 receptors was retained (IC_{50} > 100 μM) for all analogs in this series. No modifications of the remaining regions of the lead structure have been reported.

OPC-21268 displaces ^{3}H-AVP from rat liver V_{1a} receptors with a K_i of 140 nM, and the interaction was shown to be competitive. No binding data was reported for oxytocin receptors or for AVP receptors from other species. OPC-21268 was also shown to be a competitive and specific antagonist of AVP-induced pressor responses in the pithed rat after

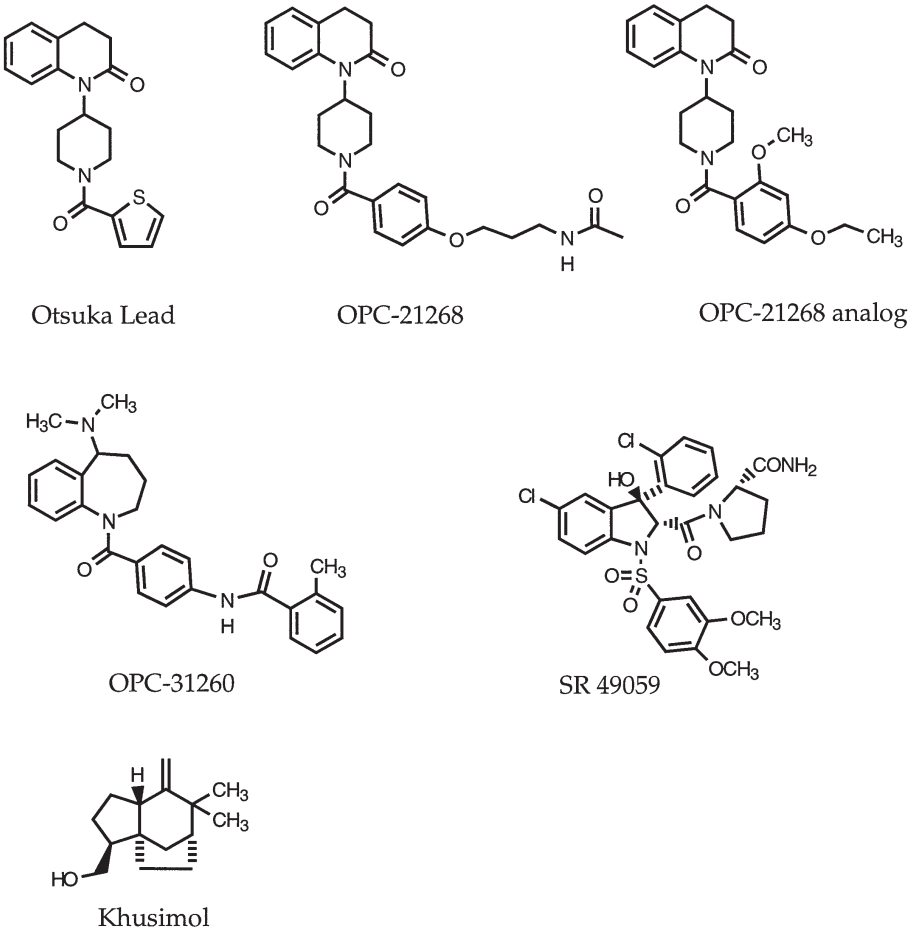


Figure 3. Nonpeptide vasopressin antagonists.

intravenous administration with a potency in the 100 to 300 μg/kg range. A hypotensive response for OPC-21268 was demonstrated in stroke-prone spontaneously hypertensive rats, suggesting a possible role of AVP in some forms of hypertension.[29] In addition, OPC-21268 improved renal function in a rat model (5/6 nephrectomized) of renal failure.[30] Significantly, the compound is orally active with an ID_{50} of 2 mg/kg for inhibiting AVP-induced vasoconstriction in conscious rats and has been shown to produce drug plasma levels after oral administration to humans.[31] In preliminary studies, it was reported that orally administered OPC-21268 produced some blockade of AVP-induced vasoconstriction in human forearm vasculature[31a] and transiently reduced blood pressure in a patient with diabetic renal failure.[32] OPC-21268, which has recently been reviewed,[33,26] will be a useful tool for studies of AVP physiology and may lead to orally active therapeutic agents.

Marked species differences in the AVP-V_{1a} receptor binding properties of OPC-21268 have been reported.[34] While high affinity for the rat liver V_{1a} receptor was confirmed (K_i = 26 nM), affinity for rhesus monkey and human liver and platelet V_{1a} receptors was considerably lower (K_i = 14,000 nM). This species difference has been confirmed independently[35] and has also been observed comparing rat liver V1a receptors and the human receptor cloned from the mesenteric artery.[8b] Recently, OPC-21268 was reported to show higher affinity for V_{1a} receptors in cultured human aortic smooth muscle cells (K_i = 138 nM) than in homogenates of various human tissues.[36] The authors suggested the existence of human AVP V_{1a} receptor subtypes from their data. In contrast to the early clinical studies, 1 μM OPC-21268 had no effect on AVP-induced contraction in human arterial coronary bypass graft internal mammary arteries.[37] These findings emphasize the differences which can occur in receptors across species and may have important implications for the therapeutic potential of OPC-21268. Interestingly, the peptide antagonist atosiban has significantly higher affinity for human liver V_{1a} receptors than rat liver V_{1a} receptors.[34,38] The basis for the species differences in pharmacology for the AVP-V_{1a} receptors will no doubt be found in their structures; the human AVP-V_{1a} receptor shows only a 72% overall amino acid identity with the rat receptor.[8a,b] Mutagenesis studies are ongoing in several laboratories[39] and will provide important clues to the structural requirements for ligand binding. In contrast to the AVP-V_{1a} antagonists, no major species differences have been observed to date for small molecule OT antagonists (see discussion below).

An alternative class of nonpeptide AVP receptor ligand represented by SR 49059 (Fig. 3) has been described by Sanofi researchers.[35] This compound is the most potent nonpeptide AVP-V_{1a} receptor ligand yet reported with K_i's for rat and human tissues ranging from 1.1 to 6.3 nM. It presumably originated from a screening lead, however, structure-activity studies have not yet been published. SR 49059 has at least 100-fold weaker affinity for AVP V_{1b} and V_2 and OT receptors. It is interesting that it does not display the great species differences seen with OPC-21268 and shows high affinity for both rat and human AVP-V_{1a} receptors.[35,40] SR 49059 is a pure, competitive antagonist in both rat (pA2, 9.42) and human tissues (pA2, 9.76)[36,37] and is orally active in rats with a long duration of action. The compound produced a potent, concentration-dependent inhibition of vasopressin-induced contraction of human coronary bypass graft internal mammary arteries. With this combination of properties, SR 49059 entered clinical trials for hypertension and congestive heart failure and represents an important new pharmacological tool.

A third structure class of nonpeptide AVP V_{1a} receptor ligand has been reported by Sanofi.[41] The sesquiterpene alcohol khusimol (Fig. 3), which was isolated from the root of *Vetiveria zizanioides,* was found to competitively inhibit the binding of vasopressin to rat liver V_{1a} receptors with a K_i = 50 μM. No other studies have been reported for this structure class.

B. AVP V_2 Selective Antagonists

The Otsuka group has also reported a different class of nonpeptide AVP receptor ligand represented by OPC-31260[42] (Fig. 3). This compound also was derived from a screening lead,[43] but structure-activity studies have not been published. In contrast to the AVP-V_{1a} selectivity of OPC-21268, this new compound binds selectively to the rat V_2 receptor. It competitively displaces ^{3}H-AVP binding to rat liver V_1 and rat kidney V_2 receptors with IC_{50}'s of 1200 nM and 14 nM, respectively. No information on oxytocin receptor binding or binding to AVP receptors from other species has been made available. The compound potently inhibited the antidiuretic action of exogenous AVP in rats within the dose range of 10 to 100 μg/kg i.v., but showed no indication of partial agonist (i.e., antidiuretic) activity on its own. Oral activity of OPC-31260 was demonstrated in normal rats in the dose range of 3 to 30 mg/kg, producing an increase in urine volume and reduction in urine osmolality. OPC-31260, therefore, exhibits the properties of an aquaretic agent as predicted from blockade of receptors. Accordingly, OPC-31260 was demonstrated to correct the water imbalance in experimental models of cirrhosis and SIADH[44] and has also been shown in several human studies to be be an effective aquaretic agent after both the i.v. and oral routes.[45] OPC-31260 represents the first orally bioavailable AVP-V_2 antagonist and has considerable potential as a novel therapeutic for the treatment of hyponatremic, water-retaining diseases.[33]

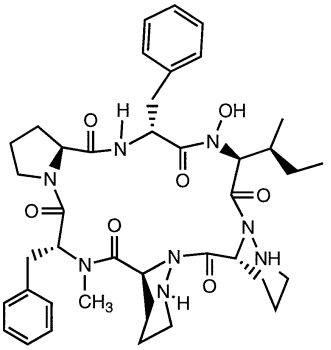
III. SMALL MOLECULE OXYTOCIN RECEPTOR SELECTIVE ANTAGONISTS

A. Cyclic Hexapeptides

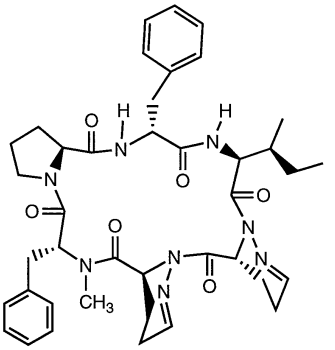
Researchers at Merck reported the first selective oxytocin antagonists from a structure class different from OT/AVP-derived antagonists.[46] The initial lead compound was discovered through a receptor-based screening program. Novel substances were sought which would inhibit the binding of ^{3}H-OT and/or ^{3}H-AVP to rat uterine OT receptors and rat liver AVP-V_{1a} and rat kidney AVP-V_2 receptors. A broth extracted from *Streptomyces silvensis* inhibited this binding, and the major active component was identified as the unusual cyclic hexapeptide L-156,373 (Fig. 4). This compound has five unusual amino acids including D-phenylalanine, *N*-hydroxy-L-isoleucine, D-piperazic acid, L-piperazic acid, and *N*-methyl-D-phenylalanine. The sixth residue is L-proline. L-156,373 has moderate affinity for rat uterine OT receptors (K_i = 150 nM) and about 20-fold selectivity versus the vasopressin receptors.

Initial chemical studies of this lead focused on the *N*-hydroxy-isoleucine residue, which was believed to be a potential source of toxicity. It was quickly found that the N—O bond could be selectively cleaved with aqueous methanolic $TiCl_3$. The resultant analog was oxidized with tert-butylhypochlorite to the more stable di-dehydro derivative L-365,209 (Fig. 4). A substantial increase in OT, but not AVP receptor affinity accompanied these changes. L-365,209 has a K_i = 7.3 nM for OT receptors and AVP-V_{1a} and AVP-V_2 selectivities of 51- and 100-fold, respectively. The compound was characterized *in vitro* and *in vivo* as a potent, competitive OT antagonist with no agonist activity. L-365,209 also exhibited AVP-V_{1a} and AVP-V_2 antagonist activity *in vitro* but was considerably weaker versus OT antagonist potency.

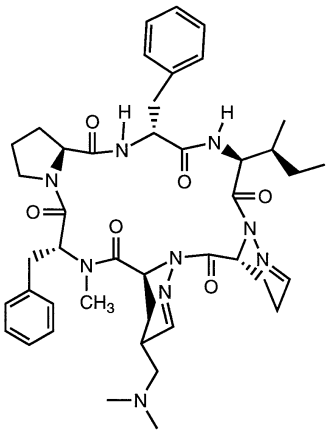
The cyclohexapeptide class of compounds represents a structural departure from all previous peptide OT and AVP antagonists. The prototype L-365,209 has three fewer amino acid residues resulting in smaller molecular size. In addition, four secondary amino acids are present, which could potentially result in better oral bioavailability by analogy to cy-



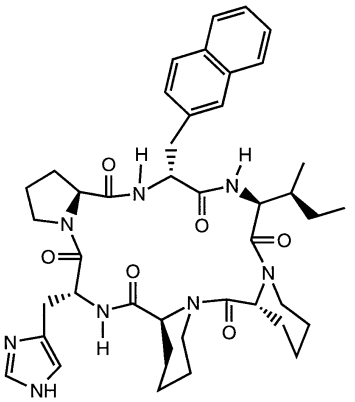
L-156,373



L-365,209



L-366,670



L-366,948

Figure 4. Cyclic hexapeptide oxytocin antagonists.

closporin.[47] Little structural homology to previous OT antagonists is obvious, however, an early working hypothesis was that the D-Phe-Ile dipeptide would mimic D-Tyr(Et)-Ile of atosiban in binding to the OT receptor.

Based on the promising properties of L-365,209, a systematic chemical modification program was initiated to identify improved OT antagonists. Specific goals were oral bioavailability and adequate aqueous solubility for intravenous formulation in a single compound. Numerous analogs from modification of the natural product[48] and compounds prepared by total synthesis[49] were studied. Each approach was successful in identifying potent, selective, and soluble antagonists, and L-366,670 and L-366,948, respectively, are optimal examples (Fig. 4). Synthetic chemical methodology had to be worked out in each case in order to prepare these analogs with facility. The key step in the former case utilized selective dimethylaminomethylation of the L-dehydropiperazic acid of L-365,209

with Eschenmoser's salt, while improved conditions for coupling highly hindered amino acids were needed in the latter instance.[50]

These structure-activity studies supported the original hypothesis that the dipeptide containing a D-aromatic amino acid followed by isoleucine is important for oxytocin receptor binding. Only substitutions for D-Phe such as D-Trp, D-2-naphthylalanine, and D-Tyr(Et) retained high receptor affinity. All substitutions for Ile resulted in lower affinity. Even the central amide bond of this dipeptide was important. The ψ [CH_2NH] compound had much reduced affinity while, in contrast, the thioamide was one of the most potent analogs (K_i = 1.1 nM). X-ray crystal structure analysis of the latter compound revealed a markedly different conformation from des-amino oxytocin including no classical beta turn.[51] The Pro and D-dehydropiperazic acid were also shown to be important for high affinity while the L-dehydropiperazic acid and N—Me—D—Phe positions accommodated a number of modifications including solublizing side chains. The preferred compounds L-366,670 and L-366,948 were excellent OT antagonists *in vitro*[52] and *in vivo* by the intravenous route with no agonist activity,[53] however, they did not display measurable activity after intraduodenal administration to rats. Interestingly, certain other compounds of this structural class unexpectedly exhibited a reasonably potent *agonist* activity at uterine bradykinin receptors in addition to their OT antagonist properties.[54]

B. Spiro Indanylpiperidines and Tolylpiperazines

In a search for alternative structures with potential oral activity, the Merck group reported the first nonpeptide oxytocin antagonists.[55] The lead nonpeptide compound L-342,643 (Fig. 5) was discovered from screening of a synthetic chemical collection and has some structural relationship to opioids such as meperidine. L-342,643 has an IC_{50} of 4 μM for displacing binding of ^{3}H-OT with some selectivity with respect to AVP receptors. Structure-activity studies produced the improved antagonist L-366,509 (Fig. 5), a derivative of camphorsulfonic acid. This water soluble compound has K_i's in the 500 nM range for rat, rhesus monkey, and human OT receptors and shows good selectivity for OT versus AVP receptors. It is a readily reversible, competitive antagonist in rat uterine tissue (pA2, 7.32) with no detectable agonist-like activity or antagonist activity against a variety of other contractile agonists. L-366,509 also antagonizes OT-stimulated uterine contractions by intravenous as well as by intraduodenal and oral routes in rat and rhesus monkey, respectively, with a long duration of action. The properties of L-366,509 indicated that water soluble, orally active OT antagonists could be obtained from this structural class; however, the potency was less than that required for further development for potential therapeutic use. Efforts were thus focused on structural modification of this analog to obtain more potent antagonists.

Initially, introduction of substituents on the spiropiperidinylindene and the camphorsulfonyl or simplification of the latter were not fruitful. Reduction of the indene to indane had little effect on receptor affinity in several analogs. In spite of the L-366,509 precedent, none of these modifications produced compounds that had good oral bioavailability, although more potent acidic compounds were identified. Eventually, the research focused on solubility- and potency-enhancing substitutions in the camphor-2-endo position.[56] From several general series of amino or aminoalkyl camphors, the endo acylamino compounds have the best overall properties. For example, L-367,773 (Fig. 5), which incorporates an imidazolacetylamino group, has K_i's of 26 and 61 nM for rat and human oxytocin receptors, respectively. It has good selectivity versus vasopressin receptors, and has suitable water solubility. L-367,773 is a potent, competitive pure antagonist in functional assays and is orally bioavailable in rat, dog, and rhesus monkey.

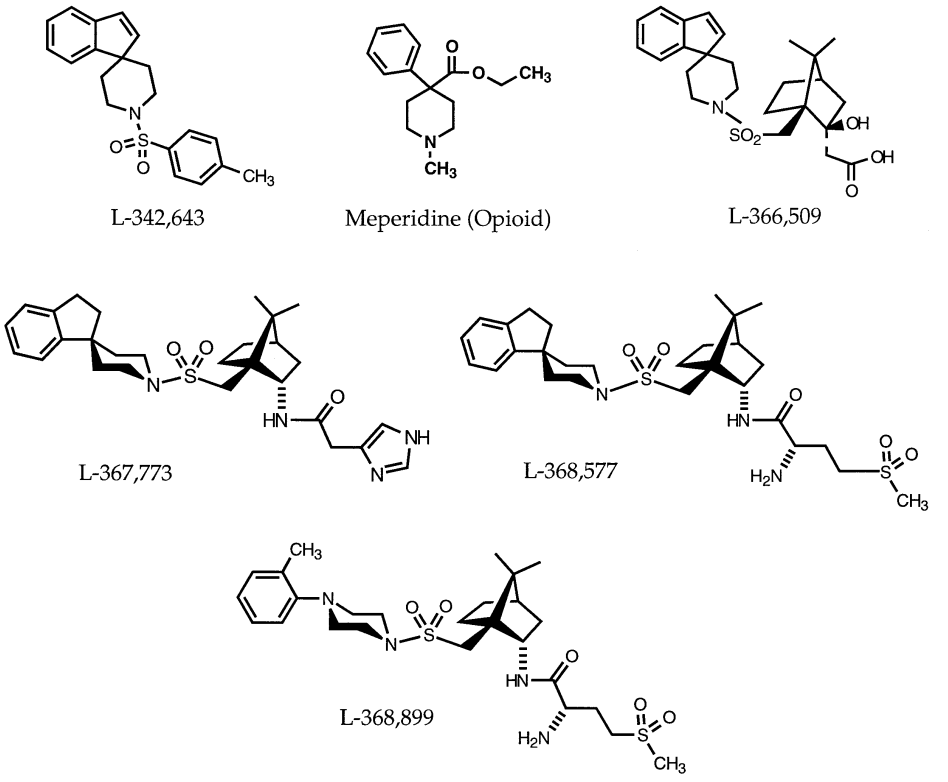


Figure 5. Nonpeptide oxytocin antagonists.

Further elaboration of the camphor 2-endo position gave a number of even more potent analogs[56,57] including the methionine sulfone analog L-368,577, K_i = 6 nM (rat), (Fig. 5). Comparison of a number of analogs suggests that the potency increase can be attributed to a new hydrogen bonding interaction with the receptor. Unfortunately, the oral bioavailability and pharmacokinetic profile of L-368,577 were not satisfactory. One of the few useful modifications of the spiropiperidinylindene to be discovered led to the optimal analog. It was found that camphorsulfonyl ortho-tolylpiperazines in almost all cases gave compounds comparably potent to the spiro derivatives.[58] Phenylpiperazines or meta- or para-substituted phenylpiperazines, however, are much less potent. X-ray structural and modeling studies indicate that the ortho substituent enforces a preferred conformation for this system that closely mimics the spiro system. This molecular arrangement along with the rigid camphor substructure is very important for good OT receptor affinity. The L-methionine sulfone analog in the tolylpiperazine series L-368,899 (Fig. 5) has the best combination of *in vitro* and *in vivo* potency (K_i = 3.6 and 13 nM, rat and human OT receptors, respectively), selectivity, aqueous solubility, oral bioavailability, and pharmacokinetics[59,60] with no agonist activity. L-368,899 was also found to be a potent OT antagonist in the near-term pregnant rhesus and to block the spontaneous nocturnal labor-like uterine activity that precedes and culminates in parturition in this species. Based on the overall attractive profile, Phase I human clinical trials of this compound in the treatment of premature labor were initiated. In these studies, L-368,899 was found to be oral-

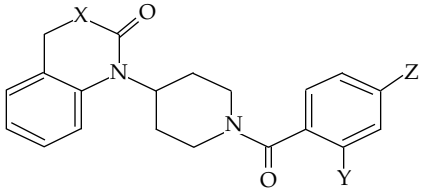
ly bioavailable and to be a reasonably potent OT antagonist in postpartum women[61] with a potency predicted by studies in the pregnant rhesus. The pharmacokinetics and oral bioavailability, however, were suboptimal, and further clinical evaluation was not undertaken. Efforts continue toward an optimal compound, and it must be emphasized that systematic investigation of structural diversity in all domains of the lead structures is crucial for solving the difficult problem of obtaining a potent, selective, and orally bioavailable antagonist with sufficient aqueous solubility for intravenous administration.

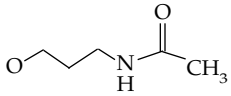
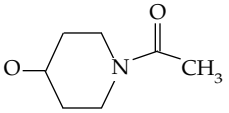
C. Benzoxazinylpiperidines

The vasopressin antagonist OPC-21268 (Fig. 3) has been examined further, especially for its affinity for rat and human uterine oxytocin receptors. Interestingly, the compound displays moderate affinity for both and is actually an oxytocin-selective ligand for human receptors (Table I).[62]

Based on these observations, the investigators at Merck undertook structure-activity studies to improve the human oxytocin receptor affinity and selectivity of OPC-21268. Insertion of an oxygen into the dihydroquinolinone to produce a benzoxazinone gave a two-fold enhancement in affinity. Constraint of the acetamidopropyloxy chain with a piperidine ring gave a further six-fold improvement. Finally, addition of an ortho-methoxy substituent on the benzoyl segment gave five-fold greater affinity. The compound L-371,257 containing all of these enhancements was then synthesized and found to have an attractive profile (Table I). It has high affinity for human uterine oxytocin receptors (K_i = 4.6 nM), but its affinity for vasopressin AVP-V_{1a} and AVP-V_2 receptors is nearly 1000- and 10,000-fold weaker, respectively. L-371,257 exhibits the same species differences in binding as OPC-21268. It has highest affinity for rat AVP-V_{1a} receptors (K_i = 3.7 nM), slightly lower affinity for rat OT receptors, and very weak affinity for rat AVP-V_2 receptors.

TABLE I
Rat and Human OT/AVP Receptor Binding of OPC-21268 and L-371,257



Compound	*X*	*Y*	*Z*	K_i (nM) OT Rat Uterus Human Uterus	AVP-V_{1a} Rat Liver Human Platelet	AVP-V_2 Rat Kidney Human Kidney
OPC-21268	CH_2	H		230 ± 35	32 ± 5.2	≥30,000
				170 ± 49	52,000	>81,000
L-371,257	O	OCH_3		19 ± 2.1 4.6 ± 0.25	3.7 ± 0.79 3200 ± 82	≥30,000 37,000 ± 5,500

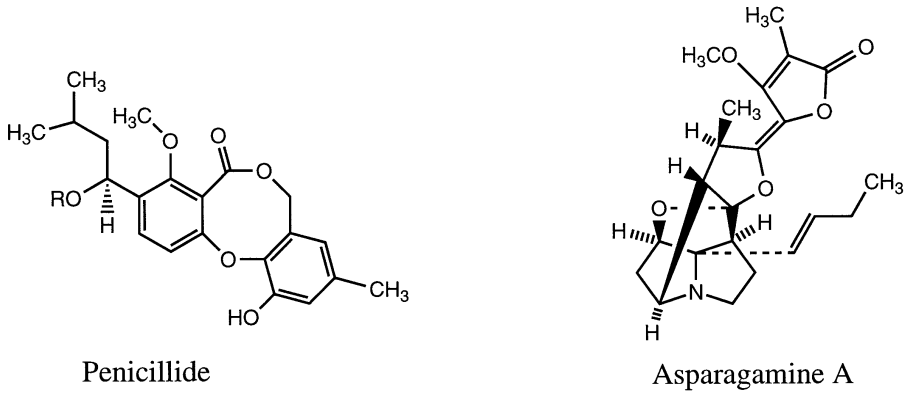


Figure 6. Natural product nonpeptide oxytocin antagonists.

L-371,257 was also found to be a potent antagonist of OT-stimulated contractions of the rat uterus *in vitro* and *in vivo* (pA_2 = 8.44, AD_{50} = 0.55 mg/kg, respectively). Importantly, significant oral activity was demonstrated in the rat.

D. Penicillide

This fungal metabolite (Fig. 6, R = H) was identified as a weak OT receptor ligand (K_i = 27 μM, rat uterus) from a fermentation extract of *Taloromyces flavus* through receptor-based screening.[63] A monoacetate derivative of penicillide had improved OT receptor affinity (Fig. 6, R = $COCH_3$; K_i = 3.4 μM, rat uterus) and was shown to be an OT antagonist on the isolated rat uterus.

E. Asparagamine A

This alkaloid (Fig. 6) was isolated as a major constituent from the roots of *Asparugus racemosus* Willd. (Liliaceae).[64] The plant has been used in Asian folk medicine and is known to have antiabortifacient properties. Asparagamine A had an inhibitory effect on oxytocin-induced contraction of rat diestrus uterus. No further studies have been reported for this class of structures.

F. Structural Comparisons of Cyclic Hexapeptide and Nonpeptide Antagonists

Molecular modeling studies with L-368,899 and its analogs have led to the hypothesis that the tolylpiperazine (or spiropiperidinylindane) camphorsulfonamide portion of the molecule functions as a mimetic of the D-Phe^2-Ile^3 or D-Nal^2-Ile^3 dipeptides found in the cyclic hexapeptide OT antagonists L-365,209 and L-366,948, respectively. As already described, structure-activity studies in the cyclic hexapeptide OT antagonist class have demonstrated the importance of this dipeptide for obtaining good receptor affinity, and this dipeptide may relate to the important D—AA^2—Ile^3 (AA = aromatic amino acid) dipeptide found in many potent OT-derived antagonist analogs. In L-368,899, structure-activity studies indicate that the camphorsulfonyl tolylpiperazine has both important binding and conformational properties for good receptor interaction. A superposition of a low-energy conformer of L-368,899 with an NMR-consistent, low-energy conformer of L-366,948 is given in Figure 7. Good overlaps of important hydrophobic and hydrophilic

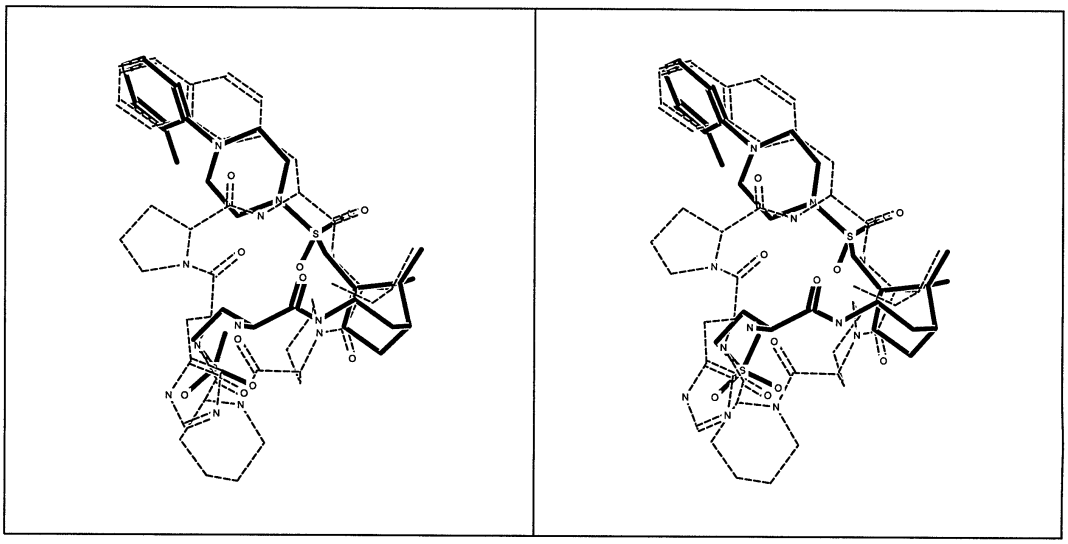


Figure 7. Comparison of L-366,948 (dashed) and L-368,899 (solid) structures.

elements from each molecule are seen, i.e., the D-Nal and Ile side chains align with the tolyl and camphor groups, respectively, and the 2,3-position amide bond oxo group aligns with one of the camphorsulfonamide oxo groups. Potency-enhancing camphor C2 *endo* substituents in this alignment are oriented toward other regions of the cyclic hexapeptide structure. For example, the flexible methionine sulfone portion of L-368,899 has several available low-energy conformations in which it can occupy the space of the 5,6-dipeptide, a region of the cyclic hexapeptide structure that is known to tolerate numerous amino acid substitutions. The specific conformation of the methionine sulfone group shown in Figure 7 provides a good alignment of important hydrophilic groups from each molecule while orienting the methionine sulfone α-amino group out toward the D-His side chain. Receptor binding results with more conformationally constrained side chains are in accord with this alignment.[65] Another possibility is that the more flexible methionine sulfone region of L-368,899 could access binding sites on the OT receptor not utilized by the cyclic hexapeptides. It is to be noted that this analysis is retrospective and did not play a pivotal role in developing either class of antagonists. Application of this hypothesis to further novel antagonist design is being attempted.

IV. CONCLUSION

In the last five years, there has been considerable progress in the development of small molecule, selective, and orally bioavailable antagonists for OT and AVP receptors. No such ligands with agonist activity have been reported, however, as has been the case for several other peptide receptors.[66] Nonpeptide antagonists selective for OT, AVP-V_{1a}, or AVP-V_2 are now known, and at least one compound in each case has entered human clinical trials. These novel structures offer the potential for advances in therapy.

All of the lead structures were discovered from natural and synthetic sources through screening efforts, not design based on known peptide structure–conformation–activity re-

lationships. Combinatorial library methodology has not yet been applied. Elaboration to compounds with optimal properties largely utilized empirical approaches. With currently available technology, *de novo* design of such structures would be highly unlikely, however, it should continue to be a future goal.

Along these lines, it is interesting to compare structural features of the different classes of OT and AVP antagonists. Possible relationships between the peptide and nonpeptide OT antagonists have already been discussed (Sect. III.F). Although 3-dimensional modeling studies comparing nonpeptide and peptide AVP antagonists have not been reported, it would appear that a similar analysis may apply. At least some elements of the peptide and nonpeptide structures may bind to similar sites on the receptor. Binding studies with specifically mutated neurokinin and CCK receptors[67] have given interesting insights on ligand-receptor interactions in these systems. Similarly, mutagenesis studies with the OT and AVP receptors using examples of the small molecule antagonists of OT/AVP described herein should provide valuable information on the structural requirements for ligand binding. Initial studies with peptide AVP ligands and mutated AVP-V_{1a} receptors have already suggested amino acid residues involved in the binding site of this receptor.[39a,b,68]

There are interesting structural similarities among the nonpeptide OT and AVP antagonists. A core benzofused 5-, 6-, or 7-membered ring is found in all cases. In most examples, a piperidine or piperazine is attached to this ring. These comparisons suggest that there is a common binding region for the core structure on all of the receptor subtypes. Selectivity for the subtypes is then achieved from the different core structure modifications which would take advantage of differences in the receptor structures. Since this type of core structure is found in selective ligands for other receptors as well (e.g., opioid), this analysis can be extended beyond OT and AVP receptors. No AVP V_{1b} selective nonpeptide ligands have yet been reported. It will be most interesting to learn if this core structure can be used as well in this case.

Elucidating the role of structure of these selective ligands and their receptors will provide for many future research challenges. Comparable advances in understanding bioavailability and pharmacokinetic problems will also be needed. These studies will facilitate efforts to design novel receptor ligands and will lead to new pharmacological tools and therapeutic agents. In the OT/AVP field in particular, there is much more to be done.

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