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Discovery and optimization of highly ligand-efficient oxytocin receptor antagonists using structure-based drug design

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ABSTRACT

A novel oxytocin antagonist was identified by 'scaffold-hopping' using Cresset FieldScreen molecular field similarity searching. A single cycle of optimization driven by an understanding of the key pharmacophoric elements required for activity led to the discovery of a potent, selective and highly ligand-efficient oxytocin receptor antagonist. Selectivity over vasopressin receptors was rationalized based on differences in the structure of the natural ligands.

The cyclic nonapeptide hormone oxytocin **1** acts at the seven-transmembrane oxytocin (OT) receptor and is associated with numerous physiological roles including control of uterine contractions, regulation of sexual response, behaviour and emotions.[1,2] The endogenous oxytocin peptide is used clinically to induce labour in pregnant women,[3] and the oxytocin antagonist atosiban is an established acute treatment to delay the onset of pre-term labour.[4] Although there are no confirmed OT receptor subtypes, OT receptors show close structural similarity to vasopressin receptors (particularly V1a), and the oxytocin **1** and vasopressin **2** peptides only differ by two amino acids (Fig. 1).[1] This similarity is demonstrated by the binding affinity of oxytocin **1** at the two receptors (OT Ki = 6.8 nM; V1a Ki = 34.9 nM).[5]

Atosiban **3** (Fig. 2) is non-selective (OT Ki = 397 nM; V1a Ki = 4.7 nM),[5] and requires dosing by continuous infusion for up to 48 h,[4] hence there has been significant interest in developing an orally active, selective OT antagonist.[2,6]

A number of chemotypes have been investigated (Fig. 2), including triazole **4** discovered by researchers at Pfizer,[7] and diketopiperazine (DKP) GSK221149 (**5**) which shows greater than 1400-fold selectivity over vasopressin receptors, and a greater than 10-fold increase in potency at the oxytocin receptor compared to atosiban, despite a greater than 50% reduction in molecular weight.[8]

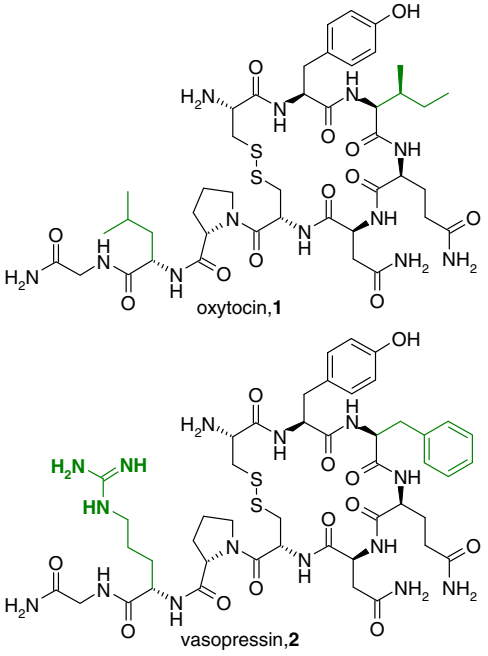

Figure 1. Comparison of structures of oxytocin and vasopressin.

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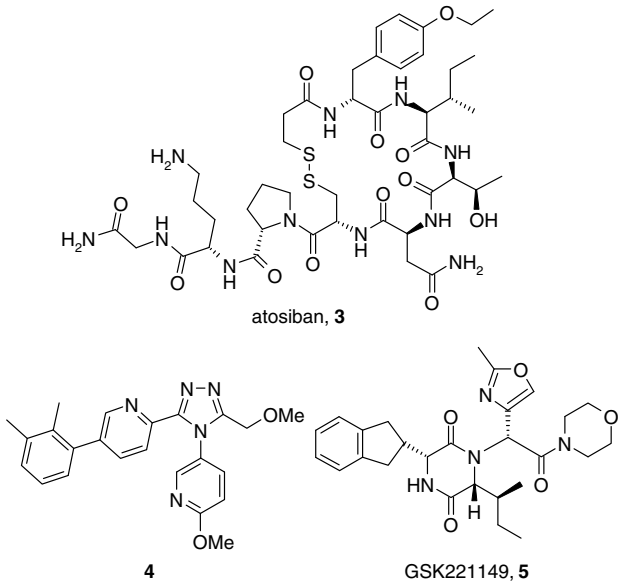


Figure 2. Oxytocin antagonists.

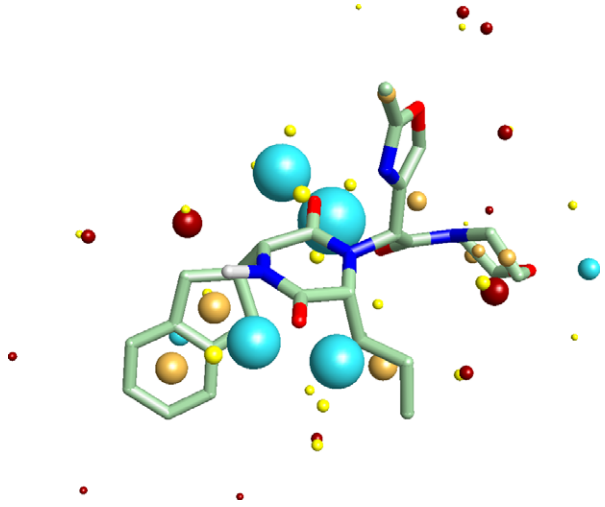


Figure 3. Cresset FieldScreen representation of GSK221149. Blue field points (spheres) highlight energy minima for a positively charged probe, red for a negative probe. Yellow spheres represent an attractive van der Waals minima for a neutral probe and orange spheres represent hydrophobic centroids. Oxygen atoms are shown in red, nitrogen in blue. The size of the points is related to the strength of the interaction.

A virtual screening approach was adopted to search for a novel chemical series of small molecule antagonists. The extended electron density representation offered by the Cresset XED forcefield provides a way to characterize the calculated field around a molecule. In this approach, probes are used to identify interaction minima and depicted as molecular field points showing the location and significance of the minima.[9]

An appropriate conformation of GSK221149 was required in order to generate molecular field points with which to perform a virtual screen. Docking studies were performed to generate a putative binding mode for the compound in a rhodopsin based homology model of the oxytocin receptor. The initial ligand conformation was based on the small molecule crystal structure of GSK221149 and fitted to the receptor model. Receptor–ligand complexes were then optimized with molecular mechanics and the preferred hypothesis selected. The resulting conformation of the ligand was used to perform a virtual screen of two million compounds in the FieldScreen system provided by Cresset (Fig. 3). The resulting hit list of optimized overlays was ranked using the similarity score of the field points and after being filtered to remove compounds with undesirable functionalities and properties, the ranked list was inspected visually in 3D. Two thousand five hundred overlays were assessed in the context of the SAR established for the DKPs which showed that a variety of groups were tolerated in the morpholine position, while the SAR around the indane group is relatively tight.[8] 219 compounds were selected for screening.

The biaryl amide, **6,** was one of fourteen active oxytocin antagonists identified from this search, and demonstrated a fpKi of 7.3 in a functional FLIPR assay using recombinant human oxytocin receptor CHO cells. The low molecular weight (335) and very low polar surface area (29.5)[10], made this an extremely attractive template with good ligand efficiency (LE = 0.40, BEI = 22, SEI = 25).[11] The synthetic tractability of the compound also made further optimization readily accessible.

The field print image (Fig. 4) used to identify **6** shows one possible overlay of this molecule with GSK221149. The amide functionality in **6** generates a molecular field point co-located with that from one of the carbonyls of the diketopiperazine (DKP). The THF moiety also has a projected nucleophilic/acceptor feature which is mimicked by the other DKP acceptor. This demonstrates an advantage of a field based approach in which it is not the contributing atoms which overlay, but the fields they present for interaction with a receptor.

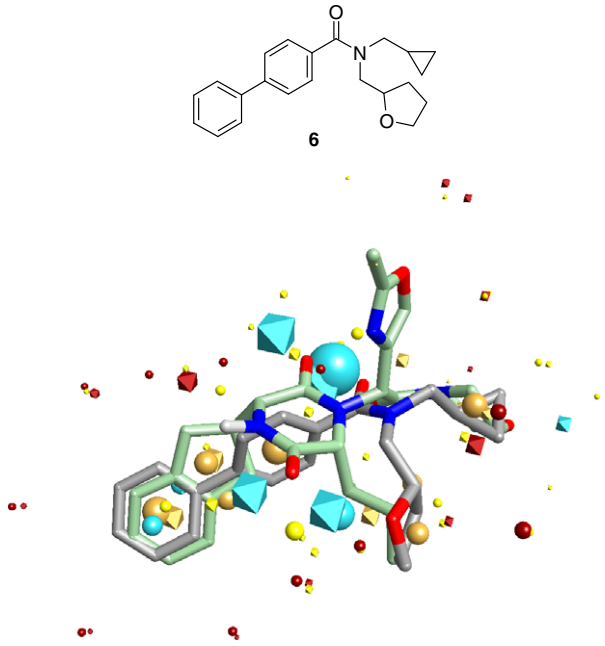


Figure 4. Cresset FieldScreen overlay of biaryl amide **6** (grey with spherical field points, structure shown at top) and GSK221149 (**5**) (green with octahedral field points).

Multiple conformations are possible for both the biaryl amide and the DKP, and before designing modifications to the lead compound **6**, alternative overlays were considered in order to be able to generate and test multiple binding hypotheses. Biaryl amide **6** was mapped to a consensus pharmacophore derived from a set of published oxytocin antagonists including triazole **4** and diketopiperazine **5**. The overlay of **6** with **5** shown in Figure 5 suggests an alternative possible alignment of these two molecules.

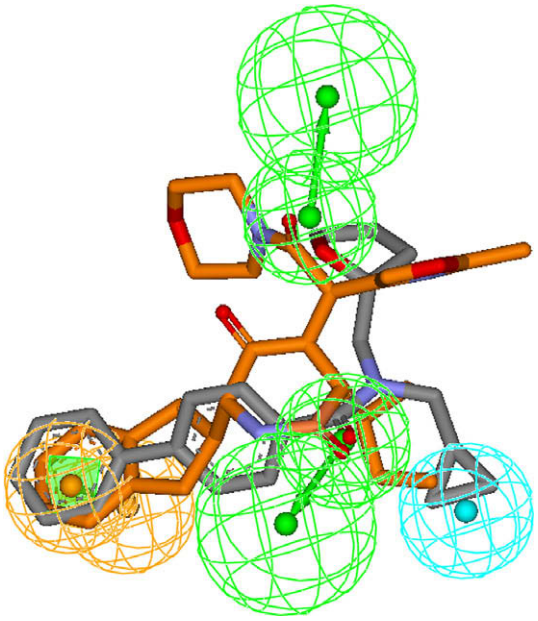
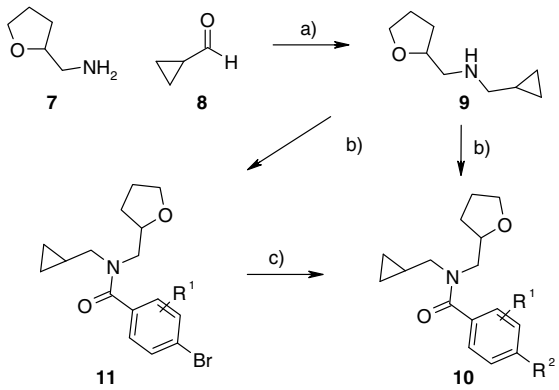


Figure 5. Mapping of the DKP compound **5** (orange) and biaryl amide **6** to a consensus pharmacophore of published oxytocin antagonists. The matched features shown are three H-bond donor features (green), aromatic pi-system (brown) and hydrophobe (cyan).

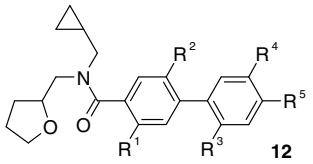
In both cases, the distal aromatic of the biaryl system overlays with the indane of the DKP. The biaryl adopts a ring-twist, suggesting that locking this conformation by incorporating a group *ortho* to the ring junction may be beneficial. The relative position of the cyclopropylmethyl and tetrahydrofuranyl (THF) groups is different in the two overlays; in the pharmacophore-derived instance the THF oxygen is superposed with the hydrogen bond acceptor of the morpholine amide of **5**, and the cyclopropylmethyl group of **6** is co-located with the isobutyl group in the DKP. It has been proposed that the isobutyl group of the DKP mimics the isoleucine group in oxytocin.[12] This is replaced by a phenylalanine in vasopressin; hence this position may be important for selectivity.

Based on these hypotheses, small arrays were designed and synthesized as shown in Scheme 1. Key SAR findings are summarized in Tables 1 and 2. The introduction of a chlorine group in the distal ring (**12a**) or a methyl group in the proximal ring (**12e**) in positions which can cause a ring-twist increase



Scheme 1. Reagents and conditions: (a) i—Na_2SO_4, DCM, 100 °C, 10 min; ii—$NaBH_4$, DCM/MeOH, rt, 1 h; (b) $ArCO_2H$, PS-EDC, HOAt, DCM/NMP/THF, rt, 2 days then add PS-NCO, MP-carbonate; (c) R^2–$B(OH)_2$, $Pd(PPh_3)_2Cl_2$, Na_2CO_3, DME/EtOH/H_2O, 140 °C, 15 min.

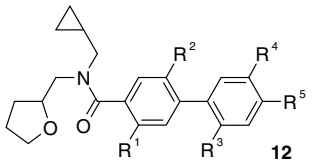
Table 1
Effect of biaryl substitution on potency



12

	R^1	R^2	R^3	R^4	R^5	fpKi
6	–H	–H	–H	–H	–H	**7.3**
12a	–H	–H	**–Cl**	–H	–H	**8.5**
12b	–H	–H	**–OMe**	–H	–H	**7.2**
12c	–H	–H	**–SO2Me**	–H	–H	**6.2**
12d	–H	–H	**–CN**	–H	–H	**5.7**
12e	–H	**–Me**	–H	–H	–H	**9.1**
12f	**–Me**	–H	–H	–H	–H	**6.9**
12g	–H	–H	–H	**–Cl**	–H	**8.1**
12h	–H	–H	–H	–H	**–Cl**	**7.1**

Table 2
Effect of nitrogen incorporation on potency



12

	X	A^1	A^2	A^3	fpKi	ΔfpKi vs 6
6	O	–CH–	–CH–	–CH–	7.3	–
12e	O	–CH–	–C(Me)–	–CH–	9.1	+1.8
13a	O	–N–	–CH–	–CH–	6.1	−1.2
13b	O	–CH–	–N–	–CH–	5.5	−1.8
13c	O	–CH–	–CH–	–N–	<5.5	>−1.8
13d	N(Et)	–CH–	–C(Me)–	–CH–	<5.5	>−1.8

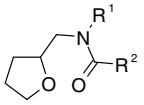
potency. Small lipophilic groups are tolerated in other positions but have a smaller effect (**12f–h**). Polarity in general appears to be poorly tolerated within the biaryl portion of the molecule (**12c,d, 13a–c**). Replacement of the THF–oxygen of compound **12e** with *N*-ethyl (**13d**) results in a complete loss of activity (fpKi < 5.5).

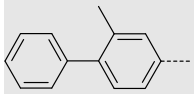
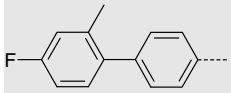
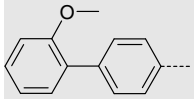
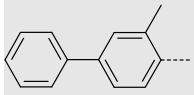
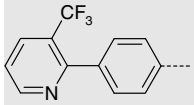
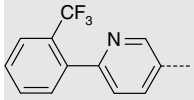
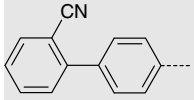
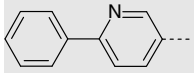
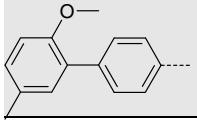
Compound **12e** was selected for further profiling and shows excellent selectivity versus vasopressin V1a in the functional assay (Table 3). The replacement of cyclopropylmethyl with benzyl conferred a reversal of selectivity, with **14** showing higher potency at V1a than OT. This effect is consistent across all analogues prepared as shown in Chart 1 and Table 3. The data supports the proposal above that this group can be superposed with the DKP isobutyl group, the isoleucine group of oxytocin or the benzyl group of vasopressin.

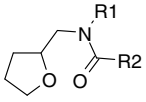
The binding affinity of **12e** at oxytocin, and selectivity versus multiple vasopressin receptors was investigated using a filtration binding assay.[13] 10-fold selectivity was observed versus V1a, and the compound showed greater selectivity versus V1b and V2 (Table 4).

In summary a novel, low molecular weight oxytocin antagonist **6** has been identified using molecular field print similarity searching. Multiple binding hypotheses were used to design an array which led to the discovery of **12e**, an OT receptor antagonist with nM potency and selectivity over vasopressin receptors in only one cycle of optimization. The molecule has low molecular weight and polar surface area making it a highly efficient ligand (LE = 0.48, BEI = 26, SEI = 31).

Table 3
Effect on vasopressin V1a selectivity of replacement of methylcyclopropyl group with benzyl



R^2	R^1 = benzyl				R^1 = methylcyclopropyl			
		OT fpKi	V1a fpKi	OT – V1a		OT fpKi	V1a fpKi	OT – V1a
	**15a**	7.4	8.5	−1.1	**12e**	9.1	7.1	2
	**15b**	7.8	8.6	−0.8	**16b**	8.5	7.6	0.9
	**15c**	7.2	7.9	−0.7	**12b**	7.2	6.1	1.1
	**15d**	6.2	7.3	−1.1	**12f**	6.9	5.7	1.2
	**15e**	6	7.1	−1.1	**16e**	6.7	5.1	1.6
	**15f**	7.1	8.4	−1.3	**16f**	6.5	5.4	1.1
	**15g**	6	6.8	−0.8	**12d**	5.7	5.4	0.3
	**15h**	5.5	6.3	−0.8	**13b**	5.5	5.4	0.1
	**15i**	5.2	5.8	−0.6	**16i**	5.2	5	0.2



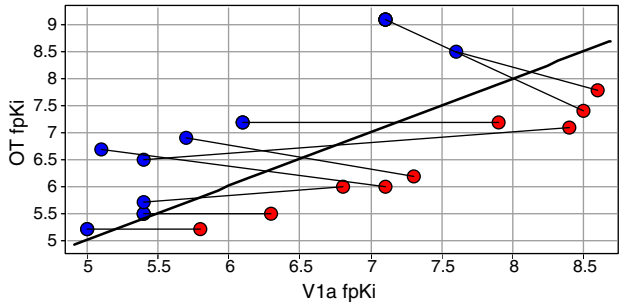


Chart 1. Plot showing OT and V1a potency where R^1 = methylcyclopropyl (blue) and R^1 = benzyl (red). Lines link molecules with the same R^2 groups. Structures shown in Table 3.

Table 4
Selectivity profiling of compound **12e**

	OT	V1a	V1b	V2
Filtration binding pKi	8.9	7.9	<4.9	6.6
Functional FLIPR fpKi	9.1	7.1	5.9	–

Acknowledgement

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13. Filtration binding was determined by displacement of 3H-oxytocin from recombinant human oxytocin receptors stably transfected in CHO cells.