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Non-peptide oxytocin agonists

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Abstract—A library of compounds targeted to the vasopressin/oxytocin family of receptors was screened for activity at a cloned human oxytocin receptor using a reporter gene assay. Potency and selectivity were optimised to afford compound **39**, $EC_{50} = 33$ nM. This series of compounds represents the first disclosed, non-peptide, low molecular weight agonists of the hormone oxytocin (OT).
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1. Introduction

Oxytocin (OT) is a cyclic nonapeptide that acts at the OT receptor, a member of the super-family of G-protein coupled receptors (GPCRs). OT and the closely related hormone arginine vasopressin (AVP) are neurohypophyseal hormones secreted by the posterior pituitary gland.[1] AVP acts at the vasopressin receptor of which three subtypes, V_{1a}, V_{1b} and V_2 have so far been identified. The OT receptor is approximately 10-fold selective for OT over AVP whereas the vasopressin receptor subtypes show stronger selectivity, up to 400-fold, in favour of AVP.[2,3] Within the periphery the OT receptor is localised in a number of different organs including the uterus and mammary glands. In the uterus it is involved in the onset and progress of labour. As such it has been shown that OT antagonists have utility in the treatment of pre-term labour and the peptide OT antagonist atosiban (Tractocile) is marketed as a treatment for this indication.[4] The OT receptor has also been localised in central tissues such as the paraventricular nucleus where it is involved in regulation of both male and female sexual response.[1,5] It is clear that OT has a range of physiological roles that have not been fully elaborated. Thus there is a need for new, potent, selective and efficacious OT receptor agonists that may be used as pharmacological tools and as potential drugs. Furthermore, the peptide vasopressin and oxytocin analogues such as carbetocin (Duratocin) are poorly absorbed due to their high hydrophilicities.[6] Thus there is a need to develop non-peptide compounds that may have potential to be orally bioavailable. These may have utility as drugs where OT function is compromised, in particular in the treatment of various sexual disorders including male erectile dysfunction. Also in promoting labour, controlling post-partum bleeding and increasing milk let-down.

A number of non-peptide antagonists of OT have been discovered.[7–9] However, it is generally perceived to be more difficult to identify non-peptide agonists, principally because of the much reduced molecular size compared to the parent peptide hormone.[10,11] However,

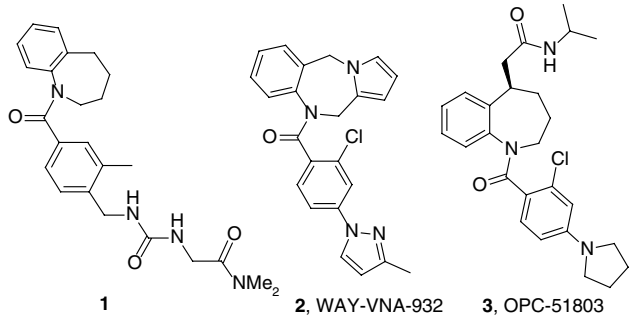

Figure 1. Known non-peptide V_2 agonists.[12–14]

Keywords: Oxytocin agonist; Non-peptide; Male erectile dysfunction.
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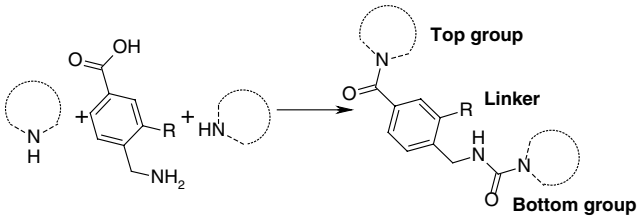


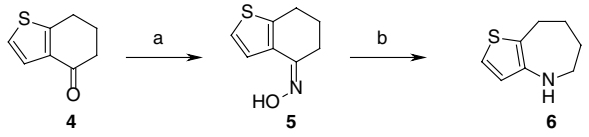
Scheme 1.

non-peptide agonists of the V_2 receptor have already been reported by us (compound **1**), as well as by Wyeth-Ayerst (WAY-VNA-932, **2**) and Otsuka (OPC-51803, **3**), the structures of which are shown in Figure 1.[12–14] Given the close relationship between OT and AVP, we hypothesised that a library designed around V_2 agonists would yield OT agonists. To test this hypothesis these V_2 agonists were assayed for their OT activity in a functional reporter gene assay, vide infra. Compounds **2** and **3** showed <10% response at 3 μM. However, **1** gave a response relative to OT of 33% at 3 μM. Therefore, a library of 50,000 structures related to **1** was screened and the resulting OT agonists are described in this paper.

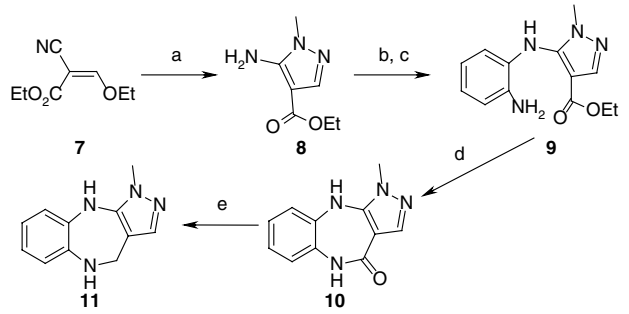
2. Chemistry

The compounds related to **1** were prepared through the synthesis of 'building blocks', which were coupled together at the benzoyl-amide and at the urea as shown schematically in Scheme 1.

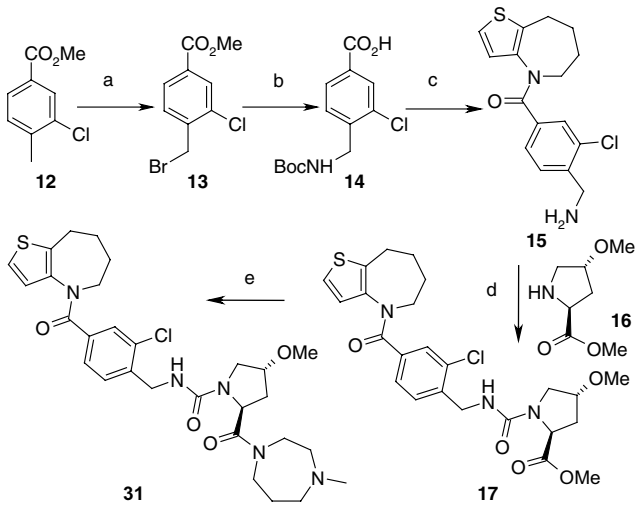
The synthesis of the top groups is shown in Schemes 2 and 3. The building block 5,6,7,8-tetrahydro-4*H*-thieno[3,2-*b*] azepine, **6**, present in compounds **28**–**38** was



Scheme 2. Reagents and conditions: (a) $HONH_2$, NaOH, EtOH, H_2O, 80%; (b) DIBAL, CH_2Cl_2, 78%.



Scheme 3. Reagents and conditions: (a) $MeNHNH_2$, EtOH, 85%; (b) 2-fluoronitrobenzene, NaH, THF, 62%; (c) H_2, Pd/C, MeOH, 92%; (d) $NaCH_2SOMe$, DMSO, 75 °C, 2.5 h, 40%; (e) $LiAlH_4$, THF, 98%.

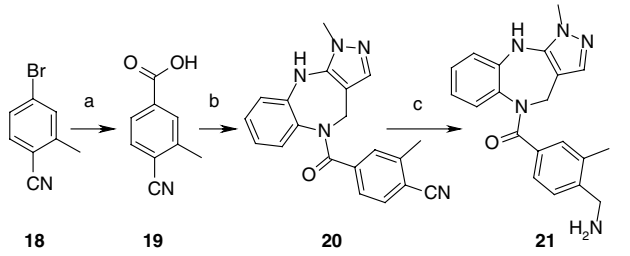


Scheme 4. Reagents and conditions: (a) NBS, AIBN, CCl_4, reflux, 84%; (b) (i) NH_3, EtOH, (ii) $(Boc)_2O$, NaOH, dioxan, H_2O, 67%; (c) (i) **6**, WSC·HCl, Et_2N^iPr, DMAP, CH_2Cl_2, (ii) 4 N HCl/dioxan, 73%; (d) CDI, Et_2N^iPr, DMF, 98%; (e) (i) LiOH, H_2O, THF, (ii) 1-methylhomopiperazine, WSC·HCl, HOBt, Et_3N, CH_2Cl_2, 76%.

prepared via a DIBAL mediated Beckmann rearrangement from commercially available 4-keto-4,5,6,7-tetrahydrothianaphthene **4**.[15]

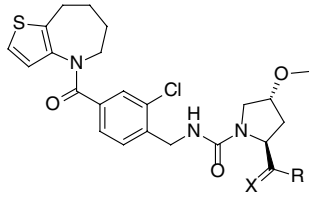
The cyclisation of **9** that led to the synthesis of the fused azepine tricycle **11** could be carried out in refluxing 2-propanol in the presence of acetic acid over 14 days in a yield of 80%. More conveniently, the cyclisation was carried out in basic conditions to achieve a yield of 40% in just a few hours.[16]

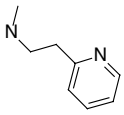
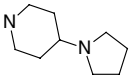
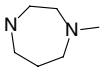
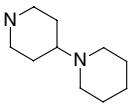
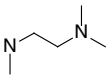
The synthesis of the chloro substituted linker **14** is shown in Scheme 4 and utilised a Boc protecting group for the benzylic amine. It was coupled to the top group by activation of the carboxylic acid using water soluble carbodiimide hydrochloride (WSC·HCl) and deprotected to give **15**. This was elaborated by coupling to methoxy-substituted proline **16** (prepared using literature procedures) with carbonyl diimidazole (CDI) to give the urea **17**.[17] The ester was hydrolysed and amide couplings were carried out using WSC·HCl to provide the amides in Table 1, as exemplified with 1-methylhomopiperazine, which provided **31**.



Scheme 5. Reagents and conditions: (a) (i) *n*-BuLi, THF, −78 °C, (ii) solid CO_2, −78 °C, 73%; (b) (i) $SOCl_2$, toluene, (ii) **11**, Et_3N, CH_2Cl_2, 87%; (c) $NaBH_4$, $CoCl_2{\cdot}6H_2O$, MeOH, 64%.

Table 1. Activities at the OT and V_2 receptors of compounds containing a basic amine

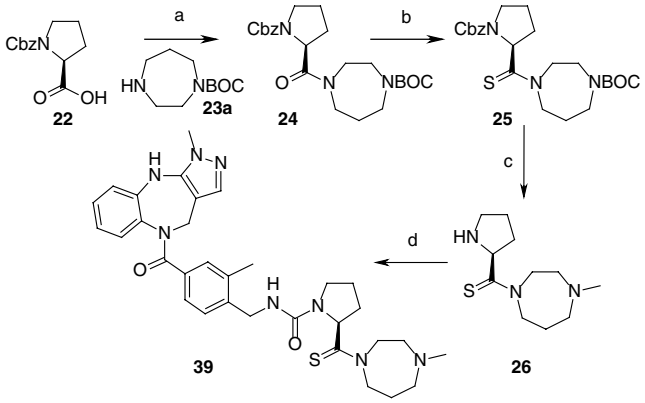


Compounds	R	X	OT EC_{50} (nM)	V_2 EC_{50} (nM)
29		O	1500 ± 1300	570 ± 0.11
34		S	470 ± 330	200 ± 79
30		O	2300 ± 1400	1100 ± 270
35		S	480 ± 150	100 ± 35[a]
31		O	2800 ± 2100	1300 ± 210
36		S	430 ± 220	230 ± 5[a]
32		O	2200 ± 700	1200 ± 330[a]
33		O	>10,000	1800 ± 450[a]

[a] Reported as mean ± range.

The synthesis of the methyl substituted linker **19** is shown in Scheme 5 and in this case the amine was masked as a nitrile group. Carboxylation was carried out at −78 °C, which was followed by an amide coupling *via* an acid chloride to the top group **11**. Unmasking of the nitrile group was carried out using sodium borohydride activated by cobalt(II) chloride to give **21**.[18]

The thioamides shown in Tables 1 and 2 were prepared as exemplified for compound **39**, Scheme 6. Cbz protected proline **22** was coupled to Boc-protected homopiperizine **23a** and the resulting amide **24** was treated with Lawesson's reagent. Removal of the Boc-group, *N*-methylation and removal of the Cbz-group afforded the thioamide **26**. Coupling to the amine **21** with CDI gave the urea **39**.[19]



Scheme 6. (a) HOBt, WSC·HCl, Et_3N, CH_2Cl_2, 100%; (b) Lawesson's reagent, toluene, 85 °C, 67%; (c) (i) 4 N HCl/dioxan, (ii) HCHO, $NaCNBH_3$, MeOH, AcOH, (iii) HBr, AcOH, 88%; (d) **21**, CDI, Et_2N^iPr, DMF, 63%.

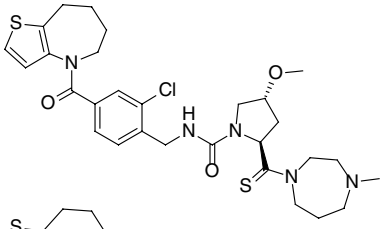
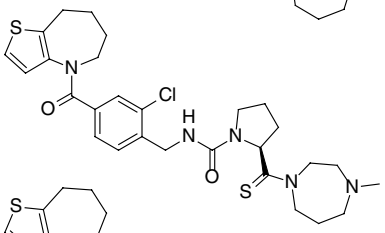
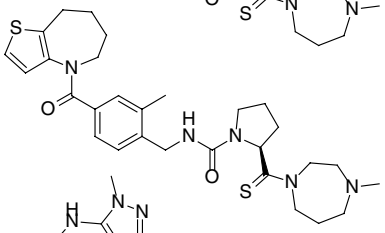
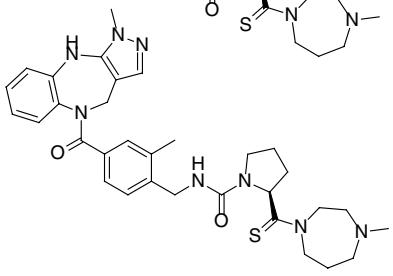
3. Biology

The human OT and V_2 receptors were cloned from ovary total RNA and kidney total RNA (BD Clontech) using the reverse transcriptase polymerase chain reaction (RT-PCR). The receptors were subcloned into the KpnI and EcoRI sites of pcDNA3. The cAMP response element (CRE) and the nuclear factor of activated T-cells (NFAT) element was subcloned into the BamHI and BglII sites of pGL3 promoter (Promega), which contains the Firefly Luciferase gene. Plasmids were amplified in Novablue *E. coli* (Merck Biosciences Ltd) and plasmid DNA was purified using UltraMobius™ Endotoxin free plasmid purification kits (Merck Biosciences Ltd).

The cell lines CHO (Chinese hamster ovary) and 293 (human embryo kidney) were maintained in Dulbecco's Modified Eagles Medium (DMEM) without phenol red, supplemented with penicillin and 10% foetal calf serum.

Cells were dual transfected with the receptor and reporter constructs using Genejuice (Merck Biosciences Ltd) and incubated at 37 °C with 5% CO_2 for 24 h before plating into 96 or 384 well white plates. CHO cells were

Table 2. Activities at the OT and V_2 receptors of analogues of the thioamide **36**

R		OT EC_{50} (nM)	V_2 EC_{50} (nM)
	**36**	430 ± 220	230 ± 5[a]
	**37**	810 ± 270	1800 ± 330[a]
	**38**	640 ± 340	890 ± 280[a]
	**39**	33 ± 15	850 ± 280

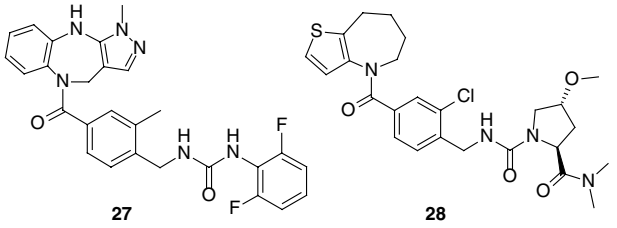
[a] Reported as mean ± range.

transfected with OT receptor and the NFAT-luciferase constructs, 293 with the V_2 receptor and CRE-luciferase constructs. The plates were incubated for a further 24 h at 37 °C with 5% CO_2 before testing for functional agonism.

Compounds were prepared in DMSO to a concentration of 10 mM and dilutions thereof to give the appropriate concentrations when added to the cells. Final DMSO concentrations were 0.1%. Levels of luciferase synthesis were measured following 5 hours incubation at 37 °C using luclite kits (Perkin Elmer). The data were fitted to a 4-parameter logistic equation and EC_{50} values determined. In most cases data are reported as mean ± standard deviation ($n \geqslant 3$). Where n=2 the data are reported as mean ± range of data. OT returned a value of $EC_{50} = 0.18 \pm 0.069$ nM ($n = 20$) and AVP of $EC_{50} = 0.14 \pm 0.062$ nM ($n = 56$).

4. Results and discussion

Screening of our own in-house assembled vasopressin targeted library for OT activity identified two hits, **27** and **28** (Fig. 2), which were related to **1** (Fig. 1). Common to all three compounds was the substituted benzoyl linker and a bicyclic or tricyclic fused azepine as a top group. A urea functionality attached to an aryl ring or an amino acid amide formed the bottom group. The



27 **28**

Figure 2. Structures of the initial screening hits.

relative responses compared to OT of these hit compounds, determined at a concentration of 3 μM, were 41% for **27** and 80% for **28**. Thus **28** was selected for hit-to-lead investigations. The bottom group was selected for analoging because the amide could be prepared rapidly. More than one hundred diverse amides were prepared and screened (not shown) to provide initial SAR. The majority of these compounds were active at the V_2 receptor but gave only a poor response at the OT receptor. Some compounds were approximately equipotent to both receptors and these appeared to contain a basic amine in the bottom group (Table 1, compounds **29**–**33**). It was found that potency at the OT receptor could be improved by preparing the thioamide analogues, compounds **34**–**36**. However, potency at the V_2 receptor was also improved. Selectivity for the V_2 receptor ranged from 5-fold for compound **35** to 2-fold for

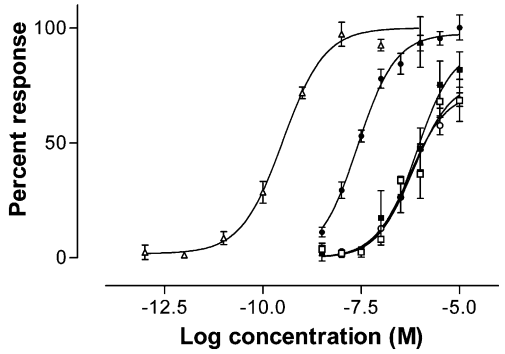


Figure 3. Dose response curves in the human OT reporter gene assay for OT (△) and compounds **36** (□), **37** (■), **38** (○) and **39** (●). Percent response is quoted relative to the OT response. Each data point is mean ± SD ($n = 3$).

compound **36** and **36** was selected for further investigation as shown in Table 2.

Compound **37** (Table 2), where the stereogenic methoxy group on proline had been removed, showed a reduction in potency compared to **36**. However it was 2-fold selective for the OT receptor and this was the first compound to show OT selectivity. Replacement of the chloro substituent by methyl was investigated to give **38**, $EC_{50} = 640$ nM but this showed no improvement. Finally, attention turned towards the top end group. Re-examination of the screening data led to the hypothesis that the fused azepine tricycle present in **27** may be responsible for some of the efficacy of this compound in the OT assay. Therefore it was incorporated into the new thioamide series to give compound **39**. This compound showed a significant increase in potency at the human OT receptor to give an $EC_{50} = 33$ nM. Furthermore, this compound maximally stimulated the OT receptor to the same degree as OT (Fig. 3). There was no such increase in potency at the V_2 receptor thus selectivity improved significantly to >25-fold. No agonist activity was detected in reporter gene assays utilising human V_{1a} or V_{1b} receptors.

The compounds herein are the first reported non-peptidic, small molecule agonists of the hormone oxytocin. The successful screening strategy demonstrates the effectiveness of a functional assay for screening for agonists. The [6,7]-fused ring template, exemplified by 2,3,4,5-tetrahydro-1*H*-benzo[*b*]azepine in compound **1** and by 5,6,7,8-tetrahydro-4*H*-thieno[3,2-*b*] azepine **6** and the fused tricycle **11**, appears to be important for activity at the AVP and OT receptors. Thus it may be considered a 'privileged structure'.[10] It remains to be seen whether this structure is privileged for other GPCRs. Compound **39** is potent against the OT receptor and selective against the V_2 receptor. It is currently under further investigation as a pharmacological tool in animal models of OT activity, to be reported in subsequent papers, and as a potential drug for diseases where oxytocin activity is compromised.

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18. Satoh, T.; Suzuki, S.; Suzuki, Y.; Miyaji, Y.; Imai, Z. *Tetrahedron Lett.* **1969**, *10*, 4555.
19. Analytical data for **39**: NMR (270 MHz, $CDCl_3$) δ 1.78–2.84 (13H, m), 3.20–4.46 (14H, m), 4.50–4.68 (1H, m), 4.98–5.16 (1H, m), 5.88 (1H, d, $J = 14.6$ Hz), 6.58–6.70 (2H, m), 6.72–7.22 (5H, m), 7.22 (1H, s) ppm. MS *m/z* 601.3 $[M+H]^+$.