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Identification of Potent and Selective Oxytocin Antagonists. Part 2: Further Investigation of Benzofuran Derivatives

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Abstract—The paper covers continuing efforts to discover novel, potent and selective oxytocin antagonists. Further benzofuran derivatives with potent oxytocin antagonist activity and good pharmacokinetic parameters are reported. Efforts to improve the in vivo activity of the series are described.

Introduction

In the search for oral agents that could significantly delay preterm birth[1] (the major cause of infant mortality and morbidity), potent and selective non-peptide oxytocin (OT) antagonists have been identified, but none has entered clinical development.[2,3] In the first paper of this series, use of amino moiety **1** (R = H) of L-371,257 **2** as a starting point for a chemical programme was reported.[3] A number of oxytocin antagonists were identified, such as the 2-substituted benzofuran derivatives **3**, with the most potent compounds having general structure **4** (R^1 = amide-containing saturated or aromatic heterocycles). Although potent OT antagonists, the saturated heterocyclic derivatives did not have suitable pharmacokinetic properties to warrant further development. This paper reports efforts to optimise the activity and pharmacokinetic parameters of the aromatic heterocyclic derivative **4a**, through modification of the benzoxazinone group (**5**), the benzofuran ring (**6**) and the amide linker from the benzofuran ring to the terminal pyridone of **4a**.

Modification of the pyridone of 4a

To optimise the activity and pharmacokinetic properties of **4a** its pyridone moiety was elaborated using base mediated coupling (NaH or polymer supported BEMP[4]) of bromide **4** (R^1 = Br) with a set of heteroaromatic amides selected to contain the hydrogen bond acceptor of **4a**. A significant increase in antagonist activity at the hOT receptor was obtained by substituting pyridone and other nitrogen heterocycles with both polar groups (e.g., **4b**,**f**,**g**) and lipophilic groups (e.g., **4c**,**h**,**i**) (Table 1). Also bicyclic heterocycles showed potent activity **4d**,**e**. The generality of the effect suggested that the activity increase was caused by space filling in the receptor site rather than any specific interactions. The compounds investigated all had selectivity over the vasopressin 1a (V_{1a}) receptor. Although the increase in hOT activity was independent of the nature of the substituents, it did nevertheless affect the

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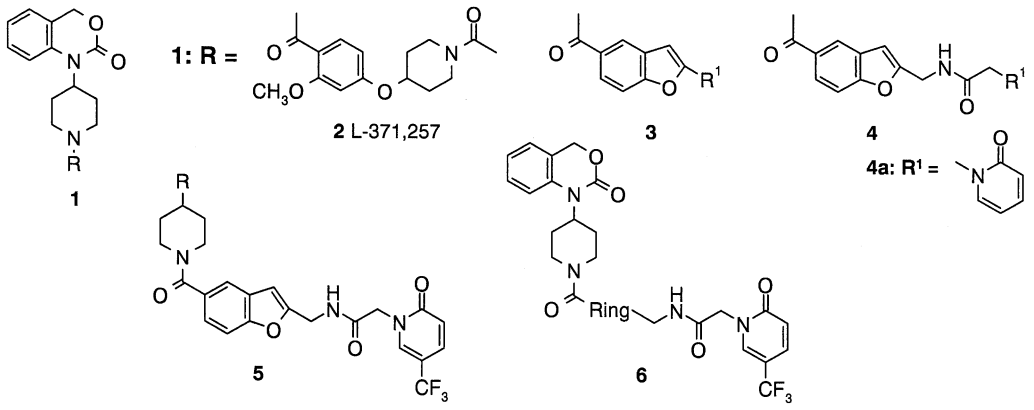
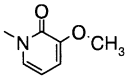
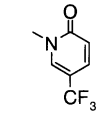
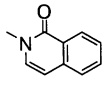
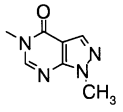
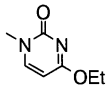
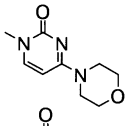
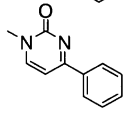
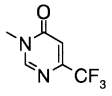


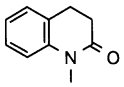
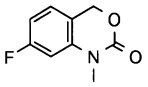
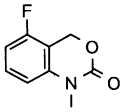
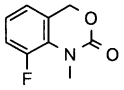
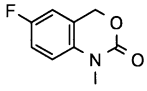
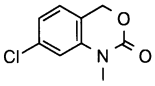
pharmacokinetic properties of the compounds. Polar groups such as the ethers **4b**,**f** gave good dog bioavailability, but conversely good rat bioavailability required the introduction of lipophilic groups such as CF_3 **4c**,**i**. Attempts to introduce water solublising groups such as morpholine **4g** resulted in a loss of bioavailability in both dogs and rats. From this set of compounds, **4c** was identified as the compound with the best all round properties, especially as rat bioavailability could be increased to 30% by changing the dosing formulation (self-emulsifying formulation, 20% v/v transcutol, 20% v/v labrafil, 60% v/v labrasol). Therefore **4c** has been used

Table 1. Inhibition of the binding of OT with human OT (hOT) and V1a (hV1a) receptors,[a] and rat and dog pharmacokinetics

Compd	R	hOT pK_i	hV1a pK_i	Rat			Dog		
				$t1/2$ (h)	Cl (mL/min/kg)	F%	$t1/2$ (h)	Cl (mL/min/kg)	F%
2		8.2	<5.7	1.1	51	55	1.1	13	29
4a		8.2					0.5	34	
4b		8.6	5.8	1.2	22	3	1.1	19	68
4c		8.7	5.9	1.0	10	16	1.5	8	51
4d		8.2		0.8	10	17	1.1	6	
4e		8.9					1.0	11	36
4f		8.7	5.8	0.8	35	2	1.9	12	43
4g		9.3		0.8	17	<1	1.2	11	2
4h		9.0					1.3	9	
4i		8.4	5.4	0.8	20	14	1.6	6	34

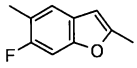
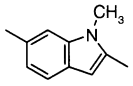
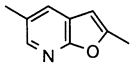
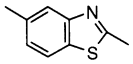
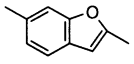
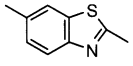
[a]Displacement of ^{3}[H] oxytocin from hOT or vasopressin from hV_{1a} by the test compound.[5]

Table 2. Inhibition of the binding of OT with human OT receptor (hOT)[a] and rat IV half-life and bioavailability

Compd	R	hOT pK_i	$t1/2$ (h)	Cl[b]	F%	Compd	R	hOT pK_i	$t1/2$ (h)	Cl[b]	F%
5a		7.6	1.1	13	44	**5d**		8.2			
5b		7.0				**5e**		8.1	1.2	11	67
5c		8.2	0.9	17	18	**5f**		8.5	1.0	21	35

[a]Displacement of ^{3}[H] oxytocin from hOT by the test compound.[5]
[b]Clearance (mL/min/kg).

Table 3. Inhibition of the binding of OT with human OT receptor (hOT)[a] and rat IV half-life and bioavailability

Compd	Ring	hOT pK_i	$t1/2$ (h)	Cl[b]	F%	Compd	R	hOT pK_i	$t1/2$ (h)	Cl[b]	F%
6a		8.1	1.3	21	15	**6d**		7.3			
6b		8.5	0.6	26	2	**6e**		7.1			
6c		7.0				**6f**		8.0			

[a]Displacement of ^{3}[H] oxytocin from hOT by the test compound.[5]
[b]Clearance (mL/min/kg).

as the lead structure in the rest of the paper to exemplify further structure–activity relationship investigations.

Modification of the benzoxazinone ring

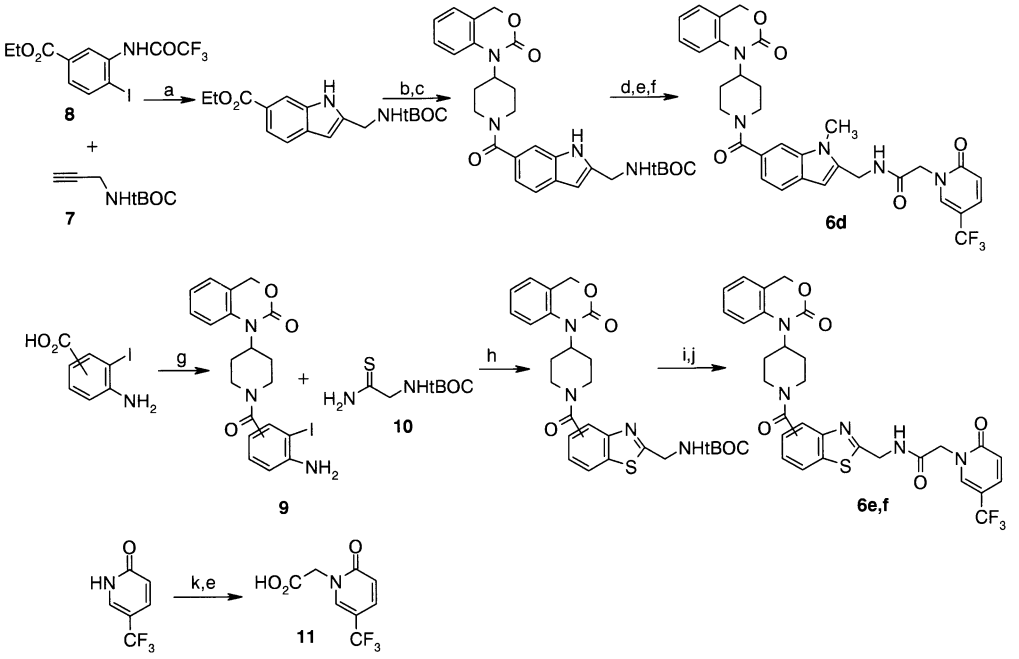
To improve the pharmacokinetic parameters of **4c** modifications to the benzoxazinone ring were investigated (Table 2). Although, amide **5a** did have improved bioavailability over **4c**, its 10-fold loss of activity illustrates the substantial reduction of activity observed for many analogues. The only compounds that retained significant levels of activity were fluoro-derivatives **5c**–**e** and the chloro-derivative **5f**. Only **5e** had significantly increased bioavailability compared to **4c**, but this was off set by a 5-fold loss of activity.

Modification of the benzofuran ring

Fluorine substituted benzofurans (e.g., **6a**) and aza-benzofurans (e.g., **6b**) (Table 3) were synthesised to block potential metabolism of the ring and for the latter case to improve water solubility. They were synthesised using analogous chemistry to that used to synthesise **4c**, involving the Sonogashira coupling of an appropriate iodo-phenol or iodo-pyridol with acetylene **7**[6] (Scheme 1). Although these modifications gave compounds with potent activity they did not increase bioavailability, however, incorporation of nitrogen into the ring **6b** did increase the water solubility 10-fold compared to **4c**. Other heterocycles such as the benzofuran isomer **6c**; indoles (e.g., **6d**), synthesised from the palladium coupling of the appropriate iodo-trifluoroacetamido-benzene **8** with **7**; and benzothiazoles **6e**,**f**, from the palladium coupling of iodo-anilines **9**[7] with thioamide **10**[8] (Scheme 1), all proved to be significantly less active.

Protein binding and in vivo activity

Due to its promising pharmacokinetic properties, **4c** was progressed into early pre-clinical development. However, **4c** proved to be less active in vivo in an OT induced rat uterus contraction model[9] ($IC_{50} = 3$ μM) than would have been predicted from its in vitro potency at the rat OT receptor[10] ($IC_{50} = 2$ nM). A possible reason for this observation was that the concentration of the compounds available for interaction at the OT receptor in vivo was limited by a high degree of binding to plasma proteins. To investigate this possibility, in vivo and in vitro studies were carried out using a selection of compounds of varying potency and physicochemical properties, including the peptide atosiban® (Tractocile),[11] L-368,899,[12] **2**, **6b** and **4c**. Protein binding



Scheme 1. (a) CuI, $PdCl_2(PPh_3)_2$, HN=CH(NCH_3)$_2$, DMF, 65 °C; (b) KOH, MeOH, H_2O; (c) **1,** R = H, TBTU, 1-hydroxybenzotriazole (HOBT), *i*Pr_2EtN, DMF; (d) NaH, MeI, DMF; (e) 4 N HCl, dioxan, ether; (f) TBTU, HOBT, *i*Pr_2EtN, DMF; (g) 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide HCl (EDC·HCl), HOBT, *i*Pr_2EtN, DMF; (h) Tris(benzylideneacetone)-palladium (0)-chloroform, 1,1′-bis(diphenylphosphino)-ferrocene, CaO, DMF, 60 °C, DMF; (i) 4 N HCl, dioxane; (j) **11**, EDC·HCl, HOBT, *i*Pr_2EtN, DMF; (k) $BrCH_2CO_2tBu$, NaH, DMF.

in both rat and human plasma was determined for **4c**, **2** and atosiban®. Binding of the compounds to purified human serum albumin (HSA) was also measured,[13] as was their potency at the human OT receptor in the presence and absence of physiological concentrations of HSA.[14] Rat serum albumin (RSA) was also examined. The binding of compounds to HSA (or RSA) was similar to that observed with rat and human plasma proteins (data not shown). When HSA was added to the in vitro human OT potency screen a loss of activity (HSA shift) was observed for many of the compounds and this shift was similar when RSA was added. For technical reasons HSA was used routinely. A close correlation was observed between the extent of binding to HSA, increasing lipophilicity, the HSA shift, and the difference between the rat in vitro and in vivo activities (Table 4). This led to the conclusion that a high degree of plasma protein binding was responsible for the relatively poor efficacy of **4c** in the OT induced rat uterus contraction model. Although we identified compounds with potent activity and moderate HSA shifts (e.g., **4g**, HSA shift = 9-fold), these compounds also had poor rat oral bioavailabilites and so were not suitable for progression.

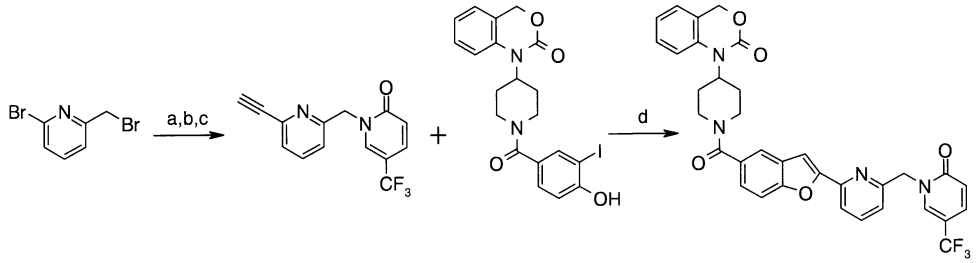
Table 4. Comparison between in vitro and in vivo activity (rat) and correlation with plasma protein binding

Compd	Atosiban®	L-368,899	**2**	**6b**	**4c**
Rat in vivo IC_{50} (nM)[a]	136	164	640	744	3040
Rat in vitro IC_{50} (nM)[b]	40	10	25	20	20
Protein binding (%)[c]	42.6	82.4	83.7	86.2	96.2
CHI logD pH2[d]	0.81	1.71	1.66	1.85	2.16
pK_i (human OT)[e]	7.4	7.6	8.1	8.5	8.7
HSA shift[f]	1	4	9	19	46

[a]Displacement of ^{3}[H] oxytocin from rat OT by the test compound.[9]
[b]Serum concentration for 50% inhibition of uterine contractility response to oxytocin in rat.[10]
[c]Human serum albumin binding.[13]
[d]Lipophilicity measure.[14]
[e]Displacement of ^{3}[H] oxytocin from hOT by the test compound.[5]
[f]HSA shift = Ratio of displacement of ^{3}[H] oxytocin from hOT by the test compound in the presence and absence of 50 mg/mL human serum albumin.[15]

Modification of the amide linker

The accumulated data for the series suggested that improved rat bioavailability was related to increasing logD of the compounds, although, this also correlated with increasing protein binding and HSA shift. Conversely, reducing polar surface area (PSA)[16] was associated with an increase in rat bioavailability, but not as closely with increasing protein binding and HSA shift (data not shown). To exploit the latter finding molecules were designed with reduced PSA values compared to **4c**. As the previously described modifications of **4c** were detrimental to the properties of the series, the amide linker between the benzofuran and pyridone ring was investigated. As previous investigations suggested this group was not involved in hydrogen bonding to the receptor, non-amide containing replacements were sought. Catalyst™[17] was used to search for fragments in the corporate compound database that bridged the 3-D space between the 2-position of the benzofuran ring and the pyridone ring nitrogen using three low energy conformations of **4c**. Representative structures from this exercise, together with ideas generated within the group, were modelled against the conformers of **4c**. A number of compounds were then selected for synthesis based on parameters such as; fit to one or more of the



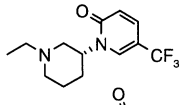
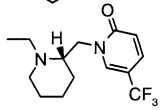
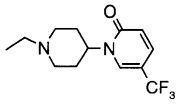
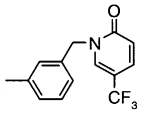
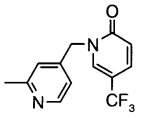
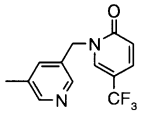
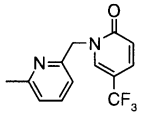
Scheme 2. (a) NaH, 5-trifluoromethyl-2-pyridone, DMF, 37%; (b) $HC{\equiv}CSi(CH_3)_3$, Et_3N, CuI, $PdCl_2(PPh_3)_2$, DMF, 45 °C, 53%; (c) tetrabutylammonium fluoride, THF, rt, 34%; (d) Et_3N, CuI, $PdCl_2(PPh_3)_2$, DMF, 65 °C, 44%.

pharmacophores; lower PSA values than **4c**; and the presence of basic functionality. Some of the compounds are shown in Table 5.

As expected, because the modelling was carried out against a number of conformations of **4c**, a range of activity was observed for the synthesised analogues. Compounds with potent activity **3a**,**b**,**g** were identified, but these tended to have high HSA shifts and poor pharmacokinetic parameters, whereas closely related structures (**3c**) with good pharmacokinetic parameters had moderate levels of activity.

The aromatic linkers **3d**–**g**, synthesised using the methodology exemplified for **3g** (Scheme 2), gave a range of activity dependent on the presence and position of the pyridyl nitrogen atom. The greater activity of **3g** over the other analogues can be rationalised by a conformational restriction caused by repulsion of the benzofuran oxygen and the pyridine nitrogen. This observation is supported by the lack of activity of the isomer **3e**. Although **3g** had good activity and reasonable pharmacokinetic parameters it had a high shift in the presence of HSA. The difference in the HSA shift seen for **3g** and the isomer **3f** suggested that the magnitude of the shift

Table 5. Inhibition of the binding of OT with human OT receptor (hOT),[a] effect of protein binding on in vitro activity[b] and rat pharmacokinetics

Compd		pK_i hOT[a]	HSA shift[b]	Protein binding[c]	$t1/2$ (h)	Cl (mL/min/kg)	AUC (h. ng/mL)	%F	PSA[d]
4c		8.8	46	93	1.5	10	1600	19	116
3a		7.6	222	96	0.6	28	150	2	86
3b		8.0	90	98					82
3c		6.1	4	93	1.7	6	12000	79	93
3d		6.1		98	2.2	7	5700	>45	78
3e		<5.5		96					90
3f		7.2	17	96	1.4	7	5400	41	91
3g		8.0	87	98	1.2	12	1500	22	89

[a]Displacement of ^{3}[H] oxytocin from hOT by the test compound.[5]
[b]Ratio of displacement of ^{3}[H] oxytocin from hOT by the test compound in the presence and absence of 50 mg/mL human serum albumin.[15]
[c]Human serum albumin binding.[13]
[d]PSA calculated polar surface area.[16]

was not just due to gross physicochemical properties, but also had a structural element to it. Although not suitable for progression, these highly conformationally constrained systems have given important structural information for further compound design.

Conclusion

The compound **4a** was used as a lead to identify potent and selective oxytocin antagonists in vitro. Investigations demonstrated that much of the molecule was intolerant to modification. However, a significant increase in activity and improvement of pharmacokinetic parameters was achieved by optimisation of the pyridone moiety of **4a**. The in vivo activity of the compounds was significantly lower than their in vitro activity, possibly due to protein binding. This loss of activity was paralleled in vitro by the addition of human serum albumin to the hOT binding screen, allowing selection of candidates for in vivo evaluation. This later finding and the structural information obtained during these studies will be valuable in the design of oxytocin antagonists with potent in vivo activity.

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10. Rat Oxytocin Binding Assay. Rats that had littered within 12 h were sacrificed. The myometrium removed to a cold assay buffer (50 mM HEPES, 10 mM, $MgCl_2 \cdot 6H_2O$, pH to 7.4 with a saturated solution of Tris base), cut into short lengths (<5 mm) and homogenised in homogenisation buffer (250 mM sucrose; 40 mM L-histidine; pH 7.3) on ice (20 mL) using a Braun (Teflon/glass) homogeniser (20 strokes at 1000 rpm at 4 °C). The suspension was centrifuged (500*g*) for 10 min at 4 °C. The supernatant was removed and retained on ice. The pellet was resuspended in homogenisation buffer, homogenised and then centrifuged as above. The pooled supernatants were centrifuged at 48,000*g* for 20 min at 4 °C. The pellet was resuspended in 8 mL assay buffer per two rats. Radioligand binding was determined using a filtration binding assay. Each well contained membrane (100 μL), [^{3}H]oxytocin (50 μL, 1 nM, 130 Ci/mmol, Amersham UK) in the presence of competing ligands. All reagents were prepared in assay buffer (total assay volume 200 μL). The plates were incubated for 1 h at room temperature before being filtered through dry Whatman GF/C filters, using a Brandel Cell harvester. The filters were washed with ice cold assay buffer (4×1 mL) to remove unbound ligand, punched out into 5 mL counting vials and Scintillant added. After >4 h to digest the filter, bound [^{3}H]oxytocin was measured using a Packard Liquid Scintilation Counter. A four parameter logistic fit was used to estimated IC_{50} and Hill slope (by non-linear least squares).
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