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Structure–Activity Relationship Investigations of a Potent and Selective Benzodiazepine Oxytocin Antagonist

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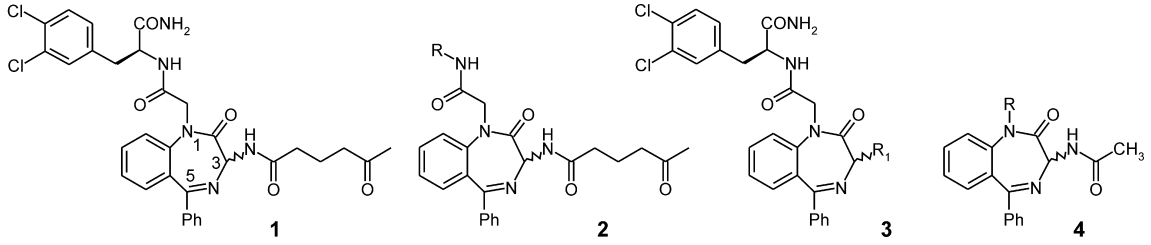
Abstract—We have investigated the structure–activity relationships of the 1- and 3-substituents and replacements of the 5-phenyl group of GW405212X **1**, a potent selective oxytocin antagonist. The effect of these modifications on oxytocin binding antagonism and on pharmacokinetic parameters is reported.

Introduction

Preterm birth, between 24 and 36 weeks of gestation, affects approximately 10% of all births in the USA and is the main cause of infant morbidity and mortality. Fetal survival rates increase from 15% at 24 weeks gestation to 95% at 32 weeks gestation, so clearly, delaying premature labour could dramatically reduce mortality, morbidity and healthcare costs.[1] At present, there is no satisfactory treatment to prevent premature labour, as lack of efficacy and maternal or neonatal side effects limit the usefulness of the currently used drugs.[2]

Oxytocin (OT), a potent contractor of the human uterus, plays an important role in the onset of labour and preterm labour.[3–5] Therefore, the development of orally active OT antagonists should provide a novel therapy for preterm labour with few side effects. Atosiban, a peptide OT antagonist, shows therapeutic promise, but is not suitable for maintenance of gestation, as it is not orally active.[6] A number of non-peptide OT antagonists have been reported, but none have undergone extensive clinical evaluation.[7]

GW405212X **1** synthesised as part of a Fully Encoded Differential Release Library was active in an OT receptor scintillation proximity assay.[8] Further studies demonstrated **1** to be a potent and selective OT antagonist.[9] However, **1** is not a drug candidate because of its poor pharmacokinetic profile.[9] We have investigated the SAR of the 1- and 3-substituents of **1** (structures **2** and **3**) and replacements of the 5-phenyl group (not shown). Using the results from these studies, we reinvestigated the 1-substituent, structure **4**. This paper



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reports the effect of these modifications on OT binding antagonism and on pharmacokinetic parameters.

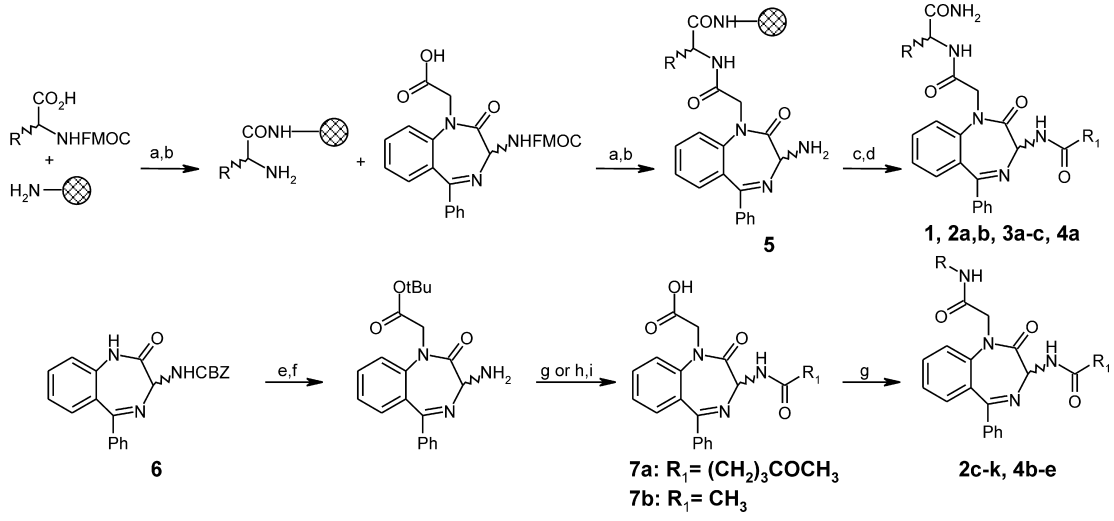
Modification of the 1-Substituent

A library of compounds was made to investigate the structure–activity relationship around the 3,4-dichlorobenzyl moiety of **1** using solid-phase FMOC chemistry outlined in Scheme 1.[13] Initial results demonstrated the need for a substituted phenylalanine in the 1-position of **1**. Replacement of the 3,4-dichloro-phenyl group of **1** with alkyl or cycloalkyl such as **2a** gave compounds that were at least 100-fold less active than **1** (Table 1). Deletion of the chloro-groups (not shown) or replacement with fluorine **2b** caused a loss of activity.

Investigations into whether the primary amide could be removed without significant loss of activity required a change in chemical strategy, as it was the point of attachment during the solid-phase synthesis of the initial libraries. To address this, a solution-phase library was made by individually coupling acid **7a** (Scheme 1) with 77 amines and the crude products were tested for OT antagonism at three 10-fold dilutions. Acid **7a** was synthesised in good overall yield from benzodiazepine **6**.[14] Some compounds (**2c**–**k**, Table 1) were remade and purified for pK_i determinations and pharmacokinetic investigations. Removal of the primary amide **2c** and the 4-chlorine atom **2d** gave a modest loss of activity, probably due to an increase in conformational flexibility rather than loss of receptor binding. The des-chloro analogue **2e** was significantly less active than **2d** as was the 2-chloro derivative **2f**. Extending the ethyl chain of **2d** to give **2g** or attempting to constrain it (**2h** or **2i**) caused a loss of activity. The naphthyl-derivatives **2j** and **2k** were as potent as **2d**.

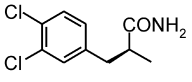
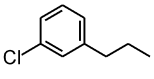
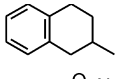
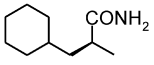
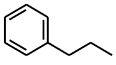
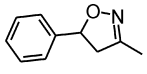
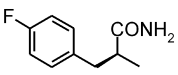
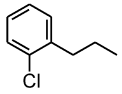
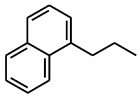
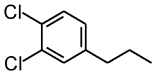
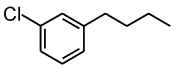
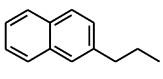
Modification of the 3-Substituent

Deletion of the 3-(5-oxo-hexanoamide) moiety **3**, $R_1 = H$ caused a substantial loss of activity (pK_i 5.7), so a series of solid-phase libraries was made from amine **5** (Scheme 1) to investigate amide, sulphonamide, and urea derivatives of amine **3**, $R_1 = NH_2$. Some examples



Scheme 1. (a) *O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU), *i*Pr$_2$EtN, DMF; (b) 20% piperidine, DMF; (c) RCO$_2$H, HATU, *i*Pr$_2$EtN, DMF or RCOCl, Et$_3$N, CH$_2$Cl$_2$; (d) 95:5 TFA/H$_2$O; (e) NaH, *t*-butyl bromoacetate, DMF, 88%; (f) H$_2$, Pd/C, ethanol, 100%; (g) RCO$_2$H, HATU, *i*Pr$_2$EtN, DMF; (h) RCOCl, Et$_3$N, CH$_2$Cl$_2$; (i) 1:1 TFA/DCM.

Table 1. Inhibition of the binding of OT with human OT receptor (hOT): comparison of **1** and **2a**–**k**[a]

	R	pK_i hOT		R	pK_i hOT		R	pK_i hOT
1		8.1	**2d**		7.7	**2h**		>5.3
2a		6.1	**2e**		6.5	**2i**		>5.5
2b		7.1	**2f**		6.7	**2j**		7.8
2c		7.6	**2g**		7.0	**2k**		7.8

[a]Displacement of ^{3}[H]oxytocin from hOT with the test compound.[9]

were resynthesised for accurate pK_i determination (Table 2). The acetamide **3a** proved to be the most active of the amide derivatives. However, the morpholino-acetamide **3b** was interesting as it lowered the log D and allowed the formation of salts to increase water solubility. A further library of amino-acetamides was made to investigate this finding, but no compounds of greater potency than **3a** were found. The sulphonamide and the urea libraries failed to give significantly active compounds, as illustrated by the methanesulphonamide derivative **3c**.

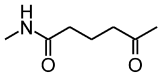
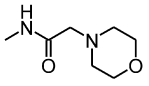
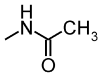
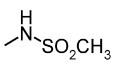
Modification of the 5-Substituent

Replacement of the 5-phenyl substituent of **1** with cyclohexyl (pK_i 7.5) gave a modest loss of activity. More radical modifications such as methyl (pK_i 6.7) or heteroaromatics, such as CH_2N-imidazole (pK_i 6.1), resulted in a more significant reduction of activity. Therefore, the 5-phenyl moiety was retained for further studies.

Further Investigation of the 1-Substituent

As the acetamide **3a** was the best replacement of the 3-substituent of **1**, template **4** was used to further investigate the structure–activity relationship of the 1-substituent. The epimer **4a** of **3a** was synthesised using the solid-phase chemistry outlined in Scheme 1 and it proved to be significantly less active than **3a**. The phenethyl derivatives **4b**–**4e** were synthesised from acid **7b** using the solution-phase chemistry outlined in Scheme 1. The substituted phenethyl derivatives **4b** and **4c** and naphthyl derivative **4d** had potencies equal to the cumulative loss of activity associated with the sequential deletion of the primary amide and the hexanoate moieties. The tertiary amide **4e** was over 10-fold less active than **4c**, suggesting the compounds bind in the *trans*-amide conformation. As the conformation of the 3-chlorophenyl group seemed crucial for potent activity, an increase in activity was sought by replacing the flexible *N*-ethyl-acetamide group with conformationally constrained heteroaromatic linkers. A library of oxadiazoles, synthesised by condensing acid **7b** with 78 amidoximes **8** (Scheme 2), was tested without purification, but failed to give any compounds of interest. The oxadiazole **4f** (Table 3) was synthesised as a pure compound to confirm the result. Similarly, the thiazoles prepared from the reaction of thioamide **10** (from nitrile **9**[15]) and a set of α-bromoketones (Scheme 2) gave no compounds with significant activity (e.g., **4g**).

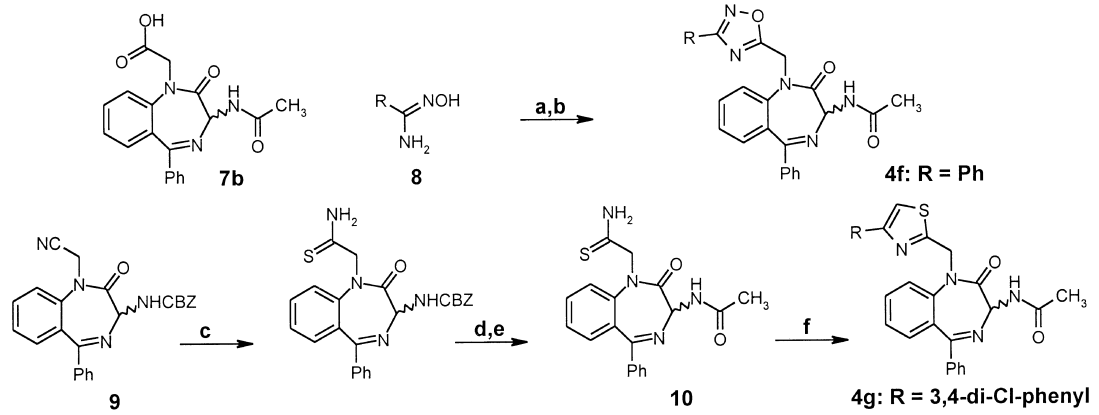
Table 2. Inhibition of the binding of OT with human OT receptor (hOT): comparison of **1** and **3a**–**c**[a]

	R_1	pK_i hOT		R_1	pK_i hOT
1		8.1	**3b**		7.4
3a		7.6	**3c**		6.3

[a]Displacement of [^{3}H]oxytocin from hOT with the test compound.[9]

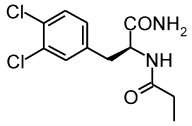
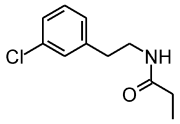
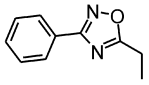
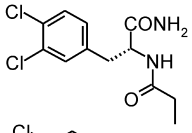
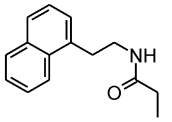
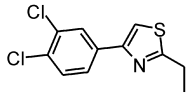
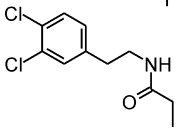
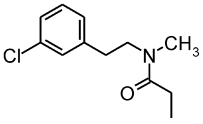
Pharmacokinetic Profile of Selected Compounds

A selection of compounds was dosed to dogs [intravenous (iv) and oral] to investigate the effect of the modifications to **1** on the pharmacokinetic profile of the series (Table 4). Removal of the primary amide from **1** to give **2c** and **2d** resulted in a substantial decrease of plasma clearance leading to at least a doubling of the iv half-lives, but **2c** still had low bioavailability. Replacement of the phenethyl group with a bulky amine to give **2h** resulted in a further dramatic reduction in plasma clearance, suggesting the secondary amide in the 1-substituent is the most metabolically vulnerable position in the molecule. Unfortunately this modification also abolished the activity of the compound. The half-life of the morpholino-acetamide **3b** was 5 times longer than **1**, mainly due a substantial increase in the volume of distribution. Truncation of the hexanoate chain of **2d** to give acetamide **4c** did not result in an improvement of the pharmacokinetic parameters, indicating that the 3-(5-oxo-hexanoamide)-substituent was not significantly metabolically vulnerable.



Scheme 2. (a) 1-Ethyl-3-(3′-dimethylaminopropyl)carbodiimide HCl, *N*-hydroxybenzotriazole, DMF; (b) 1,8-diazabicyclo[4.3.0]undec-7-ene, heat; (c) $Ph_2P(S)SH$, *i*PrOH, reflux, 47%; (d) HBr, CH_2Cl_2, 64%; (e) AcCl, iPr_2EtN, CH_2Cl_2, 100%; (f) $RCOCH_2Br$, DMF, heat.

Table 3. Inhibition of the binding of OT with human OT receptor (hOT): comparison of **3a** and **4a–g**[a]

	R	pK_i hOT		R	pK_i hOT		R	pK_i hOT
3a		7.6	**4c**		7.0	**4f**		6.1
4a		6.1	**4d**		7.0	**4g**		>5.3
4b		7.0	**4e**		5.8			

[a]Displacement of [^{3}H]oxytocin from hOT with the test compound.[9]

Table 4. Dog pharmacokinetic profile of selected compounds[a]

	c log P	iv $t_{1/2}$ (h)	Clearance (mL/min/kg)	Vd (L/kg)	Bioavailability
1	4.1	0.4	62	0.6	>20
2c	5.2	0.9	4	0.2	12
2d	4.6	1.4	3	0.3	
2h	4.4	8.8	0.3	0.2	
3b	4.9	1.9	33	2.8	
4c	5.0	0.5	6	0.4	

[a]See ref 9 for methods.

Conclusion

Although **1** has potent selective antagonist activity at human OT receptors, it is not a drug candidate because of its high molecular weight and poor pharmacokinetic parameters. Removal of the primary amide and a chlorine atom from the 3,4-dichloro-phenylalaninamide moiety of **1** to give **2d** resulted in a slight drop in activity and significant improvement of the pharmacokinetic parameters. Replacement of the 3-(5-oxo-hexano-amide)-substituent of **1** gave at best a modest loss of activity, without giving a significant improvement of the pharmacokinetic parameters. Further modifications to the molecule gave some compounds with improved pharmacokinetic parameters, but invariably led to loss of activity. The problems encountered whilst trying to optimise **1** highlighted the difficulties commonly experienced when compounds of this size and complexity are used as leads. Our experience emphasises the need to design libraries of simple molecules with low molecular weight. Although this approach will in the main give less potent leads, it should be easier to optimise these molecules by increasing size and complexity, rather than to simplify larger molecules.

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$MgCl_2$) followed by centrifugation (500*g*, 5 min, 4 °C). The pellet was discarded and the supernatant centrifuged (48,000*g*, 30 min, 4 °C). The supernatant was discarded and the membrane pellet resuspended by trituration in ice cold binding buffer and stored at −70 °C at a protein concentration of 1–2 mg/mL. Radioligand binding was determined using scintillation proximity assay (Amersham, UK) in 96-well plates. Each well contained CHO membrane (10 μg protein), 1 mg wheatgerm agglutinin coated SPA beads, 1 nM [^{3}H]oxytocin (130 Ci/mmol, Amersham UK, custom synthesis) in the presence of competing ligands. All reagents were prepared in binding buffer (50 mM HEPES, 10 mM $MgCl_2$, pH to 7.4 with KOH) and the total assay volume was 200 μL/well. The plates were incubated for 2–4 h at room temperature before being read in a scintillation counter (Wallac Microbeta). A four parameter logistic fit was used to estimated IC_{50} and Hill slope (by non-linear least squares. K_i values were calculated using the Cheng–Prussof equation.[12] Oxytocin was obtained as the acetate salt (Sigma, UK).

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14. Compound **6** (*R*,*S*)-2-oxo-5-phenyl-3-(phenylmethoxycarbonylamino)-1,4-benzodiazepine was purchased from Neosystem Laboratoire, 7 Rue de Boulogne, 67100 Strasbourg, France.
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