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The discovery of GSK221149A: A potent and selective oxytocin antagonist

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Abstract—Optimisation of a series of oxazole diketopiperazines has led to the discovery of a very potent and selective oxytocin antagonist GSK221149A. GSK221149A has been shown to inhibit oxytocin-induced uterine contractions in the anaesthetised rat.

Preterm labour occurs in 10% of all births worldwide and is the single largest cause of neonatal morbidity and death.[1] Although it is difficult to estimate the cost of neonatal intensive care of low birth-weight babies, it is thought to exceed $5 billion/year in the US alone.[2] Oxytocin (OT), a cyclic nonapeptide neurohypophyseal hormone, binds to OT receptors stimulating contractility in human myometrium and is widely used for the induction of labour.[3] The density of oxytocin receptors in myometrium is elevated during pregnancy and it is believed that a premature increase of such receptors can initiate preterm labour by sensitising the uterus to relatively unchanged circulating levels of OT.[4,5] The design of OT antagonists as potential tocolytic agents for the prevention of premature labour has therefore been an intense area of research for many years.[6] Although the peptide OT antagonist atosiban has been approved for clinical use in Europe, it is not orally bioavailable and has low plasma stability rendering it less likely to be useful for long-term or preventative treatment.[7] Atosiban also has a greater affinity for the vasopressin V_{1a} receptor than the oxytocin receptor.[8]

We recently reported the diketopiperazine **1** to be a potent and orally bioavailable OT antagonist (Table 1).[9] Compounds of this series had been hindered by poor physicochemical properties, including low solubility and relatively high log *D*, which consequently led to relatively high plasma protein binding and cytochrome P450 interactions, with high clearance in cynomolgus monkey and human microsomes.

Structure–activity relationships (SAR) had indicated that both the indanyl and isobutyl fragments contributed to potency,[13] and hence we sought to investigate the modification of the exocyclic aryl and amide functionality. Herein, we describe our efforts around the five-membered ring heterocycles to improve the poor

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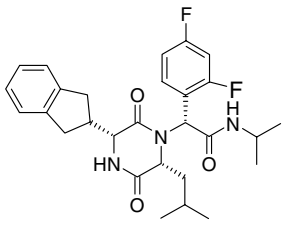
Table 1. Physicochemical properties of **1**



hOT pK_i[10]	8.9–9.2
Log D[a]	3.4
HSA[b]	93%
Sol.	<1 μg/mL
CYP450 3A4 IC_{50}	4 μM

1

[a] Chromatographic lipophilicity measurement.[11]
[b] Human serum albumin binding.[12]

physicochemical and pharmacokinetic properties of this series.

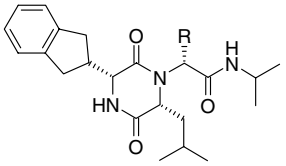
Replacing the difluorophenyl ring with unsubstituted five-membered ring heterocycles resulted in approximately a 10-fold loss in intrinsic potency (Table 2). Potency was regained on the introduction of a lipophilic group, such as methyl or bromide, in the 4 or 5 position. In an attempt to access the putative lipophilic pocket reached by these substituents, a number of trifluoromethyl heteroaryls were prepared. The CF_3 group was invariably the most potent substituent but its strongly electron withdrawing nature led to epimerisation of the exocyclic position in neutral or mildly basic media rendering the compounds less attractive for developability reasons.

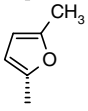
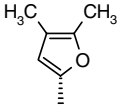
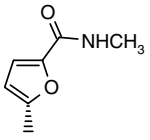
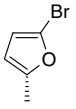
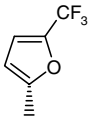
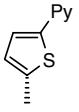
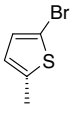
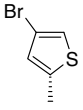
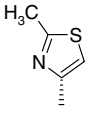
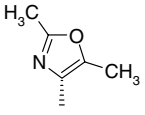
Polar groups were poorly tolerated; the methylamide **6** was significantly less potent than the parent furan 3 although large substituents, such as phenyl and pyridyl (analogue 9), were tolerated. Disubstitution in both the 4 and 5 positions did not increase potency further. Methyl substitution ortho to the ring junction was tolerated as exemplified by the dimethyl oxazole **14** having similar potency to the methyl oxazole **15**. Although the SAR trends were consistent across all heterocycles, the absolute potencies were often significantly different. The difference in potencies of similarly substituted heterocycles may therefore be a reflection of the heterocycle's ability to orientate the substituent into a lipophilic pocket of the receptor.

Although the introduction of lipophilic substituents gave favourable binding affinities, unsurprisingly this was generally accompanied by poor physicochemical properties (Table 3). Indeed, the furyl and thiophene derivatives were poorly water-soluble, relatively highly protein bound and carried undesirable CYP450 interactions (results for the 3A4 isozyme are shown in Table 3). More polar azoles were able to accommodate lipophilic substituents whilst maintaining good physicochemical properties. The oxazole analogues had good aqueous solubility, low protein binding and minimal CYP450 interaction making the series most attractive for further investigation.

Established SAR suggested that a range of amides was tolerated and we therefore envisioned using this functionality as a handle to optimise the pharmacokinetic profile. Potency and pharmacokinetic data on selected

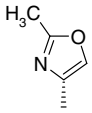
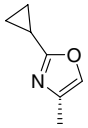
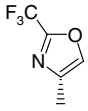
Table 2. Inhibition of the binding of OT at the human OT receptor[10]



Compound	R	hOT pK_i
2[a]		7.9
3		8.2
4		9.0
5		8.9
6		7.4
7		9.1
8		10.0
9[c]		8.6
10		8.7
11		9.0
12		8.2
13[b]		9.4
14[b]		8.5

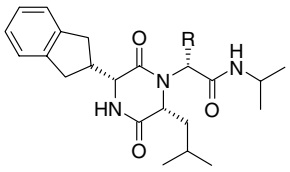
(*continued on next page*)

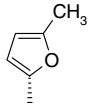
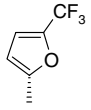
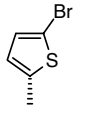
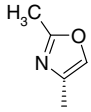
Table 2 (*continued*)

Compound	R	hOT pK_i
15		8.9
16[b]		9.1
17		9.8

[a] *tert*-Butyl amide rather than isopropylamide.[14]
[b] Dimethylamide rather than isopropylamide.[14]
[c] Py, 2-pyridyl.

Table 3. Properties of selected compounds



Compound	R	hOT pK_i	Log D[a]	HSA[b]	CYP450 3A4 IC_{50}[c]	Sol. (μg/ml)
4		9.0	3.3	96	7	34
8		10.0	3.6	97	3	<1
10		8.7	3.7	99	1	<1
15		8.9	2.5	63	>100	>240

[a] Chromatographic lipophilicity measurement.[11]
[b] Human serum albumin binding (%).[12]
[c] Lowest IC_{50} from CYP450 3A4 using substrates diethoxyfluoroscein (DEF), 7-{3-(4-phenylpiperazin-1-ylmethyl)benzyl}resorufin (PPR) or 7-benzyloxyquinoline (7-BQ).

amides are recorded in Table 4. The oxazole amides **18**–**22** had good aqueous solubility (>220 μg/mL), low plasma protein binding (<80% HSA) and no significant cytochrome P450 interactions (CYP3A4 IC_{50} > 100 μM). As expected, a range of functionality could be incorporated in the amide without having detrimental effects on potency. Compounds **15** and **18**–**22** demonstrated antagonist activity in an in vitro functional assay and were selective with respect to the vasopressin V_{1a} receptor (>10-fold). Oxazoles with polar amide substituents, such as the alcohol **18**, had poor oral exposure in the rat. Good pharmacokinetic profiles were achieved with less-polar amides. Tertiary amides **19** and **20** had moderate clearances and volumes of distribution of approximately 1 L/kg resulting in half lives of around 1 h. Both compounds had acceptable bioavailabilities in rat in excess of 30%.

The SAR of the isobutyl portion of **1** was sensitive to relatively minor modifications (Table 4) which had dramatic effects on the pharmacokinetic profile. Branching at the α-carbon was tolerated and resulted in a superior rat pharmacokinetic profile.

Indeed **22**, GSK221149A, had greater oral exposure in the rat compared to **20** in both DMSO/Peg and HPMC/Tween formulations, with good bioavailability and a half life of 1.4 h. Compound **22** also has low to moderate intrinsic clearance in microsomes from three pre-clinical species (rat, dog, cynomolgus monkey) and low intrinsic clearance in human microsomes and is more potent than **20**. Although several amide analogues of **22** were prepared, with a range of physicochemical properties, **22** offered the preferred overall profile.

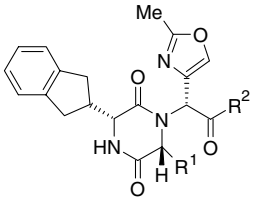
With this data in hand, **22**, GSK221149A, was selected for progression to further selectivity and in vivo efficacy models. GSK221149A is a potent and selective oxytocin receptor antagonist (Table 5) with no detectable agonist activity. GSK221149A is greater than 10-fold more potent than atosiban with a far superior selectivity profile with respect to vasopressin receptors.[15]

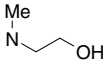
The in vivo activity of GSK221149A was evaluated in anaesthetised, non-pregnant Sprague–Dawley female rats.[15] Uterine activity was measured as an integral of force over time. Intravenous administration of GSK221149A produced a dose-dependent decrease in oxytocin-induced uterine contractions, with an $ID_{50} = 0.27 \pm 0.60$ mg/kg and $IC_{50} = 180$ nM.

The synthesis of the diketopiperazines followed previously described chemistry.[16] The isopropyl amides were conveniently prepared in three steps from the corresponding isonitrile via the Ugi reaction as outlined in Scheme 1. The intermediate mixture of diastereoisomers **23** was deprotected with 4 M HCl and subsequently cyclised by treatment with base in a one-pot procedure. Generally, a 2:1 mixture of diastereoisomers was formed with the desirable (*R*)-isomer **8** being the minor product isolable by chromatography.

GSK221149A and other tertiary amides were prepared in four steps via the Ugi reaction as outlined in Scheme 2. A 2:1 mixture of diastereoisomers **24** was formed with the desirable (*R*)-diastereoisomer being the minor product. Hydrogenation of crude **24** furnished the cyclised phenol **25**, again enriched with the undesirable (*S*)-diastereoisomer. Activation of the mixture **25** with carbonyl diimidazole followed by the addition of 2 N HCl promoted epimerisation at the exocyclic position

Table 4. Profile of selected oxazoles



Compound	R^1	R^2	hOT pK_i	Rat PK (iv 2 mg/kg, po 5 mg/kg)			
				Cl[a]	$t_{1/2}$[b]	AUC[c]	F[d] (%)
15		NH*i*-Pr	8.9			225[e]	
18			8.9			218[e]	
19			8.8	35	1.4	1070[e]	45
20			8.7	23	1.1	3633[e] 1190[f]	33
21			8.2	14	1.3	4200[e]	69
22			9.0	19	1.4	5470[e] 1800[f]	~100 42

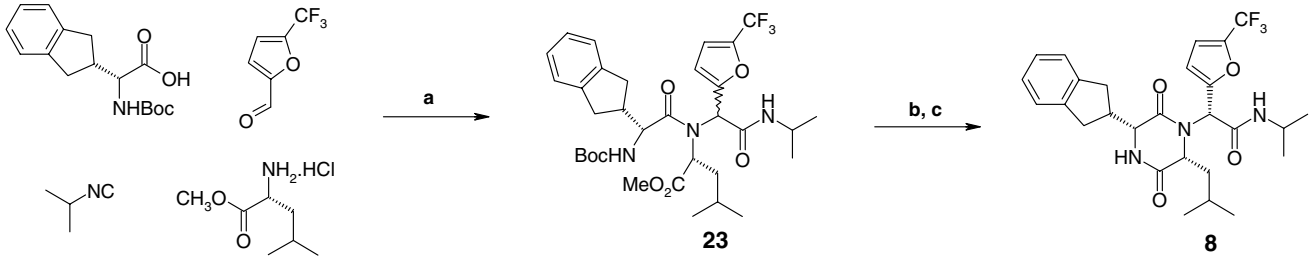
IV formulation 5:95 20% polyvinylpyrrolidone in DMSO: 35% sulfobutylether-7-betacyclodextrin in water.
[a] Clearance (mL/min/kg).
[b] Half life (h).
[c] AUC (hr ng/ml).
[d] Bioavailability.
[e] PO formulation 5:95 DMSO/PEG400 using amorphous.
[f] PO formulation: hydroxypropylmethylcellulose (HPMC)/Tween using crystalline material.

Table 5. Binding affinity at recombinant/native receptors

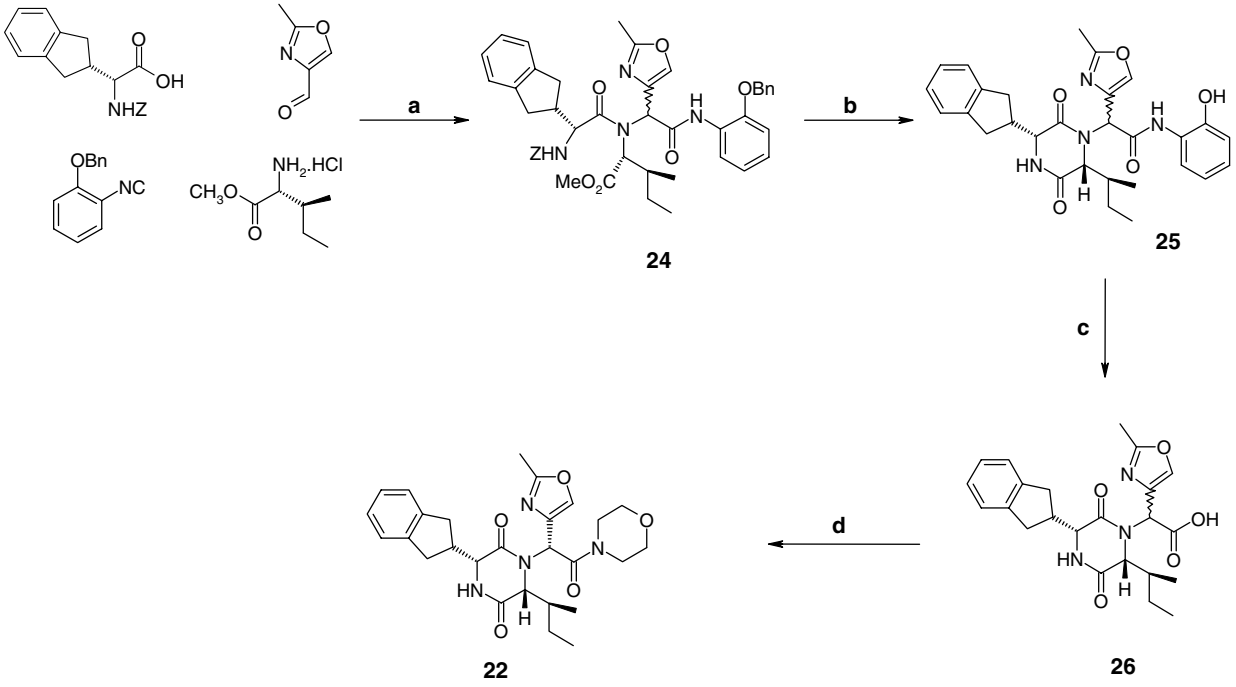
Receptor	GSK221149A K_i (nM)	Atosiban K_i (nM)
hOT	0.65	11
rOT	4.1	32
hV_{1a}	>12,000	0.15
hV_{1b}	>10,000	44
hV_2	950	330

and yielded the acids **26** with the required (*R*)-diastereoisomer as the major product. Acid activation with benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate followed by the addition of morpholine and subsequent column chromatography yielded homochiral GSK221149A.

In conclusion, GSK221149 has nanomolar affinity for the oxytocin receptor with >1400-fold selectivity over the closely related vasopressin receptors. GSK221149A has a good rat pharmacokinetic profile, low human microsomal clearance and has been shown to inhibit oxytocin-induced contraction in vivo in the anaesthetised rat.



Scheme 1. Reagents: (a) triethylamine, MeOH; (b) 4 M HCl in dioxan, MeOH; (c) triethylamine, DCM.



Scheme 2. Reagents and conditions: (a) triethylamine, MeOH; (b) H_2, Pd/C, ethanol/acetic acid; (c) carbonyl diimidazole, CH_2Cl_2 3 h then acetone/2 N HCl; (d) benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate, dichloromethane 1 h then morpholine.

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