Bioorganic & Medicinal Chemistry Letters 21 (2011) 3163–3167

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The discovery of novel 8-azabicyclo[3.2.1]octan-3-yl)-3-(4-chlorophenyl) propanamides as vasopressin V_{1A} receptor antagonists

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ARTICLE INFO

Article history:
Received 21 December 2010
Revised 21 February 2011
Accepted 23 February 2011
Available online 4 March 2011

Keywords:
Vasopressin
V_{1A}
Antagonist

ABSTRACT

The discovery of a novel series of 8-azabicyclo[3.2.1]octan-3-yl)-3-(4-chlorophenyl) propanamide antagonists of the vasopressin V_{1A} receptor is disclosed. Compounds **47** and **48** were found to be high affinity, selective vasopressin V_{1A} antagonists.

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Arginine vasopressin (AVP) is a 9 amino acid cyclic peptide produced in the hypothalamus. It is released from the posterior pituitary gland into the bloodstream or directly into the brain. AVP exerts its effects through four different G-protein coupled receptors, V_{1A}, V_{1B}, V_2 and oxytocin (OT). V_{1A} receptors are localised in the liver (glycogenolysis), vascular smooth muscle cells (vasoconstriction, uterine blood flow, contraction) and the brain (stress adaptation, memory formation, temperature, circadian rhythm). V_{1A} receptors are also localised in blood platelets and are involved in platelet aggregation. A selective V_{1A} antagonist may have clinical utility in dysmenorrhoea, preterm labour, hypertension, Raynaud's disease, depression, anxiety, hyponatremia and congestive heart failure.[1,2]

Relatively few small molecule V_{1A} antagonists have progressed into clinical development, reflecting the challenges associated with the development of non-peptidic ligands for the vasopressin family of peptide GPCRs. Relcovaptan (SR 49059, **1**), Sanofi-Aventis, is a highly potent and selective peripherally acting V_{1A} antagonist which has shown clinical efficacy for the treatment of Raynaud's disease, dysmenorrhoea and preterm labour (Fig. 1).[3–5] No further development has been reported for this compound. Johnson and Johnson have developed a brain penetrant V_{1A} antagonist, JNJ-17308616 (**2**) which was found to be active in a number of rat models of anxiety. Although JNJ-17308616 is a potent antagonist at human V_{1A} and selective against human V_{1B}, V_2 and OT, the compound's affinity for rat V_{1A} is lower and has equipotent affinity for rat V_2.[6] Azevan have developed selective V_{1A} antagonists SRX-251 (**3**) and SRX-246 (**4**), claimed to be orally bioavailable and brain penetrant.[7] SRX-251 was shown to inhibit aggression in hamsters. Both compounds have completed Phase 1 studies with Phase 2 studies planned for SRX-251 for the treatment of dysmenorrhoea.

Compound **5** (Table 1) was originally identified from an internal V_{1B} optimisation program. Routine cross screening at the human V_{1A} receptor revealed greater than two orders of magnitude higher affinity for the V_{1A} receptor over the human V_{1B} receptor (hV_{1A} $pK_i = 8.53$, hV_{1B} $pK_i = 5.90$). However, **5** suffers from high molecular weight (MWt = 641.3), high lipophilicity ($c\log P = 6.20$[8]) and high flexibility. This was accompanied by poor in vitro metabolic stability in human liver microsomes ($CL_{int} = 262$ μL/min/mg). In this publication the SAR exploration around compound **5** leading to a novel series of V_{1A} antagonists is disclosed. The initial optimisation strategy focussed upon improving the physico-chemical properties while maintaining high V_{1A} receptor affinity. Binding affinities for compounds at the human and rat V_{1A} receptors were measured in [^{3}H]AVP binding assays in CHO cells expressing

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doi:10.1016/j.bmcl.2011.02.096

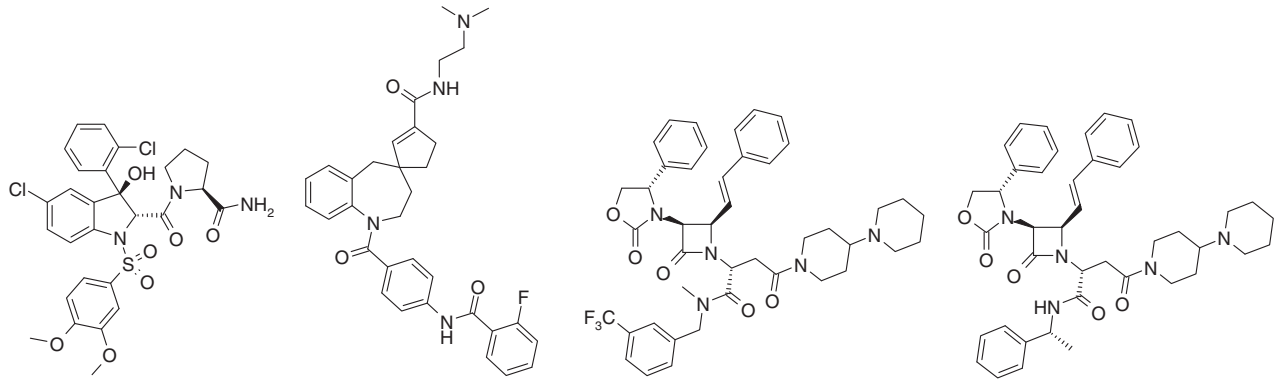
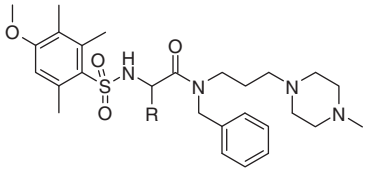


Figure 1. Structures of compounds **1**–**4**.

Table 1



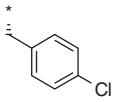
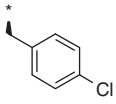
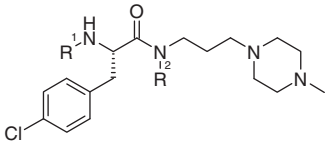
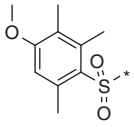
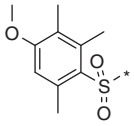
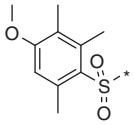
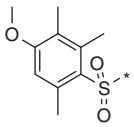
Compd	R	hV_{1A} pK_i
5		8.53
9		7.29
10		7.51
11		7.78
12		6.83
13	H	6.62

Table 2



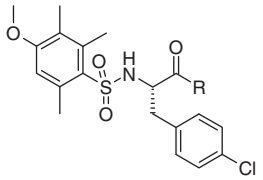
Compd	R^1	R^2	hV_{1A} pK_i
14	H	CH_2Ph	<6
15	SO_2Ph	CH_2Ph	6.63
16	SO_2CH_2Ph	CH_2Ph	6.63
17	COPh	CH_2Ph	6.03
18	$COCH_2Ph$	CH_2Ph	6.77
19		H	7.02
20		Et	7.25
21		*n*Pr	7.62
22		*i*Pr	7.82

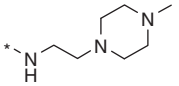
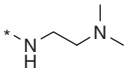
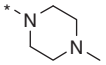
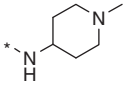
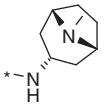
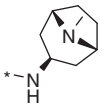
the human V_{1A} receptor or rat V_{1A} receptor, respectively. Binding affinities at human V_{1B}, V_2 and OT receptors were measured in [^{3}H]AVP or [^{3}H]OT binding assays in CHO cells expressing the human V_{1B}, V_2 or OT receptors, respectively. In all assays pK_i values were determined from a minimum of two independent experiments.

The compounds appearing in Tables 1–5 were synthesised via the general route outlined in Scheme 1. All starting materials and reagents were purchased from commercial sources unless otherwise stated. Boc-protected amino acids **6** were reacted with amines under standard amide coupling conditions to afford intermediates **7**. Amines were either commercially available or prepared by reductive alkylation according to Scheme 2. Boc-deprotection of intermediate **7** with TFA in DCM afforded amines **8** which were subsequently reacted with either a sulfonyl chloride or carboxylic acid to afford the desired sulfonamides or amides, respectively.

The initial optimisation strategy was to explore the potential importance of the substructural motifs of starting point **5** via a sequence of modifications and deletions. All compounds were initially measured for human V_{1A} affinity (Tables 1–4), subsequent compounds (Table 5) were measured for both human and rat V_{1A} affinity. Modifications around the 'amino-acid' moiety are shown in Table 1. Stereochemistry inversion to give the *R* enantiomer **9** resulted in a 17-fold decrease in V_{1A} affinity, suggesting that both enantiomers may afford high affinity V_{1A} ligands. Phenyl analogue **10** gave a 10-fold loss in affinity. The unsubstituted benzyl compound **11** showed a modest sixfold decrease in V_{1A} affinity compared to the 4-chloro benzyl starting point **5**. However, the saturated cyclohexyl methyl analogue **12** showed a 50-fold decrease in affinity. Deletion analogue **13** lost almost two orders of magnitude in V_{1A} affinity versus **5**. Table 2 shows changes to the aryl sulfonamide and the *N*-benzyl moieties. Deletion of the 4-methoxy-2,3,6-trimethylphenylsulfonyl group to afford primary

Table 3

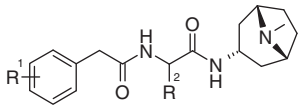


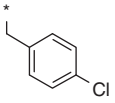
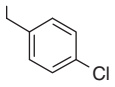
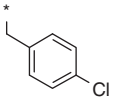
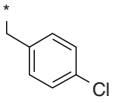
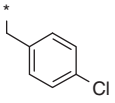
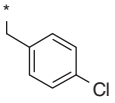
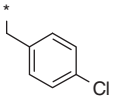
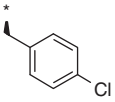
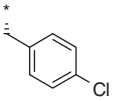
Compd	R	hV_{1A} pK_i
23	$NHCH_3$	6.47
24	$NHCH_2Ph$	8.25
25		7.90
26		6.96
27		6.74
28		6.95
29		7.31
30		6.89

amine **14** resulted in a profound loss of V_{1A} binding affinity. Unsubstituted phenyl and benzyl sulfonamides **15** and **16** showed almost two orders of magnitude decreases in affinity upon comparison with **5**. Phenyl amide **17** afforded a further decrease in V_{1A} affinity, although the benzyl amide analogue **18** showed similar affinity to that of the unsubstituted sulfonamides **15** and **16**. Deletion of the *N*-benzyl moiety to afford **19** gave a 32-fold decrease in affinity compared to the initial hit compound **5**. *N*-Ethyl analogue **20** showed a slight increase in affinity compared to the secondary amide **19**. Increasing the size of the alkyl substituent to *n*-propyl (**21**) and isopropyl (**22**) afforded further increases in affinity compared to **19**.

The initial deletion SAR indicated that removal of the *N*-benzyl moiety was slightly better tolerated than removal of the 4-chloro benzyl group. A decision was therefore taken to remove the *N*-benzyl substituent and focus on SAR exploration around **19**. Keeping

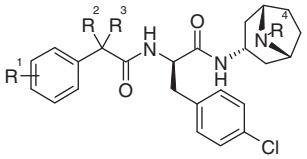
Table 4



Compd	R^1	R^2	hV_{1A} pK_i	HLM[a] CL_{int}	RLM[b] CL_{int}
31	H		7.05	23	<12
32	2-Cl		6.54		
33	3-Cl		7.25		
34	4-Cl		7.65		
35	2,3 Di-Cl		7.47		
36	3,4 Di-Cl		8.31	191	167
37	2,4 Di-Cl		7.40		
38	H		7.32	27	70
39	H		<5		

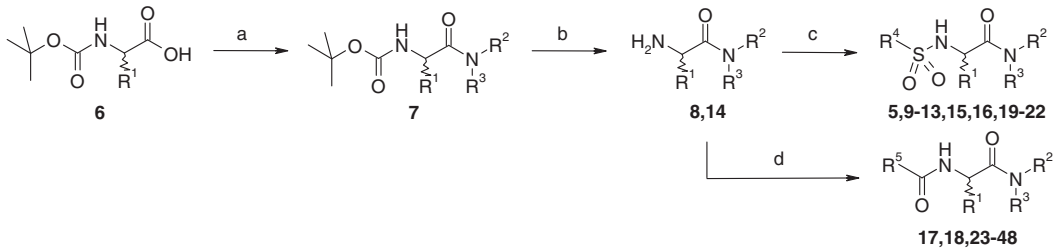
[a] In vitro human liver microsome intrinsic clearance (μL/min/mg).
[b] In vitro rat liver microsome intrinsic clearance (μL/min/mg).

Table 5

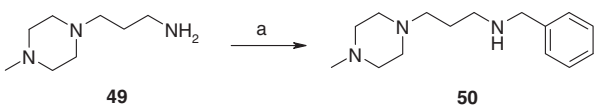


Compd	R^1	R^2	R^3	R^4	hV_{1A} pK_i	rV_{1A} pK_i	HLM[a] CL_{int}	RLM[b] CL_{int}
40	3,4 Di-Cl	H	H	H	6.89	6.71	29	42
41	4-Cl	H	H	CH_3	8.68	6.69	50	84
42	4-F	H	H	CH_3	7.57	<6	30	54
43	4-CH_3	H	H	CH_3	8.15	6.42		
44	4-OCH_3	H	H	CH_3	7.76	<6		
45	4-Cl	CH_3	CH_3	CH_3	8.50	6.88	84	119
46	4-Cl	▽		CH_3	8.78	7.26	274	259
47	4-Cl	H	H	H	9.63	7.66	<12	42
48	4-Cl	▽		H	9.25	8.38	58	55

[a] In vitro human liver microsome intrinsic clearance (μL/min/mg).
[b] In vitro rat liver microsome intrinsic clearance (μL/min/mg).



Scheme 1. Reagents and conditions: (a) R^2R^3NH, HBTU, DIPEA, DCM, rt; (b) 20% TFA in DCM, rt; (c) R^4SO_2Cl, Et_3N, DMF, rt; (d) R^5COOH, HBTU, DIPEA, DCM, rt.



Scheme 2. Reagents and conditions: (a) benzaldehyde, $NaBH_4$, MeOH, rt.

the 4-chloro benzyl and 4-methoxy-2,3,6-trimethylphenylsulfonyl groups in place, variation of the amide substituent was investigated in detail. Selected key analogues are shown in Table 3. Removal of the amine sidechain of **19** to give *N*-methyl amide **23** gave a 3.5-fold decrease in V_{1A} affinity whereas *N*-benzyl amide **24** showed a 17-fold increase in affinity. It was clear from the SAR that a basic moiety is not a requirement for high V_{1A} receptor affinity however it does positively contribute to physico-chemical properties such as solubility. The chain shortened piperazine analogue of **19**, compound **25** showed increased V_{1A} affinity although the dimethylamine equivalent **26** was of similar affinity to **19**. *N*-Methyl piperazine amide **27** and compound **28** also exhibited similar affinity to **19**. Amino tropane stereoisomers **29** and **30** both showed good V_{1A} affinity with the highest affinity residing with the *endo* isomer.

In order to further improve physico-chemical properties it was desirable to replace the sulfonamide moiety completely. Combination of 4-methoxy-2,3,6-trimethylphenyl sulfonamide replacements with different amide substituents (data not shown) led to the identification of compound **31** (Table 4), where the *endo* amino tropane amide substituent is combined with an *N*-benzyl amide sulfonamide replacement. Compound **31** shows good V_{1A} receptor affinity and importantly low intrinsic clearance in human and rat in vitro microsomal stability assays. This is perhaps consistent with the significantly lower lipophilicity of **31** ($c\log P = 2.87$) upon comparison to the starting compound **5** ($c\log P = 6.20$). In an effort to increase V_{1A} affinity yet maintain reasonable microsomal stability simple substitution around the *N*-benzyl amide was pursued (Table 4). 3-Chloro racemate **33** showed similar affinity to **31**, whereas the 2-chloro analogue **32** afforded a threefold decrease. The 4-chloro compound **34** resulted in a fourfold increase in V_{1A} affinity. Dichloro compounds **35** and **37** showed no further advantage over the 4-chloro derivative **34**. The 3,4-dichloro analogue **36** gave a 4.5-fold increase in affinity over **34**. However this was accompanied by high intrinsic clearances. Synthesis of the enantiomers of **31** yielded a surprising outcome. *R* Enantiomer **38** showed high V_{1A} affinity whereas the *S* enantiomer **39** was effectively inactive. It is speculated that the introduction of the amino tropane and benzyl amide moieties may lead to a slightly different binding mode to the sulfonamides. Human microsomal intrinsic clearance for compound **38** was similar to that of its racemate **31** but rat microsomal clearance was higher. In vivo pharmacokinetic studies in male Wistar rats for compound **38** (2 mg/kg iv and 10 mg/kg orally, 10% dimethylacetamide in water) showed 12% oral bioavailability. However, **38** ($pK_i \leqslant 5.5$) showed significantly lower rat V_{1A} receptor affinity which would compromise its use in pre-clinical in vivo models. The potential for species differences between human and rodent V_{1A} receptors for particular V_{1A} receptor antagonists has been described in the literature. Site directed mutagenesis experiments have revealed several key amino acids that contribute to the species difference, in particular a GLY337 (human) to ALA342 (rat) difference in transmembrane helix 7.[9,10]

A final optimisation cycle was undertaken in an effort to increase rat V_{1A} affinity while maintaining reasonable microsomal stabilities (Table 5). The secondary amine **40** showed decreased human V_{1A} affinity. This was accompanied by a greater than 10-fold increase in rat V_{1A} affinity compared to **38** resulting in similar affinities at the human and rat receptors whilst maintaining microsomal stability. Modifying the *N*-benzyl amide 4-position failed to make any major impact upon rat V_{1A} receptor affinity with compounds **41**–**44** all showing relatively low rat affinity upon comparison to the human receptor data. Maintaining a 4-chloro *N*-benzyl amide, substitution of the benzylic carbon was explored. Dimethyl analogue **45** maintained a similar profile to **41** whereas the cyclopropyl compound **46** demonstrated increased rat V_{1A} affinity. Combination of the 4-chloro benzyl amide and secondary amine to give compound **47** showed high human V_{1A} receptor affinity, good rat V_{1A} receptor affinity and reasonable microsomal intrinsic clearances. Compound **48** incorporating the cyclopropyl motif also showed high human and rat V_{1A} receptor affinity and moderate microsomal intrinsic clearance.

Compounds **47** and **48** were selected for further profiling. In selectivity screening versus human V_{1B}, V_2 and OT receptors both compounds showed low affinities across all three receptors ($pK_i \leqslant 5.0$). Functional antagonism in CHO cells expressing the human V_{1A} receptor was determined in a calcium flux assay. Both **47** ($pIC_{50} = 7.26$) and **48** ($pIC_{50} = 7.43$) acted as potent V_{1A} antagonists inhibiting the release of intracellular calcium induced by the vasopressin receptor agonist AVP. Furthermore **47** ($pK_i = 5.38$) and **48** ($pK_i = 5.80$) show relatively low affinity for the hERG channel in a [^{3}H]dofetilide binding assay.[11]

In vivo activity was assessed by the reversal of V_{1A} mediated AVP induced diastolic blood pressure increases in conscious rats as described in the literature.[3,12] Compound **47** (4 mg/kg, iv) decreased the AVP pressor response by 77.4 ± 12% and 58 ± 3% at 15 and 30 min post-drug, respectively ($n = 2$). Whereas **48** (4 mg/kg, iv) decreased the AVP pressor response for at least 60 min post-drug, with a maximal decrease of 50 ± 14% at 60 min ($n = 3$).[13]

Pharmacokinetic studies were performed in male Wistar rats at doses of 1 mg/kg iv and 10 mg/kg orally (Table 6). Plasma clearances were low to moderate for both compounds, reflecting improvements made in microsomal stability. Extensive distribution was also observed, resulting in promising half-lives (8–12 h). However, the oral C_{max} was very low for **47** and **48** and both showed disappointingly low oral bioavailability, suggesting poor absorption and/or extensive extra-hepatic first pass metabolism. Improving oral bioavailability would be a significant aspect of subsequent optimisation for the series.

In summary compounds **47** and **48** have been identified as novel selective vasopressin V_{1A} receptor antagonists. Both

Table 6
Pharmacokinetic parameters for compounds **47** and **48**

	47[a]	**48**[a]
CL[b] (mL/min/kg)	16	44
Vss[b] (L/kg)	10.8	17.7
$t_{1/2}$[b] (h)	11.5	8.2
C_{max}[c] (ng/mL)	6	19
t_{max}[c] (h)	1.2	3.5
F[c] (%)	<1	4

[a] 5% (v/v) cremophor EL in saline.
[b] 1 mg/kg iv dosing.
[c] 10 mg/kg po dosing.

compounds display reversal of V_{1A} mediated increases in diastolic blood pressure induced by the endogenous ligand AVP. However, both compounds show disappointing oral pharmacokinetic parameters, optimisation of which will be the subject of a subsequent publication.

Acknowledgements

We would like to thank our Analytical Chemistry colleagues for structure and purity determination.

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