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In Vitro Evaluation of New, Potent, and Selective V_2 Receptor Agonists

Kazimierz Wiśniewski, Hiroe Tariga, Glenn Croston, Diane M. Hargrove, Pierre J-M. Rivière, Gebhard Neyer, and Claudio D. Schteingart
Ferring Research Institute, San Diego, CA, 92121, USA

Introduction

The vasopressin analogue desmopressin (dDAVP, **1**) is a potent V_2 receptor agonist that also activates the related V_{1b} receptor [1]. dDAVP is approved in many countries for the treatment of diabetes insipidus, primary nocturnal enuresis, nocturia, and coagulation disorders including hemophilia A and von Willebrand's disease.

In search of novel, potent, selective and pharmacologically useful peptidic V2R agonists, we synthesized a series of *C*-terminally truncated [Val4]dDAVP (2) [2] analogs modified in positions 2, 3, 7 and/or at the disulfide bridge. The peptides were evaluated for *in vitro* potency at the humanV2 receptor (hV2R) and selectivity versus related receptors (hV1aR, hV1bR, hOTR). Here we present comprehensive *in vitro* data for the new compounds and describe synthetic methods used to prepare the analogues.

Results and Discussion

Analogues **3**-**24** (Table 1) were synthesized by a combination of solid and solution phase chemistry. The linear precursors of compounds **3**-**13**, **22** were assembled on H-Aaa-O-2-ClTrt resins by standard Fmoc chemistry using DIC/HOBt mediated couplings. The carba thioether modifications of the disulfide bridge (X or Y = CH_2, Figure 1) were introduced to the peptide sequence by coupling Fmoc-Cys($(CH_2)_3$-COOtBu)-OH or Fmoc-Hcy($(CH_2)_2$-COOtBu)-OH [3]. Fully protected peptide *C*-terminal acids were cleaved with 30% HFIP/DCM. For compounds **3**-**10** the carboxylic group was reduced to the hydroxymethyl group using mixed anhydride method [4]. For compounds **11**-**13** the *C*-terminal acids were coupled with agmatine and for analogue **22** the linear fragment was coupled with H-D-Arg(Pbf)-NEt_2. The protecting groups were removed with the TFA/TIS/H_2O 95/2.5/2.5 cocktail and the linear peptides were cyclized in DMF using HBTU/DIPEA method. The linear precursor of peptide **14** was assembled on 1,4-diaminobutane-2-ClTrt resin. After cleavage from the resin the *C*-terminal amino function was temporarily protected with the TFA resistant Z(2-Cl) group. The peptide was deprotected and cyclized as described above. The Z(2-Cl) group was removed with TMSBr/thioanisole/TFA (1/1/6) [5]. The linear precursors of compounds **15**-**21**, **23** and **24** were synthesized on BAL resin which was reductively alkylated with an appropriate primary amine prior to the peptide assembly. The peptides were cleaved with concomitant side chain protecting group removal using the TFA cocktail and cyclized as described above. All peptides were purified by preparative HPLC and lyophilized.

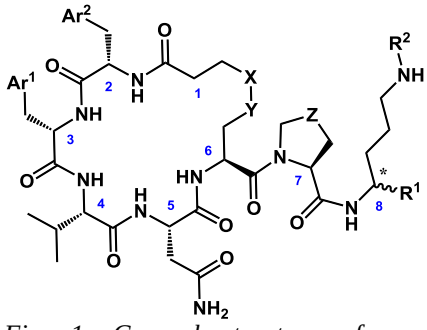

*Fig. 1. General structure of new analogues **3**-**24**. Sequence positions numbered at α-carbons.*

The pharmacological profile of **1** was determined in *in vitro* assays and was consistent with the literature data [1]. **1** was particularly selective vs. the $V_{1a}R$ (>1000-fold) presumably due to the desamino modification [6]. The Val^4 analogue of **1** ([Val^4]dDAVP, **2**) has been reported to be more potent and selective than **1** in rat *in vivo* models [7]. This profile is consistent with our *in vitro* studies at the human receptors (Table 1). Manning, et al. investigated the impact of the *C*-terminal Gly residue removal on the antidiuretic activity of **1**, **2** and related peptides in rats [7]. The corresponding desglycine analogues retained 10 - 50% initial antidiuretic activity, suggesting *C*-terminal truncation could be a good strategy to design novel V_2R agonists.

*Table 1. Structure and in vitro pharmacological profile of analogs **1-24**.*

Analog	Structure[a]			hV_2R		Selectivity[b] vs. receptor		
	Ar^1	R^1	Config of *C	EC_{50} (nM)	E_{max} (%)	$hV_{1a}R$	$hV_{1b}R$	hOTR
1	dDAVP			0.20	100	>5000[c]	55	550
2	[Val^4]dDAVP			0.05	89	>20000 [c]	480	7000
3	Ph(4-OH)	CH_2OH	S	0.10	92	>10000 [c]	520	2300
4	Ph(4-OH)	CH_2OH	R	0.08	93	>12000 [c]	720	2000
5	Ph(4-Cl)	CH_2OH	R	0.10	102	>10000 [c]	1400	>100000 [c]
6	Ph(4-Cl)	CH_2OH	R	0.08	115	>12000 [c]	800	>120000 [c]
7	Ph(4-Cl)	CH_2OH	S	0.14	96	>7100 [c]	2700	>71000 [c]
8	Ph(4-Cl)	CH_2OH	R	0.35	102	>2800 [c]	620	>28000 [c]
9	Ph(4-Me)	CH_2OH	R	0.39	104	>25000 [c]	2000	>25000 [c]
10	Ph(4-Et)	CH_2OH	S	0.35	110	>2800 [c]	>28000 [c]	>28000 [c]
11	Ph(4-Cl)	H	NA	0.31	89	>3200 [c]	450	>32000 [c]
12	Ph(4-Cl)	H	NA	0.07	104	>140000 [c]	1500	>140000 [c]
13	Ph(4-Cl)	H	NA	0.12	106	>83000 [c]	1100	>83000 [c]
14	Ph(4-OH)	H	NA	0.19	94	>5200 [c]	>52000 [c]	3400 [c]
15	Ph(4-Cl)	C(=O)NHMe	R	0.27	106	>3700 [c]	1200	>37000 [c]
16	Ph(4-Cl)	C(=O)NHEt	R	0.29	92	>3400 [c]	580	>34000 [c]
17	Ph(4-Cl)	C(=O)NHPr	R	0.33	92	>3000 [c]	750	>30000 [c]
18	Ph(4-Cl)	C(=O)NHcPr	R	0.23	86	>4300 [c]	2000	>43000 [c]
19	Ph(4-Cl)	C(=O)NHiPr	R	0.45	81	>2200 [c]	1200	>22000 [c]
20	Ph(4-Cl)	C(=O)NHBu	R	0.26	98	>38000 [c]	730	>38000 [c]
21	Ph(4-Cl)	C(=O)NHiBu	R	0.22	100	>45000 [c]	950	>45000 [c]
22	Ph(4-Cl)	$C(=O)NEt_2$	R	0.25	102	>40000 [c]	2100	>40000 [c]
23	Ph(4-Cl)	C(=O)NHBzl	R	0.19	100	>52000 [c]	780	>52000 [c]
24	Ph(4-Cl)	C(=O)NHEt	R	0.10	103	>100000 [c]	1600	>100000 [c]

*[a] XY=CH_2S for analogues **3**, **5**, **7-24** and SCH_2 for **4**, **6**; Ar^2=2-thienyl for **3-7**, **9**, **10**, **12-24** and 4-fluorophenyl for **8**, **11**; R^2=$C(=NH)NH_2$ for all new compounds except for **14** where R^2=H; [b] Ratio EC_{50}(receptor)/EC_{50}(hV_2R); [c] No agonism up to 1 uM or 10 uM, the highest concentration tested.*

First, the *C*-terminal group R^1 (–C(=O)-Gly-NH_2) in compound **2** was replaced with the hydroxymethyl function and the analogues were also modified in position 2, 3 and at the disulfide

bridge (compounds **3**-**10**). The disulfide bridge modifications (X, Y) as well as the configuration of the *C chiral carbon had very little impact on potency and selectivity profiles. Compounds in this series were potent hV_2R agonists with **9** and **10** (Ar^1 = 4-alkylphenyl) being about 2-fold less potent at the V_2R than **1**. Analogues **3**-**10** displayed improved selectivity versus both the $hV_{1b}R$ (all partial agonists) and hOTR except for the Tyr^2 compounds **3** and **4** (Ar^1 = 4-hydroxyphenyl) that were less selective vs. hOTR than **2**. Peptide **8** where Ar^2 = 4-fluorophenyl was less potent as an hV_2R agonist than its 2-thienyl counterpart **5**.

Next, we investigated if the substituent R^1 is actually required to preserve high agonistic potency at the hV_2R (compounds **11**-**14**). Compound **12** (R^1 = H, Ar^1 = 4-chlorophenyl, Ar^2 = 2-thienyl) was found to be very potent and considerably more selective than **2**. The Thz^7 modification (Z = S) was found to be neutral in this series as analogues **12** and **13** had very similar pharmacological profiles. In addition we demonstrated that the guanidino function (R^2 = -C(=NH)NH_2) does not appear to be essential to preserve hV_2R agonism (compound **14**, R^2 = H). Lastly, we explored if the *C*-terminal glycine amide could be replaced with alkyl groups (R^1 = -C(=O)-NR^3R^4 ; compounds **15**-**24**). Based on the initial results the cyclic part of the new analogues was fixed with the consensus structure (Ar^1 = 4-chlorophenyl, Ar^2 = 2-thienyl, X = CH_2, Y = S). Somewhat surprisingly a variety of R^3, R^4 substituents were well tolerated and rather shallow SAR was observed. The isopropyl compound **19** (R^3 = iPr, R^4 = H) was the least potent V_2R agonist in this subset. The Thz^7 modification was advantageous as exemplified by a 3-fold increase in potency for peptide **24** (Z = S) vs. **16** (Z = CH_2). Double alkylation, e.g. compound **22** (R^3 = R^4 = Et), was also well tolerated while maintaining excellent selectivity vs. the related receptors.

In conclusion, a series of novel *C*-terminally truncated dDAVP analogues with improved *in vitro* pharmacological profile has been identified. The novel compounds retain the potent V_2 receptor agonism activity of dDAVP, **1**. Compounds **5**-**13** and **15**-**24** display substantially improved selectivities vs. $hV_{1a}R$, $hV_{1b}R$ and hOTR as compared to **1** and **2**.

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